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REVIEW ARTICLE

IL-33 and IL-33 Receptors in Host Defense and Diseases

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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. IL-33 is considered to be crucial for induction of Th2-type cytokine-associated immune responses such as host defense against nematodes and allergic diseases by inducing production of such Th2-type cytokines as IL-5 and IL-13 by Th2 cells, mast cells, basophils and eosinophils. In addition, IL-33 is involved in the induction of non-Th2-type acute and chronic inflammation as a proinflammatory cytokine, similar to IL-1 and IL-18. In this review, we summarize and discuss the current knowledge regarding the roles of IL-33 and IL-33 receptors in host defense and disease development.

KEY WORDS

allergy, autoimmunity, basophil, chronic disease, eosinophil, host defense, IL-33, mast cell, ST2

REDISCOVERY OF IL-33

Interleukin-33 (IL-33) was originally identified as "DVS27," a gene which was upregulated in vasospastic cerebral arteries after subarachnoid hemorrhage,[1] and as a "nuclear factor from high endothelial venules (NF-HEV)," which is expressed in endothelial cell nuclei.[2] In 2005, DVS27 was rediscovered as IL-33 by using computational tools to search for sequences containing the β-trefoil structure seen in IL-1- and FGF-like proteins. IL-33 (also called IL-1F11) is now regarded as the 11th member in the IL-1 family of cytokines, which includes IL-1α, IL-1β and IL-18.[3] Expression of IL-33 mRNA/protein is observed in various organs and types of cells (Table 1). In the literature, relatively high levels of IL-33 mRNA expression are observed in the brain and spinal cord of mice.[3]

IL-33 AS A NUCLEAR FACTOR

IL-33 has the closest amino acid sequence homology to IL-18 among the members of the IL-1 cytokine family.[3] In striking contrast to the other IL-1-related cytokines except for IL-1α, IL-33 is localized in the nucleus of human epithelial and endothelial cells[2] and mouse bone-marrow derived cultured mast cells (BMCMCs)[4] by binding to chromatin via a homeodomain (helix-turn-helix-like motif) and nuclear localization signal in its amino-terminus (Fig. 1).[5,6] Although the pathophysiological role of IL-33 as a nuclear factor is not fully understood, IL-33 is known to bind to the acidic pocket of a dimeric histone, H2A-H2B, on the surface of nucleosomes, resulting in suppression of gene transcription, at least in the *in vitro* reporter assay system.[6]

IL-33 RECEPTOR AND SIGNAL TRANSDUCTION

Schmitz *et al.* first identified the orphan receptor "ST2" (also called IL-1R4) as a receptor for IL-33.[3] As in the case of receptors for the other IL-1-related cytokines, IL-33 receptor (IL-33R) is formed from heterodimeric molecules, consisting of ST2 and IL-1R accessory protein (IL-1RAcP).[7,8] IL-1RAcP is a shared component of receptors for IL-1α, IL-1β, IL-1F6, IL-1F8 and IL-1F9.[9-11] In this review, the complex of ST2 and IL-1RAcP is designated as "IL-33R1."

Two major products of ST2 genes (transmembrane form ST2 [ST2 or ST2L] and soluble form ST2 [sST2]) are produced by alternative splicing under the control of two distinct promoters.[12] In addition, ST2LV and sST2V, which are other splicing variants for ST2L and sST2, respectively, have also been iden-

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Table 1 Expression of IL-33

Reference	Spices	Detected form	Specimens	Detail information
Schmitz *et al*. Immunity. 2005	mouse	mRNA	organ	High level: stomach, lung, spinal cord, brain, and skin
				Lower level: lymph node, spleen, pancreas, kidney, and heart
		mRNA	cell	Bone marrow cell-derived cultured dendric cells
				Bone marrow cell-derived cultured macrophages: with LPS
	human	mRNA	cell	Bronchial smooth muscle cell and bronchial and small airway epithelial cell
				Primary lung and dermal fibroblasts and keratinocytes: with TNF and IL-1
Carriere *et al.* Proc Natl Acad Sci U S A. 2007	human	mRNA	cell	Tonsil endothelial cells, endothelial cells from Crohn's disease intestine and RA synovium
Sanada *et al*. J Clin Invest. 2007	rat	mRNA	cell	Cardiac fibroblasts
Hayakawa *et al.* J Biol Chem. 2007	mouse	mRNA	organ	Thymus, lung, lymph node, ovary and testis (OVA-induced asthmatic model)
Verri *et al*. Proc Natl Acad Sci U S A. 2007	mouse	mRNA	organ	Plantar tissue (methylated BSA-induced cutaneous and articular mechanical hypernociception model)
Miller *et al*. J Exp Med. 2007	mouse	mRNA	organ	Thoracic aorta (ApoE-deficient mice fed a high-fat diet)
		protein	organ	Adventitia of the aorta (ApoE-deficient mice)
	human	mRNA	cell	Primary cultured HUVECs, saphenous vein endothelial cells, saphenous vein and coronary artery smooth muscle cells
		protein	cell	Heart small vessel endothelial cells
Hudson *et al*. J Leukoc Biol. 2008	mouse	mRNA	cell	Glia cells: with dsRNA, LPS, PAM3Cys or IL-1
		protein	cell	Glia cells and astrocytes: with dsRNA, LPS, LPS + ATP and/or PAM3Cys
Xu *et al*. Proc Natl Acad Sci U S A. 2008	human	mRNA	cell	Synovial fibroblasts from RA patients: with TNF or TNF + IL-1
		protein	cell	Synovial fibroblasts from RA patients: with TNF or TNF + IL-1
		protein	organ	Synovial membranes from RA patients
Küchler *et al*. Am J Pathol. 2008	human	protein	organ	Endothelial cells in vessels of skins, small intestines, umbilical veins and lungs and HUVECs
Kurowska-Stolarska *et al*. J Immunol. 2008	mouse	protein	cell	F4/80+ CCR3- alveolar macrophages
Moussion *et al*. PLoS One. 2008	human	protein	cell	Large vessel endothelial cells (colons, small intestines, stomachs, kidneys, lungs, livers, fallopians and prostates)
				Small vessel endothelial cells (livers, skeletal muscles, kidneys, prostates and skins)
				Epithelial cells (stomach, tonsillar crypts and salivary glands)
				HEV endothelial cells, fibroblastic reticular cells (interfollicular T cell area) and keratinocytes
Goh *et al*. Immunology. 2009	mouse	mRNA	cell	Bone marrow cell-derived cultured macrophages: with LPS
Sakashita *et al*. Clin Exp Allergy. 2008	human	protein	serum	Japanese cedar pollinosis
Bartunek *et al*. J Am Coll Cardiol. 2008	human	mRNA	organ	Hearts
		protein	organ	Coronary artery endothelium

Chapuis *et al*. Mol Psychiatry. 2009	human	mRNA	organ	Brain from Alzheimer's disease patients< control group
		protein		Small meningeal and superficial cortical small vessels
			cell	Brain endothelial and vascular smooth muscle cells
Palmer G *et al*. Arthritis Rheum. 2009	mouse	mRNA	organ	Artiritic inflammed lesions (collagen-induced arthritis model)
	human	mRNA	cell	Synovial fibroblasts: with IL-1, TNF or TNF + IL-1
		protein	organ	RA synovium
			cell	HEV endothelial cells and synovial lining cells from synovium of RA patients
				Synovial fibroblasts: with TNF and IL-1
Wood *et al*. Biochem Biophys Res Commun. 2009	human	mRNA	organ	White adipose tissue
			cell	Preadipocytes and adipocytes: with TNF, Preadipocytes: hypoxia (1% O2)
Matsuda *et al*. Invest Ophthalmol Vis Sci. 2009	human	mRNA	cell	Immortalized conjunctival cell lines and conjunctival fibroblasts: with IL-1
		protein	organ	Giant papillae from atopic keratoconjunctivitis patients
			cell	Giant papillae vascular endothelial and epithelial cells from atopic keratoconjunctivitis patients
				Conjunctival fibroblasts: with IL-1 or IL-1 + IFN-g (pro-IL-33)
				Immortalized conjunctival cell lines and conjunctival fibroblasts (mature-IL-33)
Pushparaj *et al*. Proc Natl Acad Sci U S A. 2009	human	mRNA	organ	Skin from atopic dermatitis patients
		protein	serum	Atopic patients with anaphylactic shock
			organ	Skin from atopic dermatitis patients
Lüthi *et al*. Immunity. 2009	human	protein	cell	THP-1 cells: with LPS, necrotic cells
Préfontaine, J Immunol. 2009	human	mRNA	organ	Lungs from asthmatic patients
			cell	Airway smooth muscle cells from asthmatic patients, primary airway smooth muscle cells: with TNF
		protein	cell	Airway smooth muscle cells from asthmatic patients, primary airway smooth muscle cells: with TNF
Kurowska-Stolarska *et al*. J Immunol. 2008	human	protein	cell	Lung epithelial cells from asthmatic patients
Seidelin *et al*. Immunol Lett. 2009	human	mRNA	cell	Colonocytes from patients with ulcerative colitis
		protein	cell	Colonocytes from patients with ulcerative colitis
Matsuyama *et al*. J Rheumatol. 2010	human	protein	serum	RA patients
			organ	Synovila fluids from RA patients
			cell	Fibroblast like synoviocytes from RA patients
Ohno *et al*. J Immunol. 2009	mouse	mRNA	cell	Peritomeal macrophages, splenic dendritic cells: with LPS
				Bone marrow cell-cultured mast cells: with IgE, IgE + antigen
		protein	cell	Peritomeal macrophages: with LPS, bone marrow cell-cultured mast cells: with IgE + antigen
Nishida *et al*. Gut. 2009	human	protein	cell	Myofibroblasts from chronic pancreatitis patients
				Pancreatic myofibroblasts: with IL-1, TNF and LPS

HEV, high endothelial venules; HUVEC, human umbilical vein endothelial cells; RA, rheumatoid arthritis.

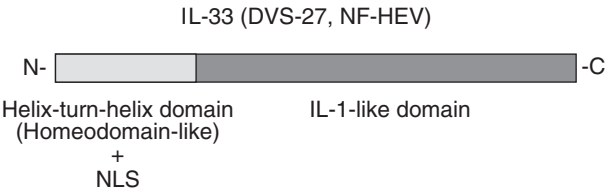

Fig. 1 IL-33, a 270-amino-acid protein, consists of two domains: a homeodomain and a cytokine (IL-1-like) domain. The homeodomain contains a nuclear localization signal (NLS).

tified in chickens and humans.[13,14] ST2 is considered to be the functional component for induction of IL-33 bioactivities, while sST2 acts as a decoy receptor for IL-33, similar to soluble IL-1Rs for IL-1.[9-11]

The signal transduction downstream of IL-33R1 is mediated by adapter molecules that are shared by other IL-1 receptor family members such as IL-1R and IL-18R. The binding of IL-33 to IL-33R1 results in recruitment of MyD88 to the Toll-interleukin-1 receptor (TIR) domain in the cytoplasmic region of ST2, leading to induction of inflammatory mediators by activating transcription factors such as NF-κB and AP-1 through IRAK, TRAF6 and/or MAP kinases (Fig. 2).[3]

Recently, it has been shown that IL-33 binds to another IL-33R different from IL-33R1. In addition to IL-1RAcP, ST2 forms a complex with another IL-1R family molecule, "single Ig IL-1R-related molecule (SIGIRR) (also called Toll IL-1R8 [TIR8])".[15] In this review, the complex of ST2 and SIGIRR is designated as "IL-33R2". SIGIRR/TIR8 is considered to act as a negative regulator for IL-1R- and Toll-like receptor (TLR)-mediated immune responses.[16] Indeed, SIGIRR/TIR8-deficient dendritic cells showed hyperresponsiveness to stimulation with IL-1, IL-18 and TLR agonists.[17] In addition, SIGIRR/TIR8-deficient Th2 cells showed augmented Th2-type cytokine production in response to IL-33.[15] In contrast to IL-33R1 (ST2/IL-1RAcP), IL-33R2 seems to act as a negative regulator of IL-33.

TARGET CELLS OF IL-33

Th2 CELLS

It is well established that IL-4 is a key cytokine for the differentiation of Th2 cells from naïve CD4+ T cells. ST2 is predominantly expressed on Th2 cells but not naïve T cells, Th1 cells, Th17 cells or regulatory T cells.[18-20] On the other hand, ST2 is not essential for Th2 cell differentiation, as shown in studies using ST2-deficient mice: ST2-deficient mice showed normal development of Th2 cells.[21,22] In support of that, although IL-33 did not induce differentiation of Th2 cells from naïve CD4+ T cells *in vitro*,[23,24] it enhanced IL-5 and IL-13, but not IL-4, production by *in vitro*-skewed Th2 cells which highly express ST2.[3,7,23,25,26] In humans, IL-33 potentiates not only Th2-type cytokine production but also production of a Th1-type cytokine, IFN-γ, by peripheral blood-derived Th2 cells,[27] although IFN-γ production is only slightly increased by peripheral blood-derived human Th1 cells.[26] In addition, IL-33 acts as a chemoattractant for Th2 cells, but not Th1 cells, in both humans and mice.[28]

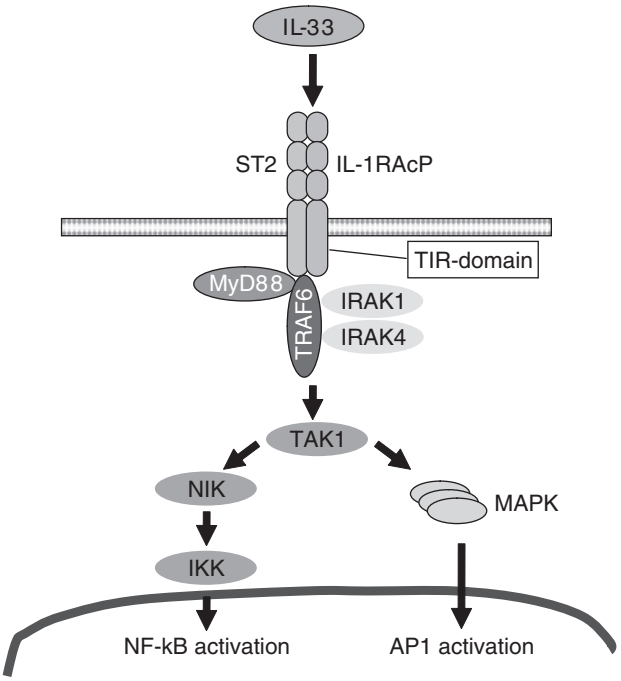

Fig. 2 IL-33 binds to IL-33 receptor, which is a dimer of ST2 and IL-1RAcP. The TIR-domain of IL-33 receptor recruits MyD88 and TRAF6, and the receptor signal results in activation of NF-kB or AP-1.

In contrast to the role of IL-33 in Th2 cell differentiation, Kurowska-Stolarska *et al*. reported that IL-33 induces differentiation of IL-5-positive IL-4-negative CD4+ T cells (IL-5+IL-4- Th cells) from naïve CD4+ T cells independently of IL-4, STAT-6 and GATA-3, which are important factors for the typical Th2 cell differentiation.[24] While ST2-expressing Th cells are also observed in IL-4-, IL-5- or IL-10-deficient mice,[19,29] two distinct populations of IL-4-producing Th cells were found: ST2-positive Th2 cells, which produce IL-4, IL-5 and IL-10, and ST2-negative Th2 cells, which produce IL-4 and IL-10, but not IL-5, in mice during *Leishmania major* infection.[30] Further evidence regarding the role of IL-33 in the differentiation of typical and atypical Th2 cells may provide new insight into the molecular mechanisms in Th2-type cytokine-mediated disorders such as allergic asthma.

In addition to CD4+ Th2 cells, it has been shown that type II CD8+ cytotoxic T cells (Tc2 cells) and IL-10-producing Tr1 cells also express ST2 on their cell surface.[31,32] However, the precise roles of IL-33 in Tc2 and Tr1 cells remain unclear.

MAST CELLS (MCs)

MCs, which express c-Kit and high-affinity IgE receptors (FcεRI) and are predominantly localized in mucosal and connective tissues, are major effector cells in the induction of IgE-mediated immune responses. After binding of antigens (Ags) to IgE-bearing MCs via FcεRI, MCs rapidly release a large variety of inflammatory mediators from their granules, thereby provoking local and systemic inflammation. Mouse MCs (i.e., BMCMCs and connective tissue-type MCs from the peritoneal cavity) and MC/basophil precursor cells and human MCs (i.e., cord blood and peripheral blood stem cell-derived cultured MCs) constitutively express ST2.[33-36] Except for IL-3 and stem cell factor (SCF, a ligand for c-kit), which are required for mast cell development at least in mice, IL-33 is the only cytokine among 45 different cytokines which can directly induce cytokine and chemokine (IL-1β, IL-6, IL-13, TNF and MCP-1) secretion from mouse BMCMCs without effecting their degranulation.[37,38] Like its murine counterpart, human IL-33 can induce cytokine and chemokine production, prolong survival and promote cell-adhesion in human cord blood stem cell-derived cultured MCs.[35,36] In addition, IL-33 can augment IgE-mediated cytokine production and degranulation by mouse BMCMCs and/or human cord blood stem cell-derived cultured MCs.[35-37,39] IL-33-mediated cytokine production by mouse BMCMCs and human cord blood stem cell-derived cultured MCs is enhanced in the presence of IL-3 and thymic stromal lymphopoietin (TSLP), respectively.[23,36]

Although the levels of phorbol ester + ionophore-induced IL-4 production and IgE + antigen-mediated histamine release from ST2-deficient BMCMCs are comparable to those from wild-type BMCMCs,[21] ST2-deficient BMCMCs do not produce cytokines in response to IL-33.[23] Therefore, IL-33-induced mast cell-derived cytokines are not involved in IL-4 production or IgE-dependent histamine release.

BASOPHILS

Basophils, which express FcεRI, but not c-Kit, on their cell surface, are considered to be a potential primary source of IL-4 in certain allergic immune responses.[40,41] Supporting this, it was recently reported that basophils express MHC class II and present Ags to naïve T cells as an Ag-presenting cell, inducing Ag-specific Th2 cell differentiation in lymph nodes that is dependent on IL-4 production and Ag-presentation by activated basophils.[42-44]

In comparison with Th2 cells and MCs, human and mouse basophils constitutively express ST2 at a relatively low level on their cell surface.[23,26,45,46] On the other hand, expression of ST2 on the cell surface of basophils is promoted by stimulation with IL-3.[26] Like the effect of IL-33 on Th2 cells and MCs, IL-33 alone can induce production of cytokines, including Th2-type cytokines, and chemokines by basophils and promote cell-adhesion and CD11b expression by human and murine basophils.[26,27,45,46] IL-33 does not induce degranulation of basophils directly, but it synergistically enhances IgE-mediated degranulation of human basophils.[26,45] In addition, IL-33 augments immune responses of human and murine basophils in humans and mice: eotaxin-mediated migration,[45] cytokine secretion in the presence of IL-3, which is a growth factor for basophils as well as mast cells,[23,26,27,45-47] and prolonged survival in the presence of IL-3 or GM-CSF.[45-47] These observations suggest that IL-33 is a potential activator of basophils by enhancing their cytokine and chemokine secretion, recruitment and adhesion.

EOSINOPHILS

Eosinophilia is found in local inflammatory sites in patients with certain IgE-mediated allergic disorders, such as asthma. Although ST2 expression was barely detectable on the cell surface of human peripheral blood eosinophils, ST2 mRNA and intracellular ST2 protein were detectable in them.[26,48,49] IL-33 can directly induce production of superoxide and IL-8 and enhance IL-3-, IL-5- or GM-CSF-mediated IL-8 production by human eosinophils.[26,48] As in the case of MCs and basophils, IL-33 enhances adhesion of eosinophils by promoting CD11b expression and survival independently of IL-4, IL-5 and GM-CSF.[49] Unlike the case of basophils, IL-33 does not influence eotaxin-mediated migration of eosinophils.[49] The role of IL-33 in the degranulation of eosinophils remains controversial. One group demonstrated that IL-33 alone could enhance degranulation (EDN release) of human eosinophils,[48] whereas another showed that IL-33 could not (assessed by EDN and LTC4 release).[49] These observations strongly suggest that IL-33 may contribute to the pathogenesis of certain allergic disorders accompanied by marked accumulation of eosinophils.

NATURAL KILLER (NK) CELLS AND NKT CELLS

As in the case of Th2 and Tc2 cells, ST2 expression was observed on the cell surface of IL-4-producing NK cells (NK2 cells), but not IFN-γ-producing NK cells (NK1 cells), which were derived *in vitro* from a freshly-isolated NK cell population of human PBMCs under Th1/Th2 cytokine-skewed culture conditions.[31] Smithgall *et al*. demonstrated that freshly-isolated NK cells from human PBMCs could produce IFN-γ in response to IL-33 in the presence, but not absence, of IL-12 or IL-23, since IL-12 and/or IL-23 enhanced the expression of ST2 mRNA in NK cells.[27] However, that study did not elucidate the role of IL-33 in Th2-type cytokine secretion by NK cells.

Smithgall *et al*. also detected ST2 mRNA expression in human invariant NKT (iNKT) cells and demonstrated a role for IL-33 in these cells: IL-33 en-

hanced TCR-dependent cytokine production (i.e., IFN-γ, IL-2, IL-4, IL-5, IL-13 and TNF) by iNKT cells after stimulation with α-galactosylceramide (α-GalCer).[27] Moreover, IL-33 enhanced IFN-γ, but not IL-4, production by iNKT cells in the presence, but not absence, of IL-12, independently of TCR stimulation.[27] As in the case of NK cells, IL-12 enhances ST2 mRNA expression in human iNKT cells.[27]

Administration of IL-33 to mice results in increased expansion of iNKT cells in the spleen and liver.[50] Thymic iNKT cells constitutively express ST2 on their cell surface, and IL-33 enhances IL-7-mediated thymic iNKT cell proliferation.[50] IL-33 alone could not induce cytokine secretion by naïve mouse iNKT cells. On the other hand, similar to the study in human iNKT cells,[27] IL-33 enhanced both IL-4 and IFN-γ production by TCR-stimulated mouse iNKT cells and IFN-γ, but not IL-4, production by mouse iNKT cells in the presence of IL-12, independently of TCR stimulation.[50] In contrast to the findings for human iNKT cells,[27] IL-12 could not enhance ST2 expression in mouse iNKT cells. These observations suggest that IL-33 may have a non-Th1/Th2 cytokine-restricted role in certain NK cell- and NKT cell-mediated immune responses.

DENDRITIC CELLS (DCs)

IL-33 is considered to promote the development of DCs from bone marrow cells.[51] It has been shown that DCs derived by cultivation of murine bone marrow cells in the presence of GM-CSF and IL-4 (that is, bone marrow-derived DCs; BMDCs) express ST2.[52] IL-33 enhances the production of IL-6, but not IL-12, by BMDCs and augments the expression of MHC class II and CD86, but not CD80, CD40 and "OX40 ligand (OX40L)", on the cell surface of BMDCs.[52] When naive $CD4^+$ T cells were co-cultured with BMDCs in the presence of IL-33 for 6 to 10 days, IL-5 and IL-13, but not IL-4 or IFN-γ, were detected in the culture supernatant even without TCR engagement. Since such cytokine secretion was not induced by IL-33 in the culture of naïve $CD4^+$ T cells alone, the effect of IL-33 seemed to be mediated by factors derived from IL-33-stimulated BMDCs through a TCR/Ag-MHC class II-independent pathway. However, the secreted cytokine profiles (IL-5 and IL-13, but not IL-4, production) in the setting (BMDCs + naïve $CD4^+$ T cells + IL-33, no Ag) are similar to those by the IL-5-positive, IL-4-negative atypical Th2 cell population observed in the culture of naïve $CD4^+$ T cells stimulated by TCR engagement plus IL-33, as described above. Thus, these observations suggest that IL-33 can enhance induction of an IL-5-positive, IL-4-negative atypical Th2 cell population from naïve $CD4^+$ T cells directly and/or indirectly from DCs via the effects of certain factors.

Like IL-33, IL-25 and TSLP are known to be Th2-prone cytokines and contribute to the induction of Th2-type cytokine-mediated immune responses.[53] In contrast to the case of IL-33, TSLP-activated DCs promote IL-4-producing Th2 cell differentiation from naïve $CD4^+$ T cells in the presence of TCR engagement through OX40L-OX40 interaction, at least in part.[54,55] IL-25 can enhance TSLP-stimulated DC-mediated Th2 cell expansion.[56] Unlike IL-33, both TSLP and IL-25 can induce differentiation of IL-4-producing Th2 cells from naïve $CD4^+$ T cells after TCR engagement, dependent on the IL-4-IL-4Rα-STAT6 pathway.[57,58] Therefore, these observations suggest that the roles of IL-33, TSLP and IL-25 in T cells and DCs may be different in Th2-type cytokine-mediated immune responses. That is, TSLP and IL-25 may be preferentially involved in the induction of antigen-specific IL-4/IL-5/IL-13-producing Th2 cell-mediated immune responses, while IL-33 may contribute, at least in part, to the induction of antigen-non-specific Th2 cell-mediated immune responses by inducing IL-5/IL-13-, but not IL-4-, and thereby producing atypical Th2 cells.

MACROPHAGES

Constitutive expression of ST2 mRNA/proteins was detected in mouse bone marrow cell-derived cultured macrophages and mouse alveolar macrophage cell lines.[59,60] Soluble ST2 expression was increased in macrophages in response to LPS and proinflammatory cytokines such as TNF, IL-1 and IL-6.[60-62] IL-33 promoted the expression of LPS receptor components, such as MD2, TLR4, soluble CD14 and MyD88.[63] Although IL-33 alone did not induce TNF, IL-1 or IL-6 production by thioglycolate-induced mouse peritoneal macrophages, it did in the presence of LPS.[63] Such effects of IL-33 on LPS-mediated activation were abolished in anti-ST2 Ab-treated and ST2-deficient macrophages. Therefore, IL-33 may be a potential activator of macrophages during bacterial infections.

In addition, both naïve and thioglycolate-induced mouse peritoneal macrophages produced IL-33 upon LPS stimulation,[4] suggesting that macrophage-derived IL-33 may autocrinely enhance LPS-mediated macrophage activation. Supporting this, LPS-mediated production of cytokines, such as IL-1, IL-6, IL-12 and/or TNF, by mouse bone marrow cell-derived cultured macrophages or mouse alveolar macrophage cell lines was inhibited by addition of soluble ST2-Fc fusion proteins.[59,60]

It is well known that macrophages are key effector cells during septic shock. It was shown that mice treated with polyclonal anti-ST2 antiserum, which had potential activity to deplete ST2-expressing cells, including macrophages,[64] were highly susceptible to LPS-induced endotoxin shock,[59] suggesting the importance of ST2-expressing macrophages for protection against this event. Consistent with the effect of soluble ST2-Fc fusion proteins on macrophage activa-

tion by LPS, mice treated with soluble ST2-Fc fusion proteins were resistant to endotoxin shock and showed reduced serum IL-6 and TNF levels after intraperitoneal LPS injection.[59]

However, in contrast with the effect of soluble ST2-Fc fusion proteins,[59,60] IL-6, IL-12 and TNF productions by ST2-deficient thioglycolate-induced peritoneal macrophages were increased in response to LPS.[65] In addition, ST2-deficient mice showed high susceptibility to LPS-induced endotoxic shock.[65] The apparent discrepancy between the results using macrophages treated with soluble ST2-Fc fusion proteins and macrophages deficient in ST2 may be explained as follows. Perhaps ST2-deficiency results in increased formation of other IL-1R family molecules, such as IL-1R (IL-1R1 and IL-1RAcP), due to the failure of formation of IL-33R1 (ST2 and IL-1RAcP), causing cytokine hyperproduction by ST2-deficient macrophages in response to IL-1, which can be produced by these macrophages after LPS stimulation. Indeed, ST2-deficient macrophages produced larger amounts of cytokines than wild-type macrophages after IL-1β treatment.[65] Although IL-1R1-deficient mice showed normal susceptibility to LPS-induced endotoxic shock,[66] IL-1R antagonist-deficient mice, which have excessive IL-1-signaling, showed high susceptibility.[67] This suggests that IL-1 is not required for induction of LPS-induced endotoxic shock, but excessive IL-1 production leads to amplified susceptibility to LPS, as seen in ST2-deficient mice.

Macrophages are phenotypically divided into two distinct populations: "classically activated macrophages (CAMϕ)/type 1 macrophages (M1)" and "alternatively activated macrophages (AAMϕ)/type 2 macrophages (M2)." CAMϕ/M1s are generated in response to IFN-γ and LPS (or TNF induced by bacterial components) and are involved in Th1-type immune responses such as host defense against viral and bacterial infections and tumor rejection by producing IL-12, IL-23 and nitric oxide. M2 macrophages are further subdivided into at least three populations by *in vitro* stimulation with distinct factors: IL-4- and/or IL-13-stimulated M2a (also called AAMϕ), immune complex plus IL-1β- or LPS-stimulated M2b (also called typeIIMϕ) and IL-10-, TGF-β- or glucocorticoid-treated M2c (also called deactivated Mϕ).[68-71]

IL-13 enhances ST2 expression in mouse bone marrow cell-derived cultured macrophages, and IL-33 amplifies polarization of M2a/AAMϕs in the presence, but not absence, of IL-13, contributing to the induction of Th2-type immune responses.[72]

CD34-POSITIVE HEMATOPOIETIC PROGENITOR CELLS

$CD34^+$ hematopoietic progenitor cells are capable of differentiating into various types of cells in the bone marrow and peripheral tissues. Recently, it was shown that mRNA and cell surface expression of ST2 as well as TSLPR were found in $CD34^+$ hematopoietic progenitor cells derived from human umbilical cord blood cells.[73] IL-33 enhances the production of various cytokines and chemokines, including IL-5, IL-13, CCL17 and CCL22, by human umbilical cord blood cell-derived $CD34^+$ hematopoietic progenitor cells in cooperation with TSLP in the presence of IL-3 and SCF.[73] Interestingly, the number of $CD34^+$ hematopoietic progenitor cells was increased in peripheral blood from allergic patients.[73] In addition, IL-5- and IL-13-producing $CD34^+$ hematopoietic progenitor cells were also detected in sputum from patients with asthma. These observations suggest that $CD34^+$ hematopoietic progenitor cells may themselves be potential effector cells by responding to IL-33 and TSLP even in the undifferentiated state and contributing to the development of allergic diseases.

NATURAL HELPER CELLS

Adipose tissue-associated Lin^- c-Kit^+ Sca-1^+ natural helper cells are a newly identified population distinct from lymphoid progenitors and lymphoid tissue inducer cells.[74] Natural helper cells constitutively express ST2 and can produce larger amounts of IL-5 and IL-13 than basophils and mast cells in response to IL-33. IL-33-mediated natural helper cell activation was shown to be important for development of goblet cell hyperplasia during *Nippostrongylus brasiliensis* infection.[74]

PRODUCERS AND RELEASE OF IL-33 AS "Alarmin"

During host defense against pathogens, innate immune cells recognize pathogen-associated molecular patterns (PAMPs) directly via Toll-like receptors (TLRs), resulting in induction of local and/or systemic inflammation. In addition, endogenous proinflammatory factors called "damage associated molecular patterns (DAMPs)" (also called "alarmin"), which are released by necrotic cells in injured tissues during trauma and/or infection, also provoke local and/or systemic inflammation by acting as an endogenous danger signal that often promoting immune responses.[75] For example, high-mobility group box 1 (HMGB1), which was initially identified as a nuclear factor acting as a transcriptional regulator, is released by macrophages in response to LPS, leading to induction of inflammation.[76] Like HMGB1, several recent lines of evidence suggest that IL-33, which is also localized in the nucleus, may also act as a DAMP/alarmin.[77]

As noted earlier, Schmitz *et al.* demonstrated that IL-33 shows the closest amino acid sequence homology to IL-18 among the members of the IL-1 family of cytokines.[3] Like IL-1β and IL-18, IL-33 is not considered to be secreted via the conventional vesicle transport pathway because its amino-terminus lacks the necessary signal sequence. Also like IL-1β and IL-18,

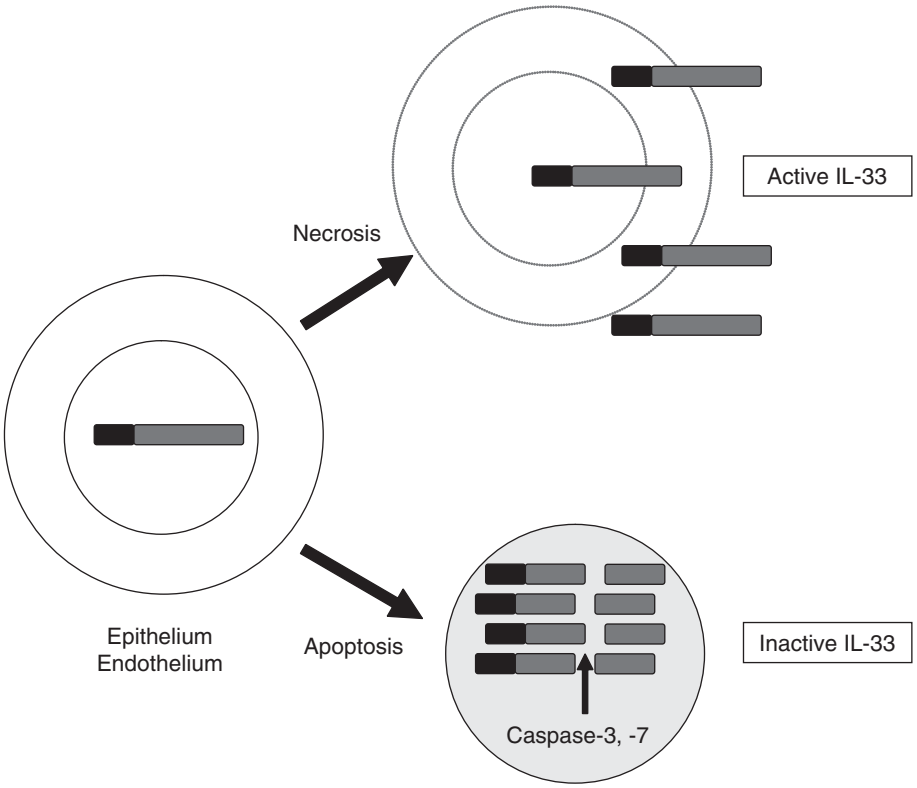

Fig. 3 Modes of cell death and IL-33 release. IL-33 is thought to be passively released by necrotic cells. On the other hand, caspases cleave IL-33, resulting in inactivation of IL-33 during apoptosis.

it was reported that IL-33 was cleaved from pro-IL-33 by caspase-1 *in vitro*,[3] suggesting that IL-33 may be secreted by activation of NACHT, LRR and PYD-containing protein (NLRP)-mediated inflammasomes. However, pro-IL-33 does not have a typical cleavage site such as seen in pro-IL-1β and pro-IL-18, and caspase-1 proteolytically cleaved pro-IL-33 in the cytokine motif, not the intermediate region between the helix-turn-helix and cytokine motifs, resulting in inactivation of IL-33.[77-79] Like caspase-1, both caspase-3 and caspase-7 are able to cleave pro-IL-33 during apoptosis, although apoptotic cells do not generally induce inflammation, and IL-33 processed by these caspases does not express biological activities via IL-33R1 (Fig. 3).[77,78] On the other hand, it is known that pro-IL-33 is released by necrotic cells without any processes by proteases such as caspase-1, -3, -7 and -8 and/or calpain.[4,77-79] In addition, like pro-IL-1α, pro-IL-33 has biological activity[78]: pro-IL-33 can induce mouse mast cell activation so that they produce cytokines via IL-33R1.[79] Therefore, these observations suggest that pro-IL-33 released by necrotic cells during tissue injury may play a DAMP/alarmin-like role in induction of inflammation. However, it remains unclear whether IL-33 can act as a potent adjuvant profoundly promoting acquired immune responses compared to other alarmin adjuvants such as ATP which are also released during necrosis.

IL-33-IL-33R IN INFECTIONS

LEISHMANIA MAJOR

It is well established that host protection against a protozoan, *Leishmania major*, is mediated by Th1 cell-mediated immune responses, but reciprocally aggravated by Th2 cell-mediated immune responses.[80]

ST2-expressing $CD4^+$ T cells accumulate in local lesions of *L. major*-infected mice.[81] Administration of polyclonal anti-ST2 antiserum, which has potential activity to-deplete ST2-expressing cells, including Th2 cells, reduced lesion development and Th2 cytokine production and increased Th1 cytokine production during *L. major* infection in female BALB/c mice, a strain that is susceptible to this pathogen.[64] On the other hand, BALB/c mice treated with anti-ST2 mAb (clone DJ8), which did not deplete ST2-expressing cells, or ST2-Fc fusion proteins showed lesion development, parasite replication and antigen-specific $ST2^+$ Th2 cell differentiation that were similar to in mice treated with control Ab, although their IFN-γ production was increased.[30] These observations suggest that ST2-expressing immune cells, but not IL-33-ST2-mediated signaling, contribute to the pathogenesis of leishmaniasis in mice.

TOXOPLASMA GONDII

Th1-type cytokine-associated immune responses are important for protection against another protozoan, *Toxoplasma gondii*.[82] ST2 mRNA expression was upregulated, but IL-33 mRNA expression was not altered, in brain lesions of mice infected with *T. gondii* compared with naïve mice.[83] ST2-deficient mice showed high susceptibility to *T. gondii* in comparison with wild-type BALB/c mice.[83] On the other hand, susceptibility to this protozoan was similar in wild-type, IL-4-deficient and IL-4Ra-deficient mice.[83] These observations suggest that the IL-33-IL-33R pathway is important for protection against *T. gondii*, independently of IL-4 and IL-13 production.

NIPPOSTRONGYLUS BRASILIENSIS

Th2-type cytokines are important for protection against *Nippostrongylus brasiliensis* and *Trichinella spiralis*, nematode parasites.[84] However, IL-4 production by splenic $CD4^+$ T cells and the serum levels of total IgG1 and IgE were normal in ST2-deficient mice on the 129 x B6 mixed background after *N. brasiliensis* infection.[21,85] On the other hand, the proportion of IL-5-producing $CD4^+$ T cells and the number of eosinophils in the lung were decreased in ST2-deficient mice on the 129 x B6 mixed background during *N. brasiliensis* infection, although the eggs of this parasite were normally cleared in these mutant mice.[85] These observations suggest that the IL-33-IL-33R pathway contributes to eosinophilia induced by *N. brasiliensis* infection, but is dispensable for protection against parasitic nematodes.

SCHISTOSOMA MANSONI

Th2 cells are considered to be key effector cells in the immune responses to a helminth parasite, *Schistosoma mansoni*.[86,87] ST2-deficient mice on the 129 x B6 mixed background showed impaired granuloma formation, characterized by eosinophil infiltration, in the lungs after the first *S. mansoni* egg injection.[22] On the other hand, these mutant mice, which had been sensitized with *S. mansoni* eggs, showed normal pulmonary granuloma formation and serum IgG1 and IgE levels after the second egg challenge, although they showed reduced IL-4 and IL-5 production by mediastinal lymph node cells in response to *S. mansoni* egg antigens.[22] These observations suggest that the IL-33-IL-33R pathway is involved in Th2 cytokine production but not antibody production during infection with *S. mansoni*.

TRICHURIS MURIS

It is also known that Th2-type cytokines are important for protection against a parasitic nematode, *Trichuris muris*.[88] During *T. muris* infection in mice, it was shown that IL-33 mRNA expression was increased in the cecum,[89] suggesting a contribution of IL-33 to host defense against *T. muris*. Indeed, administration of IL-33 to AKR mice, but not SCID mice, resulted in accelerated parasite clearance during *T. muris* infection.[89] Although T cells and/or B cells are required for IL-33-mediated parasite expulsion, as seen in SCID mice during *T. muris* infection, IL-33 induced pathological changes such as increased crypt length and intestinal epithelial cell proliferation independently of T cells and/or B cells.[89] In those settings, NK cells were increased in mesenteric lymph nodes of IL-33-injected, *T. muris*-infected SCID mice, suggesting a contribution of NK cells to the T/B cell-independent IL-33-mediated pathological changes during *T. muris* infection.

PSEUDOMONAS AERUGINOSA

ST2 mRNA/protein and IL-33 mRNA expression were increased in local inflammatory lesions infected with a gram-negative bacterium, *Pseudomonas aeruginosa*.[90,91] Administration of soluble ST2 to mice resulted in aggravation of the keratitis induced by *P. aeruginosa*,[90] suggesting that the IL-33-IL-33R pathway is important for protection against *P. aeruginosa*.

MYCOBACTERIUM TUBERCULOSIS

It is known that both Th1 and Th17 cytokines are crucial for defense against a bacterium, *Mycobacterium tuberculosis*.[92] Even though PPD-specific IFN-γ production was increased in the culture supernatants of spleen cells from *M. tuberculosis*-infected ST2-deficient mice, the survival, bacterial counts, granuloma formation and pulmonary inflammation score were comparable between ST2-deficient and -sufficient mice during *M. tuberculosis* infection.[93] On the other hand, SIGIRR/TIR8-deficient mice showed high susceptibility to *M. tuberculosis* infection.[94] These observations suggest that the IL-33-IL-33R1 pathway is not crucial for *M. tuberculosis* pathogenesis, while the IL-33-IL-33R2 pathway is important for host defense against this pathogen.

LEPTOSPIRA

It was shown that the level of soluble ST2 was increased in plasma from patients with leptospirosis due to a gram-negative *Leptospira* infection and was associated with the severity of the disease, bleeding and mortality, suggesting a contribution of the IL-33-IL-33R pathway to the pathogenesis of leptospirosis.[95] However, the precise role of the IL-33-IL-33R pathway in this disease remains unclear.

VIRUSES

The serum levels of soluble ST2 protein were increased in patients infected with dengue virus.[96] In mice, IL-33 was increased in the brain after infection with Theiler's murine encephalomyelitis virus.[97] These observations suggest a contribution of the IL-33-IL-33R pathway to the pathogenesis of certain viral infections.

Inhalation of respiratory syncytial virus (RSV) by mice that had been sensitized with recombinant vaccinia virus expressing the attachment protein G of RSV resulted in induction of Th2-type cytokine-associated eosinophilic airway inflammation. On the other hand, inhalation of RSV by mice sensitized with recombinant vaccinia virus expressing the fusion protein F of RSV led to induction of non-eosinophilic airway inflammation. In this model, administration of anti-ST2 mAb (clone 3E10) attenuated the development of Th2-type cytokine-associated eosinophilic airway inflammation, but not non-eosinophilic airway inflammation.[98] These observations suggest that the IL-33-IL-33R pathway is crucial for the development of virus-induced, Th2-type cytokine-associated eosinophilic inflammation.

IL-33-IL-33R IN ANGIOGENESIS AND TUMORGENESIS

IL-33 is constitutively expressed in nuclei of vascular endothelial cells in various human tissues such as the skin, small intestine, umbilical veins and lungs.[99] On the other hand, constitutive expression of IL-33 was not observed in human angiogenic tumor vessels.[99] Induction of IL-33 was observed during confluent growth of endothelial cells. On the other hand, expression of IL-33 was down-regulated during migration of those cells. Expression of nuclear IL-33 was rapidly down-regulated at the onset of angiogenesis during wound healing.[99] Proinflammatory cytokines (e.g., IL-1β and TNF) and proangiogenic growth factors (e.g., VEGF), which are induced during wound healing, suppressed IL-33 expression in endothelial cells.[99] In addition, IL-33 promoted angiogenesis by promoting proliferation, migration and differentiation of endothelial cells, and vascular permeability by reducing cell-cell interactions via cadherin.[100] These observations suggest that IL-33 may be involved in tumorigenesis and the development of vascular diseases.

IL-33-IL-33R IN ALLERGIC DISEASES

ASTHMA

The levels of soluble ST2 protein and IL-33 mRNA/protein are increased in sera and tissues from patients with asthma.[27,72,101-103] Genome-wide association studies identified polymorphism in the ST2 and/or IL-33 genes in patients with asthma, suggesting an association with susceptibility to asthma.[104-106] In support of that notion, intraperitoneal or intranasal administration of IL-33 to mice led to induction of eosinophilic inflammation in the pulmonary and intestinal mucosa through the IL-13 and STAT6-dependent pathway.[3,23]

The levels of soluble ST2 protein and IL-33 mRNA were increased in sera and/or lungs in a murine asthma model of airway inflammation induced by ovalbumin (OVA).[107,108] However, the roles of ST2 and IL-33 in the induction of OVA-induced airway inflammation in mice remain controversial. Respiratory function, airway pathology, eosinophil number in bronchoalveolar lavage fluids (BALFs) and/or the levels of serum total IgG1 and IgE were normal in 129 × B6 mixed and BALB/c background-ST2-deficient mice sensitized twice with OVA emulsified with alum (Table 2).[21,24,109] On the other hand, airway inflammation was attenuated in BALB/c background-ST2-deficient mice sensitized once with OVA/alum (Table 2).[24] Moreover, it is noteworthy that OVA-induced airway inflammation was exacerbated in SIGIRR/TIR8-deficient mice.[15]

Several investigators reported the effect of an anti-ST2 mAb (clone 3E10) on OVA-induced airway inflammation in BALB/c mice (sensitized twice with OVA/Alum). That mAb enhanced ST2-expressing Th2 cell proliferation and cytokine production *in vitro*, indicating that it acts as an agonistic Ab, at least on Th2 cells.[110] Despite its agonistic activity on Th2 cells *in vitro*, BALB/c mice treated with 3E10 mAb showed attenuated airway inflammation in response to OVA (Table 3).[111,112] Likewise, Th2 responses during OVA-induced airway inflammation (sensitized twice with OVA/Alum) were reduced in mice carrying a soluble ST2 gene expression vector or treated with an anti-IL-33 polyclonal Ab (Table 3).[107,113]

Adoptive transfer with *in vitro*-skewed DO11.10 Th2 cells, which express OVA-specific T-cell receptors, in mice resulted in Th2 cytokine-dependent eosinophilic airway inflammation after intranasal OVA challenge.[114] BALB/c wild-type mice or BALB/c-Rag-1-deficient mice injected with ST2-deficient DO11.10 Th2 cells showed exacerbated airway function and inflammation after OVA challenge in comparison with mice injected with ST2-sufficient DO11.10 Th2 cells (Table 4).[109] These observations suggest that IL-33 signals on Th2 cells have a regulatory function in OVA-induced airway inflammation in that animal model. In contrast with that study using ST2-deficient DO11.10 Th2 cells, administration of 3E10 (anti-ST2) mAb or soluble ST2-Fc fusion protein to mice injected with DO11.10 Th2 cells showed attenuated airway function and inflammation after OVA challenge (Table 4).[19,111] If 3E10 mAb acted as an agonistic Ab for Th2 cells *in vivo* as well as *in vitro*, the phenotypes seen in mice treated with this mAb may be consistent with the study using ST2-deficient DO11.10 Th2 cells (Table 4),[109] but inconsistent with those treated with soluble ST2-Fc fusion protein (Table 4) or other studies (Table 2, 3). The apparent discrepancy between the study using ST2-deficient mice and mice treated with anti-ST2 and ST2-Fc fusion proteins remains to be explained. However, perhaps ST2-expressing Th2 cells, macrophages and other immune cells have different roles in the induction of allergic airway inflammation, because airway eosinophilia was observed even in mice deficient in and/or

Table 2 Airway inflammation in ST2-deficient mice

Reference	Experimental protocol	Observations
Hoshino K *et al*. J Exp Med 190, 1541, 1999	129 x B6 ST2$^{-/-}$mice 0 12 24 26 28 30 31 Analyze Day 50 μg OVA in 1 mg alum i.p. 10 mg/ml OVA aerosol 30 min, 3 times at 1-h intervals	- Normal eosinophil count in BALFs - Normal pulmonary inflammation - Normal total IgG1 and IgE in sera
Mangan NE *et al*. Eur J Immunol 37, 1302-1312, 2007	BALB/c ST2$^{-/-}$ mice 0 14 28 29 30 32 Analyze Day 20 μg OVA in 2 mg alum i.p. 1% OVA aerosol, 20 min.	- Normal AHR (penh and GL) - Normal pulmonary inflammation (airway mucous cell hyperplasia, pulmonary fibrosis or peribronchial cell inflammation) - Normal OVA-specific cytokine secretion by mediastinal LN cells in vitro - Normal eosinophils, but reduced macrophages in BALFs
Kurowska-Stolarska M *et al*. J Immunol 181, 4780, 2008	BALB/c ST2$^{-/-}$mice 0 8 9 10 11 Analyze Day 100 μg OVA in 2% alum i.p. 10 μg OVA I.n.	- Reduced eosinophils and macrophages in BALFs - Reduced IL-5, but not IL-4 or IL-13, levels in BALFs - Reduced pulmonary inflammation - Normal levels of IgG1 and IgE in sera
	BALB/c ST2$^{-/-}$mice 0 12 24 26 28 30 31 Analyze Day 100 μg OVA in 2% alum i.p. 10 μg OVA I.n.	- Normal responses

Table 3 Effects of anti-ST2 mAb (3E10), soluble ST2 and anti-IL-33 Ab on mouse airway inflammation

Reference	Experimental protocol	Observations
Coyle AJ *et al*. J Exp Med 190, 895, 1999	100 μg anti-ST2 mAb (clone 3E10), or control rat IgG1 1 h before OVA sensitization and challenge, route ??? Male BALB/c-WT mice 0 7 14 21 22 Analyze Day 20 μg OVA in 1.5 mg alum i.p. 10 mg/ml OVA aerosol, 1 hr.	- Reduced eosinophil count and IL-5 level in BALFs - Reduced OVA-specific serum IgE level
Kearley J *et al*. Am J Respir Crit Care Med 179, 772, 2009	25 μg anti-ST2 mAb (clone 3E10) or control rat IgG i.v. BALB/c WT mice 0 12 18 19 20 21 22 23 25 27 29 30 Analyze Day 10 μg OVA In alum i.p. 5% OVA aerosol 20 min.	- Reduced AHR, mucus secretion and ST2+ T cell infiltration in lungs - Reduced IL-4 and IL-13 levels in BALFs - Normal IL-33 levels in lung tissues
Oikawa K *et al*. Clin Exp Allergy 32, 1520, 2002	Soluble ST2 gene transfer Female BALB/c-WT mice -1 0 6 7 13 14 15 Analyze Day 100 μg OVA in 2 mg alum i.p. 1% OVA aerosol, 30 min, twice	- Reduced eosinophils and IL-4 and IL-5 levels in BALFs - Reduced OVA-specific IL-4 and IL-5 production by spleen cells from OVA-sensitized mice in vitro
Liu X *et al*. BBRC 386, 181, 2009	150 μg anti-IL-33 polyclonal Ab, or control rabbit IgG I.p 30 min before OVA sensitization and challenge Female BALB/c-WT mice 0 14 25 26 27 28 Analyze Day 20 μg OVA in 2 mg alum i.p. 1% OVA aerosol, 30 min.	- Reduced eosinophils and lymphocytes, and IL-4, IL-5 and IL-13 levels in BALFs - Reduced pulmonary inflammation - Reduced total and OVA-specific IgE in sera

Table 4 Effects of anti-ST2 mAb (3E10) and soluble ST2 on airway inflammation in adoptive Th2 cell transfer model

Reference	Experimental protocol	Observations
Lohning M *et al*. Proc Natl Acad Sci USA 95, 6930, 1998	BALB/c-WT mice DO11.10 Th2 cell i.v. injection 20 μg anti-ST2 mAb (clone 3E10), ST2-IgG fusion protein or control IgG 1 h before OVA inhalation, route ?? Analyze 0 1 2 3 Day 50 mg/ml OVA aerosol, 20 min.	- Reduced eosinophils and IL-4 and IL-5 levels in BALFs
Coyle AJ *et al*. J Exp Med 190, 895, 1999	BALB/c-WT mice DO11.10 Th2 cell i.v. injection 20 or 100 μg anti-ST2 mAb (clone 3E10), 100 μg ST2-IgG fusion protein or control IgG 1 h before OVA inhalation, i.v. injection Analyze 0 1 2 3 Day 50 mg/ml OVA aerosol, 20 min.	- Reduced IL-4, IL-5, IL-6 and IL-13, but not IL-10 or IFN-γ, in BALFs - Reduced eosinophil count in BALFs - Reduced Penh responses and pulmonary inflammation
Mangan NE *et al*. Eur J Immunol 37, 1302-1312, 2007	BALB/c-WT mice or BALB/c-Rag-1$^{-/-}$ mice WT or ST2$^{-/-}$ DO11.10 Th2 cell i.v. Analyze 0 7 8 9 10 11 12 13 14 15 Day ±2 μg OVA In alum i.p. 2% OVA aerosol, 20 min.	- Exacerbated AHR and lung inflammation - Increased eosinophils in BALFs - Increased IL-5 expression - No differences in lung IL-33 mRNA expression - Normal OVA-specific cytokine secretion by mediastinal LN cells in vitro

depleted of T cells, B cells, mast cells, and/or NK cells after IL-33 inhalation.[23] Also, excessive IL-1 production, if it occurred in ST2-deficient mice, might contribute to the discrepant results between the effects of genetic ST2 deficiency and protein molecules on IL-33 signals, as mentioned before.

In addition, Rag-2-deficient mice, which lack B cells and T cells, including ST2-expressing Th2 cells and Tr1 cells,[32] showed aggravated airway inflammation induced by IL-33.[23] Together with the study of the adoptive Th2 cell transfer model using ST2-deficient DO11.10 Th2 cells,[109] these observations suggest that ST2-expressing T cells have a regulatory role in IL-33-mediated airway inflammation. On the other hand, IL-33 can enhance the development and activation of AAMϕ/M2a, resulting in enhanced eosinophilic airway inflammation.[72]

DERMATITIS, RHINITIS, RHINOSINUSITIS AND CONJUNCTIVITIS

In addition to in asthma, polymorphism in the ST2 and/or IL-33 genes was also found in patients with atopic dermatitis,[115] rhinitis[116] and rhinosinusitis.[117] ST2 mRNA expression is increased in skin from rats with contact dermatitis induced with 2,4-dinitrofluorobenzene.[118] IL-33 mRNA/protein levels are increased in specimens from patients with allergic conjunctivitis,[119] rhinitis[116] and atopic dermatitis.[39] These observations suggest involvement of the IL-33-IL-33R pathway in the development of such allergic disorders.

ANAPHYLAXIS

The serum levels of IL-33 were significantly increased in atopic patients during anaphylaxis.[39] In mice, IL-33 deteriorated IgE-mediated anaphylaxis.[39]

IL-33-IL-33 R IN AUTOIMMUNE AND CHRONIC INFLAMMATORY DISEASES

ARTHRITIS

The levels of soluble ST2 were increased in the synovial fluid from patients with rheumatoid arthritis (RA),[120] which is considered to be a Th17 cell-mediated autoimmune disorder. IL-33 mRNA/proteins were also elevated in the sera, synovial fluid and/or inflamed lesions of the patients.[5,121-123]

Administration of polyclonal anti-ST2 antiserum, which has potential activity to deplete ST2-expressing cells, to mice resulted in exacerbation of collagen-induced arthritis (CIA), which is considered to be a mouse model for RA.[64] On the other hand, mice treated with soluble ST2-Fc fusion proteins or anti-ST2 mAb and mice deficient in ST2 showed attenuation of CIA,[121,122,124] while mice treated with IL-33 showed aggravated disorders. These observations suggest that certain ST2-expressing cells may act as effector cells and/or regulatory cells in the development of CIA. In fact, ST2-expressing mast cells were shown to act as an effector of aggravated CIA development.[121] Inhibition of IL-1 is beneficial for treatment of RA,[11] and inhibition of the IL-33-IL-33R pathway may be similarly useful for therapy of this disease.

DIABETES MELLITUS (DM) AND ATHEROSCLEROSIS

Diabetes mellitus (DM) is divided into insulin-dependent DM (IDDM)/type 1 DM (T1D) and non-

insulin-dependent DM/type 2 DM (T2D). T1D, which is due to insulin deficiency caused by destruction of beta cells in the pancreatic islets of Langerhans by autoreactive T cells, is considered to be a chronic autoimmune disease.

Antibiotic streptozocin, which is an analogue of N-acetylglucosamine (GluNAc), is transported into beta cells via the GLUT2 glucose transporter and inhibits the activity of O-GlcNAcase.[125,126] Since metabolism of the sugar in O-linked proteins is critical for beta cells, inhibition of O-GlcNAcase leads to apoptosis of beta cells.[125,126] Thus, it is known that administration of streptozocin to rodents results in symptom of T1D.[125,126] The destruction of islet beta cells during streptozocin-induced DM is known to be mediated by TRAIL, but not Fas.[127,128] Recently, it was shown that the development of streptozocin-induced DM was exacerbated in ST2-deficient mice,[129] suggesting involvement of the IL-33-IL-33R pathway in the development of human T1D.

Some patients with T2D characterized by hyperglycemia, and hyperinsulinemia due to insulin resistance, develop atherosclerosis associated with hyperlipidemia. Apolipoprotein E-deficient mice spontaneously develop atherosclerosis that resembles human atherosclerosis. Administration of IL-33 ameliorated atherosclerosis development in apolipoprotein E-deficient mice by enhancing IL-5 production and reciprocally suppressing Th1 cell activity.[130] On the other hand, blockade of the IL-33-IL-33R pathway by treatment of apolipoprotein E-deficient mice with soluble ST2 resulted in exacerbated disease.[130] These observations suggest that IL-33 plays a protective role in the development of atherosclerosis in apolipoprotein E-deficient mice.

ALZHEIMER'S DISEASE

In addition to the role of apolipoprotein E in atherosclerosis, polymorphism of the ε4 allele of the apolipoprotein E gene is considered to be a genetic determinant of the common forms of Alzheimer's disease.[131] As another genetic candidate, it is suggested that SNPs at loci in regions associated with the IL-33 gene may be involved in susceptibility to non-ε4-type Alzheimer's disease.[132]

INFLAMMATORY BOWEL DISEASE

Crohn's disease (CD) and ulcerative colitis (UC) are representative inflammatory bowel diseases (IBD). Soluble ST2 protein and/or IL-33 mRNA were detected in endothelial cells from patients with CD and/or UC,[5,133,134] suggesting involvement of the IL-33-IL-33R pathway in induction of IBD, although the precise role of IL-33 remains unclear.

Administration of dextran sodium sulfate (DSS) to mice resulted in development of colitis associated with destruction of colonic epithelial cells even in the absence of T cells, B cells, NK cells and mast cells,[135-137] dependent on TLR signals.[138] SIGIRR/TIR8-deficient mice showed high susceptibility to development of DSS-induced colitis,[17,139] suggesting that signaling through IL-33-IL-33R2 is involved in protection against colitis induced by DSS.

SYSTEMIC SCLEROSIS

IL-33 and ST2 mRNA/protein expression was increased in the inflamed skin of patients with systemic sclerosis.[140] Repeated subcutaneous IL-33 injection resulted in development of skin fibrosis similar to that seen in patients with systemic sclerosis (scleroderma).[141] The development of IL-33-mediated cutaneous fibrosis was dependent on IL-13 (probably derived from eosinophils), but not IL-4, and it required eosinophils and T and/or B cells, but not mast cells.[141] These observations suggest that IL-33-IL-33R pathways may be important for induction of the skin fibrosis seen in patients with systemic sclerosis (scleroderma).

SYSTEMIC LUPUS ERYTHEMATOSUS

Serum levels of soluble ST2 protein were increased in patients with systemic lupus erythematosus (SLE).[142,143] MRL$^{lpr/lpr}$ mice spontaneously develop autoimmune diseases resembling human SLE. SIGIRR/TIR8-deficient C57BL/6$^{lpr/lpr}$ mice showed exacerbated lung disease and lupus nephritis.[144] These observations suggest that the IL-33-IL-33R pathway is involved in the development of SLE, but the precise roles of IL-33 and IL-33Rs in that pathogenesis remain unclear.

CARDIAC DISEASES

The serum level of soluble ST2 protein was elevated in patients with heart failure, acute myocardial infarction, aortic stenosis and congestive cardiomyopathy.[145-147] In addition, IL-33 mRNA/protein was induced in cardiac fibroblasts after biomechanical stimulation.[148] It was shown that mortality, cardiac fibrosis and cardiomyocyte hypertrophy were increased in ST2-deficient mice, but decreased in mice administered IL-33, after transverse aortic constriction.[148] Treatment with IL-33 reduced ventricular dilation, improved contractile function and improved survival in mice after myocardial infarction by preventing cardiomyocyte apoptosis.[149] These observations suggest that the IL-33-IL-33R pathway plays a regulatory role in the induction of certain cardiac diseases.

LIVER INJURY AND FIBROSIS

Both IL-33 and ST2 mRNA were increased in fibrotic livers of humans and mice.[150] Sinusoidal endothelial cells in the normal liver and activated hepatic stellate cells in fibrotic livers are the main source of IL-33.[150] Administration of carbon tetrachloride (CCl4) induces liver injury and fibrosis in mice. In general, treatment with soluble ST2-Fc fusion proteins re-

sulted in inhibition of Th2-type responses such as Th2 cytokine production. During CCl4-induced liver injury in mice, however, soluble ST2 protein treatment enhanced production of Th2 cytokines such as IL-4 and IL-13.[151] CCl4-induced liver injury developed as usual in mice treated with soluble ST2-Fc fusion proteins, while liver fibrosis was accelerated and enhanced by increased production of IL-4 and IL-13 (derived from Th2 cells, not iNKT cells) in those mice. Although the molecular mechanism of the enhancement of Th2 cytokine production by soluble ST2 protein treatment in mice during CCl4-induced liver injury and fibrosis remains unclear, the IL-33-IL-33R pathway is important for the pathogenesis of liver fibrosis.

HYPERNOCICEPTION

Such IL-1 cytokines as IL-1 and IL-18 can provoke hypernociception.[152,153] Like IL-1 and IL-18, IL-33 injection can induce nociceptor sensitization (hypernociception), and IL-33 is also involved in antigen-induced hypernociception that is dependent on TNF, IL-1 and IFN-γ, but not IL-18.[154]

CONCLUSION

Although it was initially thought that IL-33 was a crucial cytokine for Th2 cytokine-mediated host defense as well as induction of Th2-type allergic disorders, it is now known that IL-33 has a pleiotropic, not a restricted, Th2 cytokine-mediated, role in various immune responses as a proinflammatory cytokine, similar to IL-1 and IL-18. Therefore, IL-33 may have potential as a therapeutic target in various diseases. Whereas the effects of IL-33 on various cell types have been extensively investigated, further studies are required to understand the biological significance of IL-33, the cellular source(s) of IL-33, the mechanisms involved in active IL-33 production and the role of IL-33 as a nuclear factor.

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