Conflicting Vascular and Metabolic Impact of the IL-33/sST2 Axis

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Abstract

Interleukin 33 (IL-33), which is expressed by several immune cell types, endothelial and epithelial cells, and fibroblasts, is a cytokine of the IL-1 family that acts both intra- and extracellularly to either enhance or resolve the inflammatory response. Intracellular IL-33 acts in the nucleus as a regulator of transcription. Once released from cells by mechanical stress, inflammatory cytokines, or necrosis, extracellular IL-33 is proteolytically processed to act in an autocrine/paracrine manner as an “alarmin” on neighboring or various immune cells expressing the ST2 receptor. Thus, IL-33 may serve an important role in tissue preservation and repair in response to injury; however, the actions of IL-33 are dampened by a soluble form of ST2 (sST2) that acts as a decoy receptor and is produced by endothelial and certain immune cells. Accumulating evidence supports the conclusion that sST2 is a biomarker of vascular health with diagnostic and/or prognostic value in various cardiovascular diseases, including coronary artery disease, myocardial infarction, atherosclerosis, giant-cell arteritis, acute aortic dissection, and ischemic stroke, as well as obesity and diabetes. Although sST2 levels are positively associated with cardiovascular disease severity, the assumption that IL-33 is always beneficial is naïve. It is increasingly appreciated that the pathophysiological importance of IL-33 is highly dependent on cellular and temporal expression. Although IL-33 is atheroprotective and may prevent obesity and type 2 diabetes by regulating lipid metabolism, IL-33 appears to drive endothelial inflammation. Here, we review the current knowledge of the IL-33/ST2/sST2 signaling network and discuss its pathophysiological and translational implications in cardiovascular diseases.

Introduction

Interleukin 33 (IL-33) is a member of the IL-1 family of cytokines, which strongly induces production of T helper-2 (Th2)-associated cytokines. Although regulation of transcription has been recently reported as an additional mechanism of IL-33 activity (see **Novel signaling**), classically active IL-33 functions as an "alarmin" or stress-response cytokine that engages and regulates an immune response particularly at barrier sites in the body, where IL-33 is highly expressed by endothelial or epithelial cells.[1] Once released, IL-33 acts in an autocrine/paracrine manner to activate the ST2L (ST2 gene-like) membrane receptor on nearby cells, *aka* IL33R and interleukin 1 receptor like 1 (IL1RL1). A soluble truncated form of ST2L without the transmembrane and intracellular domains, sST2, is secreted by endothelial and various immune cells either constitutively or upon stimulation (in some cases by IL-33).[2] sST2 is thought to function as a decoy receptor, thereby attenuating the actions of IL-33.[2]

Evidence over the last decade has supported the conclusion that the sST2/ST2L/IL-33 triad plays an important role in CVD. IL-33 is postulated to exert for the most part beneficial actions *via* ST2L that are related to cardiac repair or attenuation of adverse cardiovascular remodeling or atherosclerotic plaque progression. In the canonical model, sST2 attenuates the cellular and beneficial actions of IL-33 in the cardiovascular system. Accumulating evidence has shown that elevated circulating levels of sST2 have evident prognostic utility for worse outcome in acute myocardial infarction (MI),[3] systemic and pulmonary hypertension,[4-6] coronary artery disease (CAD),[7] heart failure,[8] and type 2 diabetes.[9, 10] Most often, sST2, and not IL-33, was assessed due to its greater levels and stability.

New findings reveal that this view of IL-33 as strictly a protective or benign agent in CVD is over-simplistic. Neither is it established that sST2 is harmful because of its role as decoy receptor. As we assess in this review article, notwithstanding the evidence supporting the utility of sST2 as a CVD biomarker, there are gaps in our understanding of the functional significance of the IL-33/sST2 axis in cardiovascular and metabolic stress. Specifically, the focus of this

review is on the vascular and metabolic aspects of the sST2/ST2L/IL-33 triad as a diagnostic and prognostic biomarker of stable CAD, MI, atherosclerosis, stroke, obesity, and type 2 diabetes. Also, we address the complicated question of whether IL-33/ST2 signaling functions simply as an acute "alarmin" system or contributes to CVD progression under chronic or dysregulated conditions. In that context, the involvement of various immune cells and novel intracellular and extracellular signaling mechanisms in the actions of IL-33 are discussed.

Cellular expression

The membrane receptor for IL-33, ST2L is highly expressed by a wide variety of immune cells, including Th2 cells, regulatory T cells (Tregs), M2 polarized macrophages, mast cells, eosinophils, basophils, natural killer (NK) cells, invariant natural killer T (iNKT) cells, and type 2 innate lymphoid cells (ILC2s).[2] ST2L is constitutively expressed on cells of the cardiovascular system, in particular endothelial cells,[11] and can also be transiently induced in certain cases in other immune cell types, such as Th1 and cytotoxic T cells.[12] The notable actions of IL-33 on various immune cells are summarized in Table 1. In general, IL-33 is an important player in both innate and adaptive immunity as ST2L is expressed on most immune cells. By activating Th2 cells, IL-33 elicits a type 2 immune response, particularly at barrier sites. IL-33 also exerts protective and anti-inflammatory effects involving Treg and ILC2 (see **Mechanistic insights into the role of IL-33/sST2 in atherosclerosis** and **Obesity and type 2 diabetes)**. However, if exuberant or dysregulated, type 2 inflammation may lead to tissue damage likely through activation of mast cells or eosinophils, and the development of pathological fibrosis.[13] In this way, IL-33 plays an indirect role in the pathophysiology of several pro-inflammatory and auto-immune diseases including asthma, allergies, arthritis, sepsis, and inflammatory bowel disease.[14] Whether a similar scenario also occurs in CVD is not known, and in fact the immune cell-specific role of IL-33 in CVD is not yet defined.

Table 1. Principal immune cells responsive to IL-33

Immune Cell Type	Action
B Cells	• Increases circulating IL-10-producing B cells[15] • Enhances proliferation capacity of B1 B cells and IgM, IL-5, and IL-13 production[16]
Basophils	• Promotes secretion of type 2 cytokines (e.g. IL-4 and IL-13) and IL-8 in synergy with IL-3 and/or FcεRI-activation, and enhances FcεRI-induced mediator release[17] • Prevents sST2 release, which is induced by IL-3 and C5a or anti-FcεRIα antibody [17]
Dendritic cells (DC)	• Increases surface levels of maturation markers MHC-II, CD40, CD80, CD86, OX40L, and CCR7[18-20] • Increases production of pro-allergic cytokines and chemokines IL-4, IL-5, IL-13, CCL17, TNF-α, and IL-1β[19] • IL-33-activated murine DCs required for *in vitro* and *in vivo* expansion of ST2+ Tregs due to IL-2 production[21] • IL-33-activated DCs prime naive lymphocytes to produce the Th2 cytokines IL-5 and IL-13, but not IL-4 and IFN-γ[18, 20]
Eosinophils	• Regulates homeostatic development and activation during disease[22] • Enhances adhesion, CD11b expression and survival[23] • Induces superoxide anion production, degranulation, and IL-8 production[24] • Exacerbates eosinophil-mediated airway inflammation (increases IL-13, TGF-β, CCL3, CCL17, and CCL24)[25] • Enhances Siglec-8 mediated apoptosis[26]
ILC2	• Promotes type 2 cytokines production[27, 28] • Expands *in vivo*[27, 29]
Invariant natural killer T (iNKT)	• Causes expansion and activation[30] • Enhances production of several cytokines, including both IL-4 and IFN-γ and induces IFN-γ instead of IL-4 upon TCR engagement in cooperation with IL-12[30, 31]
M2 polarized macrophages	• Amplifies the expression of M2 markers[32, 33] • Enhances activation by upregulating LPS receptor components TLR4 and MD2, soluble CD14, and MyD88, thus increasing LPS-induced cytokine production[33]
Mast cells	• Induces production of inflammatory cytokines MCP-1, TNF-α, IL-1, and IL-6[34] • Enhances IgE-mediated activation[34] • Promotes survival[35, 36] • Promotes mast cell activation and maturation, and induces GM-CSF, IL-5, IL-13, CXCL8, CCL17, CCL22, and CCL2 secretions[36, 37] • Induces production of various type 2 cytokines[38-40] • Promotes Th17 response during airway inflammation[41]
Natural killer (NK) cells	• Increases IFN-γ synergistically with IL-12[30, 31]
Regulatory T cells (Treg)	• Enhances protective ability/increases immunomodulatory function[42, 43] • Expands/increases directly or *via* IL-33-induced DC production of IL-2[21, 44-50])
Th2 cells	• Increases production of type 2 cytokines IL-5 and IL-13[51] • Chemoattractant[52]

In healthy human tissues, IL-33 is mainly expressed by stromal cells, including endothelial and epithelial cells, and specialized fibroblasts.[53] IL-33 is constitutively present in the nuclei of cardiac fibroblasts, cardiac endothelial cells, cardiomyocytes, and coronary artery smooth muscle cells of human adults and is released during stress or with necrosis.[11] It is expressed only to a limited extent in mouse endothelial cells.[54] IL-33 can also be released from

cells as a consequence of the cleavage of membrane phospholipids by secreted phospholipid A2 (sPLA2) enzymes, which is relevant to how venoms and inhaled allergens elicit a type 2 immune response[55] and likely relevant to atherosclerosis as well. In addition, IL-33 is a mechanically responsive cytokine secreted by living cells in response to stretch (Fig. 1).[56] Pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-1β increase IL-33 expression.[11]

In humans, ST2L and sST2 mRNA on the other hand were reported to be expressed at low levels in cardiomyocytes, cardiac fibroblasts, and vascular smooth muscle cells, but widely present in endothelial cells of the cardiac vasculature.[11] ST2L is prominently expressed by ILC2s, mast cells, and Tregs expressing the GATA3 transcription factor, as well as by activated Th2 lymphocytes.[2] Levels of ST2L are enhanced by IL-33 in ILC2s and Tregs, but neither expresses sST2. ST2L is expressed weakly as well by dendritic cells, neutrophils, and uncommitted macrophages (and enhanced by IL-4/IL-13).[2]

Taken together these findings would suggest that the primary direction for communication of the IL-33 alarmin system is from parenchyma or endothelium to the endothelium and immune cells, with production of sST2 by endothelial cells and certain pro-inflammatory immune cells serving a protective or damping role. Uncertain, however, is how ST2L expression in cardiovascular cells is affected by disease state.

Novel signaling

Two modes of action have been identified for IL-33, an extracellular one as a cytokine or alarmin, and a nuclear one as a regulator of transcription. Pro- and anti-inflammatory actions have been attributed to both modes of action, which are cell- and context-dependent. IL-33 localizes to the nucleus due to the presence of two bipartite nuclear localization sequences in the predicted helix-turn-helix structure of the homeodomain-like N-terminus.[57] Deubiquitination of IL-33 has been implicated in its nuclear stability, yet ubiquitination of IL-33 has also been implicated in its activation of transcription.[58, 59] A better understanding of the different

ubiquitination profiles of IL-33 and their significance is needed.

IL-33 associates with chromatin to ostensibly repress gene expression *via* protein-protein interactions, involving a short chromatin-binding motif that binds the acidic pocket made by the histone heterodimer H2A-H2B at the nucleosome surface.[60] However, the nuclear actions of IL-33 are diverse and incompletely understood. Binding of IL-33 to promoter-bound homeodomain proteins, such as histone methyltransferase SUV39/HI, was implicated in IL-33-mediated suppression of IL-6 and sST2 expression in human atrial endothelial cells.[61] IL-33 was reported to induce transcription of the type 2 inflammatory cytokine IL-13 in HEK293T cells by binding a conserved noncoding sequence before the transcription initiation site.[58] In addition, IL-33 was reported to function as a transcriptional regulator of NF-κB p65 expression in endothelial cells and participate in the inflammatory response by binding the p65 promoter.[62] In contrast, IL-33 was reported to act as a transcriptional repressor of NF-κB in synoviocytes of patients with rheumatoid arthritis.[63] In some cases, IL-33/NF-κB p65 protein–protein interactions may impair NF-κB DNA binding and thus interfere with NF-κB-dependent transcription.[64] Thus, both pro- and anti-inflammatory actions have been ascribed to nuclear IL-33.[65, 66] However, in many cell types, the role of nuclear IL33 is still unknown.[67]

IL-33 is constitutively expressed in many non-hematopoietic tissues, but its expression can be induced in both non-hematopoietic and some hematopoietic cells.[2, 60] Th1 and Th2 cytokines were reported to regulate intracellular levels of the precursor or full-length IL-33 in fibroblasts of healthy human lungs by activating or inhibiting, respectively, its proteasomal degradation.[68] Notably, full-length IL-33 was found to promote inflammation in the lung, but not a Th2 response, in an ST2-independent fashion.[69] Importin-5 (IPO5) was identified as an intracellular binding partner of full-length IL-33 that protects it from proteasomal degradation, but IPO5 is not required for nuclear localization of IL-33 and does not control its secretion.[70]

Full-length IL-33 is released into the extracellular space on cell damage or necrosis, whereas caspases 3 and 7 cleave and inactivate intracellular IL-33 during apoptosis (Fig. 1).[71]

Alternative transcript splicing with deletion of exons 3 and 4 may confer cytoplasmic localization and facilitate secretion.[72] The release of IL-33 from cells in the absence of damage or necrosis is not well understood, but in bronchial epithelial cells was shown to be under the regulation of ATP-induced P2 purinergic receptor stimulation and calcium influx.[73]

Extracellular IL-33 activates the membrane receptor ST2L, which together with the co-receptor IL-1R accessory protein (IL-1RAcP) recruits MYD88, IRAK1, IRAK4, and TRAF6, followed by activation of multiple signaling pathways, including MAPK1/ERK2 and/or MAPK3/ERK1, p38α MAPK, JNK1, and NF-κB (Fig. 2).[60] An extensive quantitative phosphoproteomic analysis of IL-33-mediated signaling was recently reported.[74] There is evidence as well that extracellular IL-33 may suppress activation of the p38 MAPK and NF-κB pathways in the heart 3 days post-MI, but this is likely indirect.[75] A number of mechanisms act to localize and limit both temporally and spatially the actions of extracellular IL-33 so as to make less likely an uncontrolled Th2 inflammatory response. Unlike most IL-1 family members, IL-33 has a comparatively long pro-peptide sequence of ~110 amino acid residues at the N-terminus. Contrary to original thinking, IL-33 bioactivation does not seem to be dependent upon caspase1/inflammasome-mediated processing within the cell, nor is cleavage necessary for secretion.[76, 77] Rather, a number of extracellular proteases are involved in its activation, with the cleaved sequence targeted within the N-terminal domain or central domain being protease-specific.[71] These include proteases that are released by neutrophils and mast cells, such as neutrophil proteinase 3 (PR3), elastase, and cathepsin G. Moreover, it was recently proposed that full-length IL-33 functions as a biochemical sensor of the proteolytic activities of a large variety of environmental aeroallergens.[78]

While short term exposure enhances the activity of IL-33, longer exposure to some proteases promotes further degradation and loss of activity by targeting the C-terminus IL-1-like cytokine domain. Furthermore, IL-33 is also rapidly oxidized within the extracellular milieu resulting in the formation of two intramolecular disulfide bonds that disrupt the ST2L binding

site.[79] Besides impairing function, IL-33 oxidation might alter its immunoreactivity and confound assays that rely on antibody detection. Thus, oxidation should be taken into consideration in measuring IL-33 especially under conditions of heightened inflammation and oxidative stress, as seen for instance with cigarette smoke, a major CVD risk factor.[80, 81]

In the canonical model, sST2 functions as a decoy receptor for IL-33, thereby preventing the cellular actions of IL-33 mediated by interaction with the membrane receptor ST2L (Fig. 2). However, there are a few intriguing reports that sST2 may have actions of its own on certain cells. Evidence was reported that sST2 has direct anti-inflammatory actions on macrophages by downregulating Toll-like receptors. Treatment with an ST2-human IgG fusion protein induced cellular signaling and down-regulated expression of TLR4 and TLR1 in bone marrow-derived macrophages.[82] In addition, administration of the fusion protein to mice attenuated LPS-mediated mortality and serum levels of IL-6, IL-12, and TNF-α, while an anti-ST2 antibody worsened the toxic effects of LPS, which are known to be mediated by TLR4. Others reported that sST2 suppresses LPS-induced IL-6 production in a human monocytic leukemia cell line.[83] Evidence (based on an ST2 Fc chimera protein) was also reported to support the conclusion that sST2 may contribute to adverse aortic remodeling seen in obesity by stimulating VSMCs to produce collagen type I, fibronectin, and profibrotic factors, as well as increase activities of MMPs.[84] Note, however, that because of the IgG portion of the molecule, sST2-fusion proteins (unlike sST2) could theoretically undergo dimerization, which might impact on their actions.

IL-33 and sST2 as biomarkers in coronary artery disease and myocardial infarction

The results of several studies summarized in Table 2 support the conclusion that serum levels of IL-33 decrease with increasing CVD severity.[85-87] The opposite pattern was reported for either the pro-inflammatory cytokine IL-6 or the extracellular protease matrix metalloproteinase (MMP)-28,[85-87] supporting the proposal that combining their assessment with that of IL-33 might be useful in gauging the severity of CAD. However, the number of CAD/ACS cases were small

213 in these 3 studies (n = 83/40, 103/27, and 70/20). Others did not find a difference in IL-33
214 between patients with ACS (n = 195) and stable CAD (n = 178), but in this study the highest
215 quintile of IL-33 predicted mortality (mean follow-up 3.6 years) in patients with STEMI.[88]
216 Although the number of participants was larger, it was still relatively small and the number of
217 patients with adverse clinical events at follow-up was very small (37 deaths). Moreover, serum
218 IL-33 was undetectable in more than 50% of study participants. Accurate assessment of IL-33 in
219 human serum is difficult for a number of reasons, including lack of sensitivity and specificity of
220 available ELISA assays, interference by the presence of sST2, and the use of non-serum
221 certified kits.[89] There is also a necessity to differentiate between oxidized (inactive) and reduced
222 (active) forms of IL-33, which is now possible through the development of specific ELISAs.[79]
223 In contrast, a clear pattern of increasing serum sST2 levels with greater severity of CAD
224 events has been consistently observed (healthy < stable angina < unstable angina < non-ST
225 elevation MI (NSTEMI) < STEMI < sudden cardiac death). Several studies have reported the
226 prognostic value of sST2 in patients with stable CAD. In the Ludwigshafen risk and
227 cardiovascular health study, sST2 did not correlate with the angiographic severity of CAD;
228 however, on long-term follow-up (median time of 9.8 years), higher levels of sST2 were an
229 independent predictor on multivariate analysis for all-cause mortality and cardiovascular death
230 after adjusting for clinical variables (including age, sex, BMI, hypertension, smoking status, and
231 diabetes) and biomarkers.[7] Soluble ST2 within the normal range had prognostic value additive
232 to NT-proBNP and hs-cTnT, supporting its utility in a multimarker approach. Results of a 2 year
233 follow-up from the ARTEMIS (international Ambulatory blood pressure Registry: TEleMonitoring
234 of hypertension and cardiovascular rISk project) study, involving a study population of 1,243
235 patients and 649 controls, revealed that in multivariate analysis only sST2 and hs-CRP
236 predicted the primary endpoint of cardiac death or heart failure hospitalization in both diabetic
237 and nondiabetic patients with CAD.[90] Results of the KAROLA study showed that after
238 multivariable adjustments sST2 levels in a cohort of 1081 stable CAD patients independently

predicted both short-term (4.5 years) and long-term (12.3 years) risk for total mortality, and short-term risk for fatal cardiovascular disease-related events, but not non-fatal cardiovascular events.[91]

Circulating sST2 levels have diagnostic and prognostic value after STEMI. sST2 levels measured within 1 day post-MI correlated positively with peak creatinine kinase, an estimate of the extent of necrosis, and negatively with pre-discharge left ventricular ejection fraction (LVEF).[92, 93] Early sST2 positively correlated with infarct size and expansion, as well as greater infarct transmurality and endocardial extent, microvascular obstruction, and plasma aldosterone levels.[94] Early values were a significant predictor of cardiovascular death and heart failure over the following 30 days after STEMI, independent of baseline characteristics or NT-proBNP levels and, in combination with NT-proBNP, improved risk stratification.[93, 95] Interestingly, unlike NT-proBNP, sST2 levels on presentation were not associated with clinical conditions linked to increased LV wall stress, such as age, hypertension, previous MI, or prior MI; however, higher levels were associated with diabetes mellitus.[93]

In a recent report on multimarker risk stratification for STEMI involving upwards of 1258 patients enrolled in the Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis in Myocardial Infarction 28 (CLARITY-TIMI 28) trial, sST2 was a significant predictor of heart failure or short-term cardiovascular death (out to 30 days) along with two other biomarkers, troponin T and myeloperoxidase (MPO).[96] Soluble ST2 had greater prognostic value than hs-cTnI for 30 day cardiac mortality in both STEMI and NSTEMI patients.[97] Another study showed that elevated sST2 levels with STEMI were associated with increased all-cause mortality up to 1 year and improved risk stratification using a multi-marker approach.[98]

sST2 levels were reported to be elevated in patients with STEMI and NSTEMI, with levels markedly higher in those with STEMI. [88] In addition, the highest quintile of sST2 predicted mortality in patients with STEMI, but not those with NSTEMI. Others reported that elevated sST2 predicated long-term major adverse event in NSTEMI patients, but did not improve risk

stratification for established markers.[99] In a recent study of 1401 first-ever MI patients involving mostly (79%) NSTEMI, higher sST2 values were associated with increased risk of death and heart failure over a 5 year follow-up, independent of other prognostic indicators. In this study, higher values of sST2 were associated with age, female sex, and hypertension, in addition to diabetes mellitus.[100] Findings of a cross-sectional, population-based study revealed that sST2 also positively correlates with markers of type 2 diabetes and endothelial dysfunction, but not established cardiovascular risk factors.[10] This suggests that activated/stressed vascular endothelial cells are the source of sST2 in diabetes. While pathology-related increases in circulating sST2 have clinical value, others reported that sST2 levels in healthy men and women added little long-term predictive information for cardiovascular events or all-cause mortality.[101]

Overall, there is strong evidence for the diagnostic and/or prognostic utility of sST2 in CAD and MI (both STEMI and NSTEMI), particularly in combination with established biomarkers. Key studies supporting this conclusion are listed in Table 2. The observation that circulating levels of IL-33 and sST2 exhibit an opposite pattern of change with increasing severity of CAD event, together with MI preclinical studies (see below), underpins the conclusion that enhancing cardiovascular activity of IL-33 may be beneficial for limiting cardiovascular events.

Table 2. Utility of IL-33 and sST2 as biomarkers for cardiovascular diseases

Diagnosis	Biomarker	Outcome/Prognosis
Coronary Artery Disease (CAD) - General	IL-33	Serum levels lower in patients with stable angina, and even lower in patients with acute coronary syndrome (ACS)[85]
		Elevated MMP-28 and decreased IL-33 in CAD patients correlate with disease severity [86]
		Differential IL-33 and IL-6 expression reported for those with ACS or stable CAD[87]
		No difference in those with ACS *vs.* stable CAD, although highest quintile predicted mortality in patients with STEMI [88]
		Increased levels in patients with ACS vs. patients with stable CAD and normal controls [88]

	sST2	sST2 not correlated with stable CAD severity, but higher levels independent predictor for all-cause mortality and cardiovascular death [7]
		Only sST2 and hs-CRP predicted cardiac death or heart failure hospitalization in both diabetics and nondiabetics with CAD[90]
		Higher levels independently predicted short- and long-term risks for total mortality, and short-term risk for fatal cardiovascular events[91]
		Higher levels associated with increased all-cause and cardiovascular mortality[102]
STEMI	sST2	Levels correlated positively with heart damage[92, 93]
		Positively correlated with infarct size and expansion, as well as greater infarct transmurality and endocardial extent, microvascular obstruction, and plasma aldosterone [94]
		Early values predict increased mortality and heart failure over subsequent 30 days, independent of baseline characteristics or NT-proBNP levels; improved risk stratification in combination with NT-proBNP[93, 95]
		Predictor of heart failure or short-term cardiovascular death along with troponin T and MPO[96]
		Greater prognostic value than hs-cTnI for 30 day cardiac mortality in both STEMI and NSTEMI patients [97]
		Elevated levels associated with increased all-cause mortality out to 1 year and improved risk stratification in multi-marker approach[98]
NSTEMI	sST2	Elevated sST2 predicated long-term major adverse event but did not improve risk stratification for established markers[99]
		Elevated sST2 associated with increased risk of death and heart failure over next 5 years, independent of other prognostic indicators; higher values associated with age, female sex, hypertension, and diabetes[100]
		Higher levels associated with adverse outcomes at 30 days and 1 year; improved risk stratification in CVD and heart failure at 30 days and 1 year when levels added to established clinical biomarkers[103]
		Elevated levels predict mortality at 1 year; independent of CV comorbidities or risk factors such as age, renal function, and diabetes[104]
Stroke	IL-33	Elevated in acute ischemic stroke; lower levels associated with greater stroke severity and large infarct; levels higher in patients with favorable outcome; levels independent predictor for functional outcome[105]
	sST2	Higher sST2 at admission associated with all-cause mortality 90 days after acute ischemic stroke, but no prognostic value in multivariate analysis[106]

Atherosclerosis	IL-33	Increased expression in plaques; promotes leukocyte adhesion to endothelial cells and induces adhesion molecules and CCL2 in endothelial cells [107]
		Induces expression of CXCL1 chemokine[108]
	ST2L	Similar ST2L expression in atherosclerotic plaques of asymptomatic and symptomatic patients on T cells and endothelial cells of neo-angiogenic vessels; more ST2L in macrophages of symptomatic patients[109]
	sST2	Identified as risk factor for subclinical atherosclerosis; levels positively correlated with standard atherosclerosis risk factors [110]
Diabetes/Obesity	sST2	Blood levels positively associated with hypertension and diabetes[4]
		Levels correlate with markers of type 2 diabetes and endothelial dysfunction, but not established cardiovascular risk [10]
		Levels elevated with obesity, suggesting attenuation of beneficial actions of IL-33 in obesity [111]
		Higher levels in type 2 diabetes [4, 10, 112-114]
		Positive association of levels with risk factors for diabetes after adjusting for age and sex; highest increases associated with increased risk for diabetes [9]
		Association of hs-TnT and sST2 with cardiovascular and all-cause mortality during ~5 year follow-up among diabetics [115]
		Levels among diabetics increased further by LV diastolic dysfunction[113, 116]
		Association of severe obesity with increased expression in endothelial cells of human adipose tissue[111]
	Il-33	Levels lower in non-lean *vs.* lean individuals, and negatively correlated with BMI and body weight in those lean and overweight, but not obese; [117] negatively correlated with HbA1c in non-diabetic persons, and associated with protective lipid profile
		Severe obesity associated with increased expression in endothelial cells of human adipose tissue [111]

Pathophysiological role of IL-33/ST2 signaling in preclinical studies of myocardial infarction

The strong association between increased circulating levels of sST2 and poor prognosis in patients with MI provides circumstantial evidence for a protective role of IL-33 in the heart

under stress that is borne out by preclinical studies. Biomechanical strain induces expression of sST2 and IL-33 in both cardiac myocytes and fibroblasts, with cardiac fibroblasts being more responsive.[118] Similarly, IL-33 was mostly expressed by interstitial cells (likely myofibroblasts) in pressure overloaded mouse hearts.[118] Levels of IL-33 in human adult cardiac myocytes and fibroblasts are also increased by inflammatory cytokines.[11] IL-33 was found to protect neonatal rat cardiomyocytes from cell hypoxia-induced caspase-3 cleavage and apoptosis, and this was associated with increased expression of anti-apoptotic proteins (XIAP, cIAP1, survivin, Bcl-xL, and Bcl-2).[119] The addition of sST2 blocked these protective actions of IL-33. Others reported evidence for the attenuation of ROS generation by IL-33, and the subsequent sequential activation of PKCβII and JNK, in the protection of neonatal mouse cardiomyocytes from apoptosis after anoxia/reoxygenation.[120]

In vivo preclinical evidence also indicates that IL-33 protects the heart from infarction. IL-33 treatment was found to decrease fibrosis, infarct size, and apoptosis after ischemia-reperfusion (I/R) in the rat and improve cardiac function.[119] In addition, IL-33 reduced ventricular dilation, improved contractile function, and increased survival following coronary artery ligation in wild type, but not in $ST2^{-/-}$ mice.[119] IL-33 treatment was associated with a decrease in mast cell density in the infarct area, as well as an increase in Th2 and decrease in Th1 genes in the infarct. Many of these beneficial actions of IL-33 are probably secondary effects on the myocardium, since as discussed IL-33 by itself may have pro-fibrotic actions. In addition, IL33 activates mast cells and a reduction in cardiac mast cells was reported to attenuate myocardial contractility after MI.[121] Thus, the reduction of mast cells in the study of Seki K et al.[119] is probably secondary to general reduction of inflammation and unrelated to improved cardiac contractility.

Another study on MI (permanent LAD occlusion) in mice also reported similar beneficial effects of post-treatment with IL-33 on cardiac function and structure, as well as reduced myocardial macrophage infiltration and inflammatory cytokine production, and suppression of

p38 MAPK and NF-κB activation.[75] However, the exact involvement of p38 MAPK signaling in the cardioprotective actions of IL-33 is likely a matter of timing and model of injury. Others recently implicated activation of p38 MAPK in the anti-apoptosis and anti-inflammatory actions of pre-treatment with IL-33 in protecting the heart, including decreased expression of the cytokine/alarmin high mobility group box 1 protein (HMGB1), in a rat model of I/R-induced cardiac injury.[122]

Diabetes mellitus increases the vulnerability of the heart to I/R-induced injury. This has been attributed in part to increased PKCβII activity, which is enhanced by diacylglycerol (DAG).[123] Cellular levels of DAG are in turn regulated by DAG kinase (DGK), which catalyzes its conversion to phosphatidic acid. Diabetes-related exaggerated apoptosis and dysfunction of the myocardium that is observed with I/R was attributed to increased PKCβII activity due to reduced expression of DGK-zeta.[123] The later was linked to reduced levels of IL-33, which was shown to induce DGK-zeta expression in the heart and isolated cardiomyocytes. Thus, IL-33 may negatively regulate PKCβII activity in cardiac myocytes both by attenuating oxidative stress and by enhancing expression of DGK-zeta. Evidence was reported that the reduced IL-33 levels in the diabetic heart result from high glucose-induced secretion of HMGB1 from cardiac myocytes.[124] HMGB1 in turn stimulates TLR4 receptors on fibroblasts to reduce their IL-33 production, thereby leading to enhanced collagen production and cardiac fibrosis. However, the means by which IL-33 suppresses fibrosis in the heart is not known and likely indirect. Surprisingly, IL-33 was found not to directly inhibit collagen I/III or periostin production by adult rat cardiac fibroblasts, or their proliferation; rather, IL-33 stimulated expression of cytokines and chemokines (IL-6 and CCL-2) associated with cardiac inflammation and fibrosis, although the migratory ability of the cardiac fibroblasts was attenuated.[125] Interestingly, in a mouse infarction model, myocyte-targeted ablation of TGFβ signaling markedly augmented IL-33 expression in what appeared to be perivascular interstitial cells, but no impact on collagen deposition in the infarct was seen.[126]

In summary, despite the fact that stressed and injured cardiac myocytes may secrete IL-33, they produce factors (e.g., HMGB1) that reduce IL-33 production by cardiac fibroblasts, which may favor ROS-induced PKCβII/JNK activation, inflammatory cytokine and apoptosis gene expression. Several rodent studies reported a protective effect of IL-33 supplementation on the heart, delivered either before or after MI, which is attributable to reduced ROS production. The cell type(s) mediating the cardioprotective effects of IL-33 is (are) not yet defined. Paradoxically, Abston et al. have reported that IL-33 treatment in healthy mice induces inflammatory cytokines in the heart, and independently induces eosinophilic pericarditis and impairs heart function.[127] Strain differences or dosing regimen cannot explain the discrepant findings between this study and the ones involving MI, so other factors such as diet, surgical procedure, pre-existing injury, need to be considered. In any event, the findings of Abston et al. caution against taking a broad approach in IL-33 delivery for protecting the infarcted heart.

IL-33/ST2 signaling in the pathophysiology and clinical outcome of stroke

In patients (n = 206) who suffered acute ischemic stroke, serum IL-33 levels were elevated; however, lower levels were associated with greater stroke severity and large infarction volume. Levels were higher in patients with a favorable outcome, and IL-33 levels were an independent predictor for functional outcome.[105] On the other hand, higher sST2 at the time of admission was reported to be associated with all-cause mortality 90 days after acute ischemic stroke (n = 721), but did not offer prognostic value in multivariate analysis.[106] Larger, adequately powered and well-designed studies across multiple centers with proper controls are needed to assess the prognostic value of IL-33/sST2 in ischemic stroke.

In preclinical models, treatment with IL-33 was shown to be protective in ischemic stroke[128, 129] and spinal cord injury[130] by causing a shift towards the M2 microglial/macrophage cell phenotype and attenuating inflammation. Expression of IL-33 in oligodendrocytes and astrocytes increases with ischemic injury in the mouse, along with ST2L expression in microglia

and astrocytes. Yang et al.[131] provided evidence that the neuroprotective actions of IL-33 in ischemic stroke are due in part to its stimulation of anti-inflammatory cytokine IL-10 production by microglia cells.

In summary, despite serum IL-33 being increased in ischemic stroke, an association of lower IL-33 and higher sST2 with worse outcome was observed. Although based on a single study, this is consistent with the idea that in ischemic stroke IL-33 has protective actions that are dampened by sST2 (Table 2), as supported by animal studies. However, by themselves early serum IL-33 levels may reflect mostly the extent of injury, rather than serving as a measure of the extent of protection mounted. Paradoxically, its induced target sST2 is likely a gauge of both extent of injury and blockade of protection. For that reason and technical issues previously discussed, greater confidence ought to be placed in reported sST2 values in MI and stroke studies.

Genetic variants in IL-33 and ST2 genes and relationship with CAD

A prospective study of 2,991 Framingham Offspring Cohort participants revealed that much of the variation in sST2 production among individuals is due to genetic factors.[132] The *IL1RL1* gene encodes for both the membrane-bound receptor isoform (ST2L) and the soluble protein (sST2) through alternative promoter activation and splicing.[133] Multiple single-nucleotide polymorphisms (SNPs) within *IL1RL1* were found to correlate with sST2 levels in a genome-wide association study, and five missense variants mapping to the intracellular domain of ST2L, which is not present in sST2, correlated with higher sST2 levels.[132] Experiments on cultured cell lines expressing the intracellular variants attributed the increase in sST2 levels to an autocrine loop of increased IL-33 induction and enhanced ST2L responsiveness. Briefly, increased sST2 was ascribed to (a) increased induction of IL-33 by ST2L because of enhanced NF-κB and AP1 signaling, which also selectively activated the proximal promoter of *IL1RL1* linked to sST2 expression, and (b) a selective increase in ST2L expression due to an increase in endogenous

IL-1β levels resulting from enhanced constitutive ST2L-mediated inhibition of a counterregulatory PI3K/AKT/mTOR signaling axis that attenuates IL-1β levels. In light of the recently reported outcome of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), [134, 135] the potential synergistic interplay between IL-1β and IL-33 *in vivo* merits investigation.

An earlier study linked two polymorphisms in the distal promoter of *IL1RL1* that drives ST2L expression to enhanced CAD severity, but no sST2 measurements were made.[136] Another SNP in *IL1RL1* was linked to increased risk for CAD without defining its functional impact.[137] Yet another SNP of *IL1RL1* was associated with lower circulating sST2 levels; however, in affected individuals with CAD or peripheral artery disease, increased sST2 levels were an independent predictor of all-cause mortality by multivariable Cox regression analysis, but not for secondary endpoints of CV death, MI, hospitalization for heart failure, stroke, and amputation.[138] Unfortunately, the impact of this SNP on IL-33 levels or ST2L expression was not determined. An SNP within the promoter region of the IL-33 gene was associated with increased circulating levels of IL-33 and increased risk for CAD.[137] Another IL-33 gene polymorphism that was linked to decreased IL-33 production was associated with a decreased risk for developing premature CAD or central obesity.[139] Others reported the opposite effect of this SNP genotype on serum IL-33 levels in patients with rheumatoid arthritis and thus no causal relationship can be drawn.[140] In addition, a direct causal relationship between IL-33 levels and CAD is not established as neither of the studies on IL-33 gene variants reported values of sST2. Nonetheless, an SNP in the *IL-1RAcP* gene was also linked to CAD risk.[141]

In summary, limited reports suggest that genetic variants in or around the *IL1RL1* gene are associated with differences in expression levels of both sST2 and ST2L, as well as IL-33. Polymorphisms in the gene cluster within which *IL1RL1* resides have been associated with a number of immune and inflammatory conditions,[132] but more extensive GWAS are needed to

establish a causal link between IL1RL1 variants and CAD. This is the case for the *IL-33* gene as well.

Mechanistic insights into the role of IL-33/sST2 in atherosclerosis

Increased IL-33 expression has been detected in human atherosclerotic plaques, emphasizing the importance of IL-33 in vascular biology and remodeling (Fig. 3).[107] Atherosclerosis is characterized by a chronic arterial wall inflammation that plays a major role in atheroma formation.[142] The presence of oxidized low-density lipoproteins (ox-LDL) in the vessel induces the production of pro-inflammatory mediators like cytokines and growth factors from surrounding tissues that further fuel the inflammatory response and atherosclerosis progression.[143] Miller at al. revealed that IL-33 administration to $ApoE^{-/-}$ model of atherosclerosis in mice, induced a shift from the Th1 pro-atherosclerotic immune response to a Th2 protective and pro-resolving immune response by significantly increasing Th2 cytokine production (IL-4, IL-5 and IL-13) and decreasing IFNγ levels, a typical Th1 cytokine.[144] Th1 to Th2 polarization resulted in a reduction of aortic atherosclerotic lesions when compared to vehicle-treated group.[144] Of note, atherosclerotic plaque formation and progression is multifactorial and T cell infiltration can either increase (Th1) inflammation in plaques or decrease (Th2/Treg) it depending on the dominant phenotype.[145] In addition to polarizing effects, IL-33 increased levels of atheroprotective natural IgM type anti-ox-LDL antibodies suggesting a potential effect on B1 cells. Neutralizing IL-33 effects *via* sST2 administration to $ApoE^{-/-}$ mice resulted in aortic plaque expansion when compared to control IgG-treated group. Additionally, blocking IL-5 with a neutralizing antibody negated the protective effect of IL-33 and dampened the production of ox-LDL antibodies suggesting that IL-5 might have a key role in the atheroprotective effect of IL-33.[144] *In vitro* studies on the other hand, showed that IL-33 atheroprotection might have occurred *via* inhibition of macrophage foam cell formation through decreased acetylated LDL and ox-LDL uptake and enhanced cholesterol efflux.[146] Recently, the ability of IL-33 to protect

446 macrophage-derived foam cells from cholesterol overload was attributed to the induction of IL-
447 10, which helped IL-33 in an autocrine manner to increase expression of ATP-binding cassette
448 transporter (ABCA1), potentially promoting cholesterol efflux.[147]

449 Multiple lines of evidence support the concept that IL-33 may also be atheroprotective by
450 engaging ILC2s and activating downstream type 2 immunity, mainly IL-5 and IL-13.[148, 149] IL-5
451 may stimulate B1 cell proliferation and production of atheroprotective natural IgM antibodies
452 against the phosphorylcholine (PC) head group of oxidized phospholipids within LDL.[149-151]
453 Besides inducing the expansion of ILC2s, recent evidence indicates that IL-33 promotes the
454 egress of ILC2s from the bone marrow and possibly from secondary lymphoid organs,[152] which
455 further lends weight to the idea that administration of IL-33 at pharmacological levels may be
456 necessary to reveal its role in atherosclerosis. Consistent with this possibility, loss of either
457 endogenous IL-33 or its receptor ST2 was found to have no impact on development of
458 atherosclerosis in ApoE-deficient mice.[153] Activated ILC2s may also attenuate the progression
459 of atherosclerosis by producing IL-13, which polarize macrophages towards the “M2” like
460 phenotype.[154] In addition, the actions of ILC2s in regulating adipose tissue homeostasis and
461 limiting obesity (see **Obesity and type 2 diabetes**) may be an additional means by which IL-33
462 exerts atheroprotective effects.

463 IL-33 may also contribute to an increase in Treg cells, which exert anti-atherogenic
464 effects by limiting both adaptive and innate immune responses.[155-157] This function of IL-33 may
465 be compromised in atherosclerosis due to both increased serum levels of sST2 and reduced
466 levels of $CD4^+ST2^+$ cells.[158] Recent evidence shows that expression of ST2 is also a feature of
467 a sizable number of tissue-resident Treg cells that are important for tissue repair and promoting
468 organ homeostasis.[159] Their expansion and activation is stimulated by IL-33.[159, 160] These $ST2^+$
469 Tregs exert anti-inflammatory actions and suppress CD4 T cell proliferation through the release
470 of IL-10 and TGF-β.[161] This pool of Treg cells is especially prominent in visceral adipose tissue,
471 where Treg cells support metabolic functions and possibly adipocyte differentiation.[162, 163]

Little information is available concerning the expression pattern of the IL-33/ST2L axis within the atherosclerotic plaque. In an immunohistochemical study on endarterectomy samples, Marzullo et al.[109] observed that ST2L was expressed in atherosclerotic plaques to a similar extent in asymptomatic and symptomatic patients on both T cells and endothelial cells of neo-angiogenic vessels (much more so than the endothelial cells covering the residual lumen of the vessel). In contrast, expression of ST2L on macrophages was more remarkable in symptomatic patients. Based on these observations, the authors hypothesize that the IL-33/ST2L axis drives plaque development and eventual rupture; however, the sample size in their study was small, and causality was not studied. Others have recently suggested that IL-33 may contribute to plaque progression in part by inducing expression of the chemokine CXCL1 (see **Vascular inflammation**).[108] On the other hand, in patients with primary hypertension, a major risk factor for atherosclerosis, circulating levels of sST2 were found to be high, whereas IL-33 levels were low.[110] Moreover, sST2 was identified as a risk factor for subclinical atherosclerosis and its levels were positively correlated with the standard atherosclerosis risk factors, LDL cholesterol, C-reactive protein (CRP), and carotid intima-media thickness.[110]

The overall evidence supports the conclusion that IL-33 has atheroprotective effects. Several mechanisms may explain these actions. These include a shift in T cell polarization from Th1 to Th2 and an increase in Treg cells, increased levels of natural IgM anti-ox-LDL antibodies, inhibition of macrophage foam cell formation, stimulation of ILC2s, and polarization of macrophages towards the “M2” like phenotype.

Vascular inflammation

Several studies have reported direct pro-inflammatory effects of IL-33 on endothelial cells. For instance, IL-33 induces the secretion of the inflammatory cytokines IL-6 and IL-18 from human umbilical vein endothelial cells (HUVECs),[164] as well as the expression of chemoattractants for leukocytes (CXCL1 and CCL2).[108] Also, IL-33 promotes the adhesion of

498 human leukocytes to human endothelial cells and induces vascular cell adhesion molecule-1,
499 intercellular adhesion molecule-1, endothelial selectin, and CCL2 mRNA and protein expression
500 in human coronary artery and umbilical vein endothelial cells *in vitro* and human explanted
501 atherosclerotic plaques *ex vivo*.[107] These effects of IL-33 on endothelial cells and immune cells
502 may explain why increased IL-33 serum levels after coronary stent implantation are associated
503 with coronary in-stent restenosis,[165] as leukocyte activation is a critical step in development of
504 restenosis after PCI.[166] Interestingly, Pollheimer et al.[167] observed that the pro-inflammatory
505 actions of IL-33 on cultured HUVECs was greater in proliferating cells and correlated with ST2L
506 receptor levels. Their observations are consistent with the previously mentioned findings of
507 Marzullo et al.[109] on endarterectomy samples of human carotid atherosclerotic plaques.
508 Other studies have demonstrated that IL-33 promotes angiogenesis and vascular
509 permeability *in vitro* and *in vivo*, notably within the context of inflammation.[168-173] The pro-
510 inflammatory actions of IL-33 on the vasculature, and endothelial cells in particular, may
511 contribute to the pathogenesis of giant-cell arteritis (GCA), which is an inflammatory disease of
512 blood vessels that occurs in the elderly. The exact basis for GSA is uncertain, but ageing-
513 related alterations in the immune system in genetically predisposed individuals seem to be
514 involved.[174] Recently, increased expression of both IL-33 and ST2, chiefly in endothelial cells of
515 newly formed vessels, was found in GCA arteries.[175] IL-33 expression correlated with the
516 degree of vessel wall inflammation and was reduced in arteries from steroid-treated GCA
517 patients. In addition, a positive association was observed between IL-33 and the numbers of
518 neovessels, suggesting that IL-33 participates in the pathogenesis of angiogenesis-dependent
519 inflammation in GCA. Although no Th2 cytokines were detectable, expression levels of IL-33
520 correlated with the presence of M2 macrophages, the latter being reported to promote
521 angiogenesis *in vivo*.[176] Recently, the rs7025417 polymorphism in the IL-33 gene, which was
522 noted to be associated with increased IL-33 plasma levels, was identified as a risk factor for

GCA in a large meta-analysis involving a total of 1,363 biopsy-proven GCA patients and 3,908 healthy controls from four European cohorts.[177]

GCA and other inflammatory or infectious conditions increase the risk for having an acute aortic dissection. Other risk factors include hypertension, smoking, atherosclerosis, and certain genetic diseases. In a recent large retrospective study with a prospective validation cohort, sST2 was found to have overall superior diagnostic utility for detecting acute aortic dissection among emergency room patients with sudden-onset severe chest pain, which is easily misdiagnosed.[178] This finding and those related to GSA and diabetes (see **IL-33 and sST2 as biomarkers in coronary artery disease and myocardial infarction** and **Obesity and type 2 diabetes)** highlight the utility of IL-33/sST2 as a biomarker of vascular health.

In summary, IL-33 has been implicated in vascular inflammation *via* upregulation of adhesion molecules and chemokines for leukocytes. The pro-inflammatory actions of IL-33 on endothelial cells contribute to the pathogenesis of GCA, and are seemingly more prominent in angiogenesis. Further studies are needed to establish the role IL-33-induced endothelial inflammation in restenosis, as well as plague and post-ischemic neoangiogenesis.

Obesity and type 2 diabetes

Obesity and its common consequence, type 2 diabetes are major risk factors for cardiovascular disease that are marked by chronic systemic and vascular inflammation. [179, 180] Obese adipose tissue is characterized by an inflammatory immune environment consisting of classically activated M1 macrophages, cytotoxic T cells, and pro-inflammatory Th1-type cytokines (such as, TNF-α and IFN-γ).[181] In contrast, lean fat tissue is characterized by an anti-inflammatory environment of alternatively activated M2 macrophages, eosinophils, Th2 cells, Tregs, and ILC2s, along with anti-inflammatory Th2-type cytokines (such as, IL-4, IL-5, IL-9, and Il-13). IL-33 was recently shown to regulate white adipose tissue (WAT) homeostasis, a process that when dysregulated results progressively in the pro-inflammatory state, obesity, insulin

549 resistance, and the metabolic syndrome.[77] Production of IL-33 by WAT is stimulated by the
550 sympathetic nervous system, with IL-33 exerting positive reinforcement by inducing the
551 upregulation of tyrosine hydroxylase, a rate-limiting enzyme in catecholamine biosynthesis.[182]
552 Compared to wild type mice fed a high fat diet, ST2 knockout mice fed a high fat diet have a
553 higher body weight and greater fat mass, along with more impaired insulin secretion and
554 glucose tolerance.[183] The major orchestrators in the actions of IL-33 on adipocyte function and
555 metabolic homeostasis in both rodents and humans are ILC2s, which may actually be the major
556 source of the type 2 cytokines in WAT, rather than Th2 cells.[184, 185] IL-33 that is released most
557 likely by adipose tissue endothelial cells, and perhaps adipocytes themselves, maintains ILC2
558 cells in WAT and stimulates them to initiate a number of actions that limit adiposity by increasing
559 caloric expenditure.[77, 185, 186] The overall process is known as beiging or browning of WAT and
560 involves upregulation of uncoupling protein 1 (Ucp-1) in adipocytes.[77] ILC2 cells were proposed
561 to recruit eosinophils and M2 macrophages, which support optimal beiging of WAT through the
562 release of type 2 cytokines. ILC2 cells also produce methionine-enkephalin peptides that
563 directly act on adipocytes to promote beiging.[184] IL-33 may also exert positive regulatory actions
564 on WAT mass and milieu *via* the development and maintenance of $ST2^+$ visceral adipose
565 tissue-Treg cells, which are diminished in obese mice and implicated in preserving insulin
566 sensitivity and glucose tolerance through dampening actions on pro-inflammatory M1
567 macrophages and $CD8^+$ T cells.[162] On the other hand, while M1 macrophage-driven
568 inflammation subserves obesity-associated insulin resistance, fat-resident $ST2^+$ Treg cells have
569 been implicated in promoting age-associated insulin resistance.[187] One possible explanation
570 would be that some degree of inflammation is favorable for adipose tissue remodeling and
571 metabolic function.

572 Serum IL-33 levels are lower in non-lean individuals compared to those who are lean,
573 and negatively correlated with BMI and body weight in those who are lean and overweight, but
574 not obese.[117] In addition, IL-33 was found to be negatively correlated with HbA1c in non-diabetic

persons, but not diabetics, and to be associated with a protective lipid profile. On the other hand, severe obesity is associated with increased expression of both IL-33 and ST2 in endothelial cells of adipose tissue of both humans and mice, although the significance of this observation to endothelial function or inflammation is unclear.[111] Plasma sST2 levels are also reported to be elevated with obesity in humans, suggesting an attenuation of the beneficial actions of IL-33 in obesity.[111] Several studies report higher circulating sST2 levels in individuals with type 2 diabetes.[4, 10, 112-114] A recent study reported a positive association between sST2 levels and various risk factors for developing diabetes after adjusting for age and sex and implicated the highest increases in sST2 with increased risk for developing diabetes.[9] Among diabetic patients, only hs-TnT and sST2 were found to be independently associated with cardiovascular and all-cause mortality during a ~5 year follow-up.[115] Levels of sST2 among diabetics are increased further by LV diastolic dysfunction.[113, 116]

In summary, IL-33 has been shown to limit adiposity by increasing caloric expenditure *via* ILC2s and prevents insulin resistance and impaired glucose tolerance by taming WAT inflammation *via* WAT Tregs. Plasma sST2 levels are increased with obesity and are a risk factor for development of type 2 diabetes. Increased circulating sST2 in type 2 diabetes may be reflective of microvascular endothelial inflammation.

Unresolved issues and future directions

Accumulating evidence supports the conclusion that sST2 is a biomarker of vascular health with diagnostic and/or prognostic value in various cardiovascular diseases, including coronary artery disease, myocardial infarction, atherosclerosis, giant-cell arteritis, acute aortic dissection, and ischemic stroke, as well as obesity and diabetes. However, the role of IL-33 is more complicated, as this alarmin may have both pro- and anti-inflammatory actions depending upon which cell type is engaged (Fig. 4). Overall, the actions of IL-33 *in vivo* are pleiotropic and must be viewed in pathophysiological context.

In pursuing the pharmacological potential of IL-33/ST2, it is important to acknowledge the detrimental versus protective effects of IL33/ST2 signaling. There is a need for additional experimental studies in various contexts to better comprehend the role of IL33/ST2 signaling. For example, the cell-specific effects of IL33 *in vivo*; the impact of the microbiota; the impact of acute injury (IL33 can be secreted after MI, and atherosclerosis can be accelerated after MI; does IL33/ST2 signaling play a distinct role in this context?), the interaction with other CV risk factors (does IL33/ST2 signaling affect atherosclerosis differently in obese or diabetic conditions?), etc. Additionally, there is a need for GWAS studies to address causality between IL33/ST2 signaling and CVD. To exploit the translational potential of IL-33/ST2-based therapies, a better understanding of differences in pharmacology between sST2 and anti-ST2 is needed.[188] Also, caution must be exercised in assessing the translational relevance of studies with injection of recombinant IL33, which might not reflect endogenous levels. Several strategies that aim at blocking IL33 signaling are nowadays feasible in patients. A few pharmaceutical companies are developing anti-IL33 mAb, anti-ST2, or sST2, mainly for asthma and COPD. Obviously, these approaches may lead to potential CV side effects; it might be wise to measure natural IgM anti-oxLDL antibodies in these patients as the levels of those antibodies are inversely associated with CVD in humans.

It is increasingly appreciated that the pathophysiological importance of IL-33 is highly dependent on cellular and temporal expression. The actions of IL-33 are likely to be pleiotropic in a dose-dependent manner, depending as well on which immune cells are activated and for how long or whether endothelial cells are engaged. The final outcome would reflect the contribution of its protective and anti-inflammatory actions mediated by Treg cells, the inflammatory actions of various recruited immune cell types, and the injury-related response of stromal/parenchymal cells, all of which are modulated by the dampening actions exerted by sST2. In many cases, the levels of either ST2 or sST2 are positively affected by IL-33 in a dose-dependent manner. IL-33 may also increase levels of myeloid-derived suppressor cells (MDSC),

627 which potently suppress T cell responses.[189] Additional *in vivo* studies involving immune cell
628 type-specific knockouts and transgenics are desired to better define the role of IL-33/ST2 axis in
629 various diseases.

630 The importance of spatiotemporal context in IL-33 signaling is illustrated by the actions
631 of IL-33 on mast cells in asthma. On the one hand, IL-3 acts on mast cells *via* ST2 to increase
632 bronchial hyperresponsiveness in part by boasting FcR-mediated degranulation.[190] The
633 released proteases generate forms of IL-33 with increased biological activity, thus establishing a
634 positive feedback loop. On the other hand, mast cell sST2, which dampens the actions of IL-
635 33,[2] is strongly induced by IL-33, and long-term exposure to IL-33 also induces a mast cell
636 phenotype with decreased degranulation. Moreover, recent evidence shows that in smaller
637 peripheral airways IL-33 protects against bronchial hyperresponsiveness by inducing PGE2
638 formation by mast cells, which has relaxing effects on airway smooth muscle and anti-
639 inflammatory actions on mast cells.[191]

640 While ST2/IL-33 signaling in ILC2s, Tregs, and IL-10 producing B cells protects against
641 inflammation, IL-33 clearly contributes to pathogenesis as a regulator of a type 2 immune
642 response in certain settings (e.g., allergic diseases and asthma). Although initially beneficial in
643 dealing with certain pathogens, chronic, excessive, or dysregulated type 2 immunity may
644 contribute to tissue damage and fibrosis.[13] As an early component of tissue injury and
645 inflammation, IL-33 plays an important role in tissue repair, but in certain cases, IL-33 may
646 contribute to excessive acute sterile inflammation and tissue damage. For instance, IL-33 from
647 liver sinusoidal endothelial cells was found to exacerbate I/R-induced hepatic sterile
648 inflammation, a contributor to organ damage in liver surgeries, by stimulating neutrophil
649 extracellular trap formation.[192] Moreover, ST2 expression by neutrophils was markedly
650 increased by IL-33, thereby amplifying its inflammatory actions. Both the identity of the cell type
651 engaged and the magnitude of its response will impact on the outcome seen with IL-33.

652 Unrecognized until recently are the different potencies of the various proteolytic forms of

extracellular IL-33 that are generated *in vivo*. Which forms are actually elevated in various disease conditions is largely unknown. There are great gaps also in our understanding of the nuclear roles of IL-33 and how these are coordinated with its extracellular actions. The processes involved in the secretion of IL-33 are also poorly understood. Finally, the potential actions of sST2 on its own, independent of its role as an IL-33 decoy receptor, need to be better established.

In conclusion, IL-33 serves as an important local link between tissue injury or metabolic disturbances and a physiological response of limiting or repairing tissue damage. In CVD, IL-33 exerts beneficial actions that are attenuated by its sST2 decoy receptor, which in many cases is induced by IL-33 and can serve as a biomarker of tissue stress/damage. IL-33 supplementation is atheroprotective and may be beneficial in treating MI and ischemic stroke. IL-33 may also prevent obesity and type 2 diabetes by regulating lipid metabolism. The mechanisms behind these beneficial actions are not fully defined, but are now known to involve Treg, ILC2 cells, and type 2 immune responses. On the other hand, IL-33 appears to drive endothelial inflammation and angiogenesis, which is relevant to metabolic syndrome, type 2 diabetes, and GSA. Moreover, as in several pro-inflammatory and auto-immune diseases, exuberant IL-33 signaling may cause tissue damage due to recruitment/activation of mast cells or eosinophils. Thus, a cellular or targeted approach is needed to exploit the beneficial therapeutic potential of IL-33 in CVD.

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Figure Legends

Figure 1: Pro-IL-33 Processing: Pro-IL-33 possesses three major domains including nuclear domain, activation domain, and interleukin-1 like cytokine domain. Following expression, pro-IL-33 may be processed into three major forms: **1)** ***Inactive forms,*** following cleavage by caspases 3 and 7 at interleukin-1 like cytokine domain if the cell undergoes apoptosis, ***2) Regulator of transcription,*** following localization to the nucleus due to the presence of two bipartite nuclear localization sequences in the nuclear domain, ubiquitination of IL-33 as well as its association with chromatin via protein-protein interaction is implicated in its activation/repression of transcription, and **3)** ***Active forms***, also known as cytokine or alarmin, following cleavage by extracellular proteases including cathepsin G and elastase at the activation domain after being released extracellularly in response to cellular necrosis or stress. ***CBM****; Chromatin Binding Motif,* ***Ub****; ubiquitination.*

Figure 2: IL-33 Effects Post-Activation and Release: Active IL-33 binds to sST2 and ST2L. Upon binding to the decoy receptor sST2, the effects of IL-33 on the cardiovascular system are neutralized or diminished, promoting use of sST2 as a prognostic biomarker. Binding to ST2L receptor which together with the co-receptor IL-1R accessory protein (IL-1RAcP) recruits MYD88, IRAK1, IRAK4, and TRAF6, followed by activation of multiple signaling pathways, including ERK1/2, JNK, p38 MAPK, and NF-κB and subsequent activation and regulation of transcription. Cytokines secretion, immunomodulation, cell proliferation, activation, and survival contribute to observed effects of IL-33 on the cardiovascular system. IL-33 effects, although mostly cardioprotective, vary depending on the disease state and cell type. ***IR****; Insulin Resistance,* ***WAT****; White Adipose Tissue, I/R; Ischemia/Reperfusion, T2D; Type II diabetes, CAD; Coronary Artery Diseases;* ***HF****; Heart Failure,* ***AS****; Aortic Stenosis,* ***ROS****; Reactive Oxygen Species,* ***IBD****; Inflammatory Bowel Disease,* ***COPD***; Chronic Obstructive Pulmonary Disease.

Figure 3: Conflicting actions of IL-33 in atherosclerosis. IL-33 has a number of actions on endothelial and immune cells that promote inflammation and atherosclerosis. In contrast, evidence indicates that IL-33 can act on T cells, macrophages, and B cells to attenuate plaque development and progression. A better understanding of the temporal and spatial/cellular factors involved in regulating the actions of IL-33 is needed to reconcile its opposing actions in atherosclerosis.

Figure 4: Cell-type specific pro- and anti-inflammatory actions of IL-33. IL-33 also increases generation of sST2 by certain cells, which serves as a decoy receptor. Note that generalized responses are highlighted, and in some cases an opposite response may be elicited such as mast cell-induced bronchodilation in small airways. See text for additional details.