Minireview

Targeting IL-33 in Autoimmunity and Inflammation

Theoharis C. Theoharides, Anastasia I. Petra, Alexandra Taracanova, Smaro Panagiotidou, and Pio Conti

Laboratory of Molecular Immunopharmacology and Drug Discovery, Department of Integrative Physiology and Pathobiology (T.C.T., A.I.P., A.T., S.P.), Graduate Program in Pharmacology and Experimental Therapeutics, Sackler School of Graduate Biomedical Sciences (T.C.T., A.T.), Department of Internal Medicine (T.C.T.), Tufts University School of Medicine, and Tufts Medical Center (T.C.T.), Boston, Massachusetts; and Immunology Division, Graduate Medical School, University of Chieti-Pescara, Chieti, Italy (P.C.)

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ABSTRACT

Interleukin-33 (IL-33) belongs to the IL-1 family of cytokines. Whereas IL-1 is processed and released by live immune cells in response to infection or other triggers, IL-33 is mostly released as a danger signal (“alarmin”) from damaged cells. IL-33 may also be processed and released from activated mast cells (MCs) with subsequent autocrine and paracrine actions. IL-33 augments the stimulatory effects of IgE and substance P on MCs but can also trigger release of cytokines from MCs on its own. Blood IL-33 levels are increased in asthma, atopic dermatitis, multiple sclerosis, rheumatoid arthritis, and Sjögren’s syndrome. However, prolonged elevation of IL-33 downregulates FceRI and may be protective in atherosclerosis, suggesting different roles in immune-regulated diseases. Even though neutralizing IL-33, knocking-down its receptor, or using its soluble “decoy” receptor has resulted in anti-inflammatory effects, there appear to be different outcomes in different tissues. Hence, selective regulation of IL-33 synthesis, release, and signaling may be required to provide effective treatment options.

Introduction

Interleukin-33 Structure and Function. There are 11 known members of the interleukin (IL)-1 family. IL-33 is a new member of the IL-1 family that regulates innate and adaptive immune systems to promote inflammatory responses (Dinarello, 2009). IL-33 is mainly expressed by keratinocytes, epithelial and endothelial cells (Moussion et al., 2008), as well as by human monocytes (Nile et al., 2010) and mouse astrocytes (Kempuraj et al., 2013). IL-33 acts as an alarmin against injury-induced stress, pathogens, or cell death by activating local immune cells (Lukens et al., 2012; Lunderius-Andersson et al., 2012).

IL-1α and IL-1β are both synthesized in proforms and require proteolytic cleavage for their activation and release through what has been termed the NOD-like receptor family, pyrin domain–containing 3 (NLRP3) “inflammasome” (Schroder et al., 2010; Franchi and Nunez, 2012). The cysteine protease caspase 1 must be cleaved and activated from its proform and it is then organized into the NLRP3 inflammasome (Schroder et al., 2010). Pro–IL-1α (31 kDa) must be cleaved by caspase 1 to generate the active form (17 kDa) (Arend et al., 2008), which is normally conserved within the cell, mainly bound to the plasma membrane, or localized in the nucleus (Dinarello et al., 2012).

IL-33 is synthesized in its proform (30 kDa), but its processing does not appear to involve the NLRP3 inflammasome (Schroder et al., 2010). In contrast, caspase-1 cleaves pro–IL-33 into an inactive form (Carayol and Girard, 2009). Moreover, unlike pro–IL-1α, the proform of IL-33 is biologically active and also contains nuclear localization sequences (like pro–IL-1α) allowing it to act both as an intracellular nuclear factor and as an extracellular cytokine (Zhao and Hu, 2010). Proteases such as calpain, cathepsin G, and elastase can cleave pro–IL-33 into more potent mature forms (Lefrancais et al., 2012; Garlanda et al., 2013) (Fig. 1). It was recently reported that chymase and tryptase secreted from mast cells (MCs) can generate IL-33 forms that are 30-fold more potent than full-length IL-33 (Lefrancais et al., 2014).

IL-33 primarily induces the production of Th2-associated cytokines (Dinarello, 2002) but can also activate CD8+ cells and it is required for the activation of regulatory T cells (Tregs) (Lefrancais et al., 2012). IL-33 also promotes the differentiation of naive T cells into Foxp3+ Tregs (Korzennik et al., 2013; Atkinson et al., 2013). Moreover, IL-33 may be involved in autoimmune diseases such as multiple sclerosis (Delcayre et al., 2013), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren’s syndrome (Klein et al., 2010). In these diseases, IL-33 is upregulated in inflamed tissues and may play a role in the development of autoimmunity. IL-33 may also contribute to the development of chronic inflammatory diseases such as asthma, atopic dermatitis (AD), and allergic asthma. In these conditions, IL-33 may be responsible for the recruitment and activation of immune cells, including mast cells (MCs), neutrophils, and eosinophils. In addition, IL-33 may contribute to the development of chronic inflammatory diseases such as atherosclerosis, diabetes, and autoimmune diseases. In these conditions, IL-33 may be responsible for the recruitment and activation of immune cells, including mast cells (MCs), neutrophils, and eosinophils. In addition, IL-33 may contribute to the development of chronic inflammatory diseases such as atherosclerosis, diabetes, and autoimmune diseases.

ABBREVIATIONS: AD, atopic dermatitis; CCL2, chemokine ligand 2; hBD2, human β-defensin 2; IFN, interferon; IL, interleukin; JNK, c-Jun N-terminal kinases; MC, mast cell; MS, multiple sclerosis; NF-κB, nuclear factor-κB; NLRP3, NOD-like receptor family, pyrin domain–containing 3; SCF, stem cell factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
IL-33 induces lymphoid cell–mediated airway inflammation by also activating the mammalian target of rapamycin (Salmond et al., 2012). Despite IL-33 sharing the IL-1 receptor accessory protein with other members of the IL-1 family that are known to upregulate an inducible antimicrobial peptide human β-defensin 2 (hBD2), IL-33 downregulates serum-induced hBD2 in human primary keratinocytes; this finding may explain the increased colonization rate of Staphylococcus aureus seen in atopic dermatitis (AD) patients (Alase et al., 2012). Interestingly, hBD2 has been reported to activate human MCs (Subramanian et al., 2013).

Regulation of IL-33 Expression. IL-33 expression is induced by pathogen-associated molecular patterns and environmental triggers (Lloyd, 2010). Interestingly, extracellular ATP was reported to act as a sensor for airborne allergens (Kouzaki et al., 2011). IL-33 can also be induced by triggers of Toll-like receptors (Zhang et al., 2011b) that are also present on MCs and can be activated by bacterial and viral DNA sequences, leading to release of different cytokines (Abraham and St. John, 2010).

IL-33 and its surface receptor ST2 were upregulated by interferon-γ (IFN-γ) in keratinocytes derived from patients with AD (Seltmann et al., 2013). Tumor necrosis factor (TNF), but not IL-17, stimulates secretion of IL-33, which induces expression of IL-6, monocyte chemoattractant protein-1, and vascular endothelial growth factor (VEGF) (Balato et al., 2012). However, it appears that the type of cytokines/chemokines produced by IL-33 may depend on the particular tissue since the extent and type of such mediators varies between sensitized skin and asthmatic airways (Savinko et al., 2013).

IL-33/ST2 Signaling. IL-33 was discovered as a main ligand to ST2 (IL-1R4) receptor, which is mostly expressed on the surfaces of epithelial cells, fibroblasts, and MCs (Liew et al., 2010). The ST2 receptor is found in either the transmembrane ST2L form, which is the more abundant form, or in the cytoplasm as the soluble sST2 form, which may be acting as a decoy by binding and neutralizing IL-33 (Liew et al., 2010). The receptor complex comprises the ST2 and IL-1 receptor accessory proteins (Chackerian et al., 2007). IL-33 binding recruits the IL-1R AcP coreceptor, the adaptor protein MyD88, along with the associated protein IL-1R kinase.

**Fig. 1.** Diagrammatic representation of the proposed steps for the activation and release of IL-33. (A) Damage-associated molecular patterns (DAMPs), IFN-γ, and other triggers activate immune cells, including mast cells, to release both active pro–IL-33 and active IL-33 extracellularly, where they act as alarmins for other cells. (B) Pathogen-associated molecular patterns (PAMPs), ATP, and other environmental or innate triggers activate either NF-κB directly or the inflammasome (NLPR3) to generate IL-1. In addition, NF-κB induces the synthesis of pro–IL-33, which is then acted upon by calpain and elastase to generate active IL-33. The vertical black line is meant to distinguish (A) from (B). IL-33 is mainly expressed by keratinocytes, epithelial and endothelial cells, as well as by monocytes, mast cells, and astrocytes (the cell types are listed on the basis of the strength of the evidence). IL-33 is involved in the pathogenesis of a number of diseases that are listed in boxes with the colors of the rainbow meant to indicate the strength of the evidence. IBD, inflammatory bowel disease; RA, rheumatoid arthritis.
mitogen-activated protein kinase, but not extracellular signal-regulated kinase 1/2; however, IL-13 production in these cells apparently did not require JNKs or extracellular signal-regulated kinase 1/2 signaling (Tare et al., 2010). IL-33 can also act as a DNA binding factor (Kakkar and Lee, 2008).

**IL-33 and Mast Cells.** The effect of IL-33 on MCs was reviewed recently (Sabatino et al., 2012). ST2 is expressed on MCs, for which it acts as a chemoattractant and augments the effect of other triggers (Fux et al., 2014). MCs respond to cell injury through IL-33 (Lunderius-Andersson et al., 2012) and have therefore been considered “sensors of cell injury” (Enoksson et al., 2011). A murine MC line (MC/9) was reported to produce significant amounts of IL-33 after stimulation with IgE and antigen (Hsu et al., 2010). More recently, bone marrow–derived cultured mast cells stimulated by ovalbumin and specific IgE induced the expression and release of IL-33, which has autocrine action on IL-6 and IL-13 expression (Tung et al., 2014).

MCs are hemopoietically derived cells located close to blood vessels and nerves, where they proliferate primarily in response to stem cell factor (SCF) (Galli et al., 1995) but also nerve growth factor (Matsuda et al., 1991). MCs are important for allergic reactions but also for mastocytosis, mast cell activation disorders, and other inflammatory diseases (Theoharides et al., 2015). IL-33 has also been reported to drive maturation of human MCs (Allakverdi et al., 2007) and promote MC survival (Iikura et al., 2007). IL-33 promoted proliferation of mouse mast cell independent of c-Kit (Saluja et al., 2014). Nevertheless, IL-33 was reported to cross-activate the SCF c-Kit receptor on MCs (Drube et al., 2010). Evidently, IL1RAcP interacts with c-Kit constitutively and IL-33R binds upon stimulation with SCF leading to cytokine release (Drube et al., 2010). Apparently, inhibition of c-Kit signaling also blocked human MC release of IL-1β (Drube et al., 2012), which had been shown to occur selectively without degranulation (Kandere-Grzybowska et al., 2003).

IL-33 augmented the activating effect of IgE and SCF on MC and basophils (Silver et al., 2010). IL-33 induced release of proinflammatory cytokines, especially IL-6, without degranulation from bone marrow–derived MCs (Moulin et al., 2007), and enhanced IL-8 production from human cord blood–derived cultured mast cells stimulated by IgE/anti-IgE, but without histamine release (Iikura et al., 2007). IL-33 augmented human MC release of VEGF in response to substance P, but not on its own (Theoharides et al., 2010). Moreover, IL-33 production of IL-13 independently of FceRI stimulation (Ho et al., 2007) stimulated prostaglandin D2, but not tryptase, release from activated human MCs (Nicolletti et al., 2012). IL-33 was also able to prime murine MCs for enhanced activation by IgG immune complexes (Drube et al., 2010; Kaieda et al., 2012), and stimulated MC-dependent neutrophil influx (Hueber et al., 2011; Enoksson et al., 2013). In addition to activation through cross-linking of the high-affinity IgE receptor (FceRI) (Blank and Rivera, 2004), MCs are also stimulated by a variety of other triggers (Theoharides et al., 2007), such as the neuropeptide neurotensin (Donelan et al., 2006) and substance P (Zhang et al., 2011a).

MCs secrete numerous inflammatory mediators including histamine; leukotrienes; prostaglandins and tryptase; cytokines such as IL-6, IL-9, IL-13, TNF; chemokines like CCL2 and IL-8 (CXCL8); as well as VEGF (Theoharides et al., 2012a). MCs are the only cell type in which preformed TNF is stored in secretory granules (Olszewski et al., 2007) and released bound to heparin particles that can reach draining lymph nodes and contribute to inflammation (Kunder et al., 2009). TNF can also activate T cells (Nakae et al., 2005; Kempuraj et al., 2008). MC-derived IL-6 and transforming growth factor-β are critical for the development of Th17 cells (Nakae et al., 2007; Suurmond et al., 2011), and MCs secrete IL-17 themselves (Kenna and Brown, 2013).

IL-33 also augments the action of thymic stromal lymphopoietin (Nakae et al., 2007), which may be acting as another early immune alarmin through MCs (Kaur et al., 2012).

### Functional and Pathologic Features of IL-33 in Allergic Diseases

IL-33 is thought to contribute to disease pathology (Liew et al., 2010), especially inflammatory (Dinarello, 2002; Lukens et al., 2012; Milovanovic et al., 2012), allergic (Saluja et al., 2015), and autoimmune (Pei et al., 2014) diseases, through activation of MCs (Castellani et al., 2009).

**Allergic Inflammation.** There is substantial evidence that IL-33 is involved in airway inflammation (Oboki et al., 2011) and it appears to facilitate allergic airway responses to subthreshold exposure to dust mites (Llop-Guevara et al., 2014) and to ovalbumin in mice (Sjoberg et al., 2015). IL-33 induced Th17–mediated airway inflammation via MCs (Hsu et al., 2010). In fact, there is evidence that IL-33 modulates cross-talk between MCs and smooth muscle cells in human airways (Kaur et al., 2015). Genetic polymorphism of ST2 and ST2L provides susceptibility for asthma development (Moffatt et al., 2010). ST2L expression was significantly increased in patients with severe asthma, and multiple single nucleotide polymorphisms in IL1RL1 were found in these patients (Traister et al., 2015).

IL-33 serum levels were significantly increased compared with the control nonasthmatic individuals (Pushparaj et al., 2009; Raieszadeh et al., 2014). IL-33 was secreted from human bronchial epithelial cells after stimulation with an extract of Alternaria, a common fungus causing allergic respiratory diseases (Lefrançais and Cayrol, 2012). IL-33 also mediated maximum responses in allergen-induced airway inflammation (Kamijo et al., 2013). IL-33 is also involved in allergic rhinitis (Haenuki et al., 2012) and allergic conjunctivitis (Lin et al., 2013).

Additional studies from rodent models support the role of IL-33 in lung diseases (Eiwegger and Akdis, 2011; Kamijo et al., 2013). Intranasal administration of IL-33 triggered an immediate allergic inflammatory response in the airways, which was absent in IL-33–deficient mice (Louten et al., 2011). In another study, IL-33–deficient mice did not develop sneezing (early) or accumulate eosinophils and basophils (late) when challenged with ragweed pollen (Haenuki et al., 2012). In a model using ST2-deficient mice, ST2 receptor signaling was the main inducer of Th2 cytokines in the asthmatic airways, but receptor presence was dispensable for Th2-dependent inflammation in the sensitized skin (Savinko et al., 2013).

The IL-33/ST2 pathway appears to be involved in AD (Cevikbas and Steinhoff, 2012; Savinko et al., 2012). Serum levels of IL-33 were increased in patients with AD compared with healthy controls and were significantly reduced after...
clinical improvement of skin lesions (Tamagawa-Mineoka et al., 2014). IL-33 was reported to stimulate innate lymphoid cells in AD (Salimi et al., 2013). IL-33 was also elevated in the skin, but not in the serum, of patients with psoriasis, a chronic inflammatory condition that the pathogenesis of which is also associated with MC activation (Theoharides et al., 2010).

**IL-33 in Autoimmunity and Inflammation.** The IL-33/ST2 axis has been increasingly implicated in immunopathology and inflammation (Milovanovic et al., 2012). Blood levels of IL-33 are increased in a number of autoimmune and inflammatory (Milovanovic et al., 2012) diseases, such as rheumatoid arthritis (Kritas et al., 2013), systemic lupus erythematosus (Yu et al., 2013), Sjögren’s syndrome (Awada et al., 2014), Grave’s disease (Celik et al., 2013), and inflammatory bowel disease (Beltran et al., 2010). Genetic polymorphism studies have identified IL-33R1 as a susceptibility gene in inflammatory bowel disease (Jostins et al., 2008; McLean et al., 2014). One recent paper reported the inhibitory effect of fingolimod and newly synthesized analogs on IL-33/ST2 signaling in dendritic cells; they reported variable effects on inhibition of IL-13 and IFN-γ production possibly through regulation of intracellular calcium levels and protein phosphatase 2A activity (Ruger et al., 2014). However, long term exposure (72 hour) of human and mouse MCs to IL-33 resulted in significant reduction of MC activation by antigen, suggesting that IL-33 may have the ability to induce “a hyporesponsive phenotype” in MCs (Jung et al., 2013). Blocking IL-33 also may not be as critical as blocking ST2, as was previously reported for the TNF-α and its receptor in experimental allergic encephalomyelitis (Kassiotis and Kollias, 2001).

**Treatment Possibilities**

Targeting IL-33 has been considered as a therapeutic target for atopic (Nabe, 2014), rheumatic (Duan et al., 2013), and autoimmune diseases (Wang et al., 2012). Administration of sST2 to murine splenocytes inhibited production of Th2 cytokines (e.g., IL-4 and IL-5) but not of Th1 (e.g., IFN-γ) (Lohning et al., 1998). Additionally, when murine thymoma cells were stably transfected with ST2L and then treated with sST2, IL-33 binding to the ST2L was inhibited and so was the induction of NF-κB (Hayakawa et al., 2007).

Intranasal anti–IL-33 antibody significantly inhibited cigarette smoke–induced lung inflammation in mice, as evidenced by reduced levels of IL-33 and ST2, as well as decreased number of neutrophil and macrophage infiltration along with decreased expression of inflammatory cytokines (IL-1β, TNF, IL-17) (Qi et al., 2013). Mice challenged with ovalbumin and then treated with anti–IL-33 antibody or sST2 showed negative regulation of ovalbumin-induced allergic airway inflammation (Lee et al., 2014); both treatments decreased Th2 cytokine levels in bronchoalveolar lavage fluid and decreased the count of eosinophils (Lee et al., 2014). One company has announced the production of a humanized anti–IL-33 antibody for clinical use (www.anaplysbio.com/anti-il33). One study on human corneal epithelial cells reported that ST2 antibody or soluble anti-ST2 protein blocked IL-33–stimulated thymic stromal lymphopoietin and chemokine (CCL2, CCL20, CCL22) production from these cells at both mRNA and protein levels, (Lin et al., 2013).

On the basis of the evidence discussed above, the IL-33/ST2 signaling pathway could serve as novel biomarker or target for the development of new treatments (Kakkar and Lee, 2008; McLean et al., 2014). One recent paper reported the inhibitory effect of fingolimod and newly synthesized analogs on IL-33/ST2 signaling in dendritic cells; they reported variable effects on inhibition of IL-13 and IFN-γ production possibly through regulation of intracellular calcium levels and protein phosphatase 2A activity (Ruger et al., 2014). However, the role of ST2/IL-33 appears to differ according to the tissue target(s). Surprisingly, IL-33 was reported to reduce the development of atherosclerosis in ApoE knockout mice, in spite of the fact that it elevated serum IgE levels, and treatment with sST2 led to development of larger atherosclerotic plaques (Oboki et al., 2010). This finding appears contrary to the well known role of MCs in the development of atherosclerosis (Sun et al., 2007; Theoharides et al., 2011).

Moreover, long term exposure (>72 hour) of human and mouse MCs to IL-33 resulted in significant reduction of MC activation by antigen, suggesting that IL-33 may have the ability to induce “a hyporesponsive phenotype” in MCs (Jung et al., 2013). Blocking IL-33 also may not be as critical as blocking ST2, as was previously reported for the TNF-α and its receptor in experimental allergic encephalomyelitis (Kassiotis and Kollias, 2001).
There is also some controversy about the role of IL-33 in cancer. Some authors have reported that IL-33 promotes metastasis of gastric (Yu et al., 2015) and colorectal (Liu et al., 2014) cancers. However, other papers have reported that IL-33 may act as an adjuvant to enhance antigen-specific tumor immunity (Villarreal et al., 2014).

The natural flavonoid quercetin can block the ability of IL-1 to stimulate selective release of IL-6 from human MCs (Kandere-Grzybowska et al., 2006), and its structural analog luteolin can block the stimulatory effect of IL-33 on MC TNF release (data not shown). Quercetin and luteolin have potent antioxidant and anti-inflammatory actions (Middleton et al., 2000). They both inhibit the release of histamine, leukotrienes, and prostaglandin D2 from human cultured MCs in response to cross-linkage of FceRI (Kimata et al., 2000). Quercetin also inhibits histamine, IL-6, IL-8, TNF-α, and tryptase release from human MCs (Kempuraj et al., 2005; Park et al., 2008), as well as asthma in a guinea pig model (Moon et al., 2008) and contact dermatitis in humans (Weng et al., 2012). Luteolin has also been reported to inhibit stimulation of activated T cells (Kempuraj et al., 2005; Solanki et al., 2015) and to attenuate experimental autoimmune uveitis (Barbour et al., 2014). These apparently opposite involvement of different IL-33 isoforms (Villarreal and Weiner, 2015). Hence, some isoforms may be pathologic, whereas others may be protective.

**References**


Black ginger is rich in methoxyluteolin and luteolin analog, tetramethoxyluteolin, is more potent that luteolin can block the stimulatory effect of IL-33 on MC TNF expression (data not shown). Quercetin and luteolin have potent antioxidant and anti-inflammatory actions (Middleton et al., 2000). They both inhibit the release of histamine, leukotrienes, and prostaglandin D2 from human cultured MCs in response to cross-linkage of FceRI (Kimata et al., 2000). Quercetin also inhibits histamine, IL-6, IL-8, TNF-α, and tryptase release from human MCs (Kempuraj et al., 2005; Park et al., 2008), as well as asthma in a guinea pig model (Moon et al., 2008) and contact dermatitis in humans (Weng et al., 2012). Luteolin has also been reported to inhibit stimulation of activated T cells (Kempuraj et al., 2005; Solanki et al., 2015) and to attenuate experimental autoimmune uveitis (Barbour et al., 2014). These apparently opposite involvement of different IL-33 isoforms (Villarreal and Weiner, 2015). Hence, some isoforms may be pathologic, whereas others may be protective.

**Conclusion**

IL-33 has been reported to improve functional recovery after contusion spinal cord injury in a mouse model (Pomeshchik et al., 2015) and to attenuate experimental autoimmune uveitis (Barbour et al., 2014). These apparently opposite results may be attributable to species differences or the involvement of different IL-33 isoforms (Villarreal and Weiner, 2015).

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**Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Theoharides, Petra, Taracanov, Panagiotidou, Conti.
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Address correspondence to: Dr. Theoharis C. Theoharides, Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Suite 304, 150 Harrison Ave., Boston, MA 02111. E-mail: theoharis.theoharides@tufts.edu