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The IL-33/ST2 pathway: therapeutic target and novel biomarker

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Abstract

For many years, the interleukin-1 receptor family member ST2 was an orphan receptor that was studied in the context of inflammatory and autoimmune disease. However, in 2005, a new cytokine — interleukin-33 (IL-33) — was identified as a functional ligand for ST2. IL-33/ST2 signalling is involved in T-cell mediated immune responses, but more recently, an unanticipated role in cardiovascular disease has been demonstrated. IL-33/ST2 not only represents a promising cardiovascular biomarker but also a novel mechanism of intramyocardial fibroblast–cardiomyocyte communication that may prove to be a therapeutic target for the prevention of heart failure.

The interleukin-1 (IL-1) receptor family has several members, including the classical interleukin-1 receptor (IL-1R) and the interleukin-18 receptor (IL-18R)[1]. In 1989, one member of the family, ST2, was identified as an orphan receptor[2]. Investigation into the function of ST2 revealed its participation in inflammatory processes, particularly regarding mast cells, type 2 CD4+ T-helper cells and the production of Th2-associated cytokines[3]. In fact, ST2 was characterized as a specific cellular marker that differentiated Th2 from Th1 T-cells[4]. Clinical and experimental observations led to the association of ST2 with disease entities such as asthma, pulmonary fibrosis, rheumatoid arthritis, collagen vascular diseases and septic shock[5–9].

In 2005, the discovery of interleukin-33 (IL-33) as an ST2 ligand provided new insights into ST2 signalling[10]. IL-33 is clearly a potential mediator of diverse inflammatory diseases[11]. However, despite its heritage in the investigation of classical inflammatory diseases such as asthma and urticaria, IL-33 has now also been shown to participate in cardiovascular pathophysiology. As will be discussed in this Review, myocardial production of IL-33 can protect cardiac function in response to pressure overload[12]. Furthermore, the IL-33/ST2 system may play a part in the progression of atherosclerotic vascular disease[13].

Competing interests statement

The authors declare competing financial interests: see web version for details.

Beyond its role as a therapeutic target, the soluble form of ST2 has also emerged as a biomarker for disease. For example, serum levels of ST2 are elevated in patients with acute exacerbations of bronchial asthma[5]. Furthermore, in emergency-room patients presenting with shortness of breath, serum levels of ST2 can discriminate between heart failure and non-cardiovascular aetiologies[14]. Thus, ST2 represents a promising biomarker for cardiac injury.

This Review will discuss the discovery of the IL-33/ST2 system as a mediator of inflammation, but will focus on its emergence as a novel cardioprotective paracrine system and the therapeutic potential of targeting this pathway in the prevention of cardiac fibrosis and heart failure.

Basic biology of ST2

Structure

ST2 (also known as IL1RL1, DER4, T1 and FIT-1) is a member of the Toll-like/IL-1-receptor superfamily. Members of this superfamily are defined by a common intracellular domain, the Toll/Interleukin-1 receptor (TIR) domain. This domain of ~160 amino acids is composed of a central five-stranded β-sheet surrounded by five α-helices located on the cytosolic end of the protein[15]. The Toll-like/IL-1-receptor superfamily can be divided into three subfamilies based on their extracellular domains: the IL-1 receptor-like subfamily, the Toll receptor subfamily and a family comprised of their adaptor proteins. The IL-1 receptor-like subfamily is characterized by three linked immunoglobulin motifs on the extracellular domain of the protein. Members of this family include the type I and II IL-1Rs (IL-1R1 and IL-1R2), the IL-18R, their accessory proteins IL-1RAcP and IL-18RAcP, and ST2, among others. The Toll receptor subfamily is characterized by extracellular leucine-rich repeat motifs and is represented by the Toll-like receptors TLR-1–12 (reviewed in REFS 16,17); these receptors serve as gateways to proinflammatory signalling pathways. A family of five adaptor molecules comprised of myeloid differentiation factor 88 (MyD88), MyD88-adaptor-like protein (MAL, also known as TIRAP), TIR-domain-containing adaptor protein inducing IFNβ (TRIF, also known as TICAM1), TRIF-related adaptor molecule (TRAM, also known as TICAM2) and sterile α- and armadillo-motif containing protein (SARM) has also been identified (reviewed in REF. 18) (BOX 1).

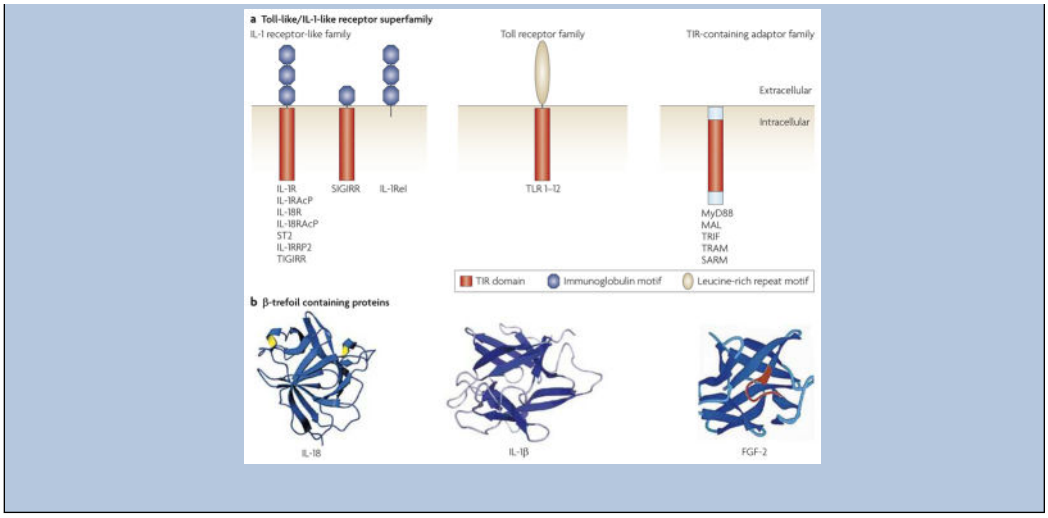
Box 1

The Toll-like/IL-1-receptor superfamily

The Toll-like/Interleukin-1 (IL-1) receptor superfamily members share a Toll/Interleukin-1 receptor (TIR) sequence. There might be significant differences between TIR domains of different TIR-containing molecules at both the primary and tertiary structural level, with sequence conservation being roughly between 20–30%. However, the basic common TIR structure is that of a central five-stranded β-sheet surrounded by five α-helices, as elucidated by X-ray crystallography by Xu and colleagues in 2000 (REF. 15). The TIR domain mediates the interaction between members of the superfamily, specifically receptor–receptor, adaptor–adaptor and receptor–adaptor protein dimerization.

The Toll-like/IL-1 receptor superfamily can be further subdivided into the IL-1 receptor-like family, the Toll receptor family and the TIR-containing adaptor protein family (see figure, part **a**). The IL-1 receptor-like subfamily shares the common feature of linked immunoglobulin motifs within the extracellular domain. Members of this family include the type I and II IL-1 receptor (IL-1R1 and IL-1R2), the IL-18 receptor (IL-18R), their accessory proteins IL-1RAcP and IL-18RAcP, ST2 and others (such as IL-1RRP2 and TIG1RR)[157]. As their name implies, they serve as transmembrane receptors for various interleukins, modulating leukocyte responses upon cytokine stimulation and activation[9]. The Toll receptor subfamily members are type-1 transmembrane proteins characterized by extracellular leucine-rich-repeat motifs linked to an intracellular TIR containing domain. This subfamily is represented by the Toll-like receptors (TLRs). The founding member of this family, Toll, was identified in the fruitfly, and noted to be necessary for dorso-ventral polarity during embryonic development. TLRs are conserved across most species and to date, 13 mammalian TLRs have been identified (10 in human, 12 in mice[158]). They are expressed on various cell types and are modulated in response to extracellular stresses and cytokine-mediated intercellular crosstalk (reviewed in REF. 159). The TIR-containing adaptor subfamily is a family of five molecules and includes myeloid differentiation factor 88 (MyD88), MAL (MyD88-adaptor-like, also known as TIRAP), TRIF (TIR-domain containing adaptor protein inducing IFNβ, also known as TICAM1), TRAM (TRIF-related adaptor molecule, also known as TICAM2) and SARM (sterile α- and armadillo-motif containing protein).

The IL-1 cytokine family. IL-1α (IL-1F1), IL-1β (IL-1F2), IL-1 receptor antagonist (IL-1Ra or IL-1F3), IL-18 (IL-1F4), IL-1F5–10 and IL-33 (IL-1 F11) make up the IL-1 family of cytokines. Members of this family display similar genomic intron–exon sequences and protein tertiary structure, specifically twelve interwoven β-sheets in a structure known as the β-trefoil[160] (reviewed in REFS 9,161). In humans, most of the members of this family (excluding IL-18) reside on chromosome 2 (2q13–q21), within an ~450 kb gene cluster[162–164]. In 2005, Schmitz and colleagues used a generic β-trefoil protein sequence derived by superposition of IL-1 and FGF protein structures to uncover a novel member of the IL-1 family which was subsequently named IL-33 (IL-1F11)[10]. Depicted here (see figure, part **b**) are the structures of IL-18 (REF. 166), IL-1β[160,164,165] and human basic fibroblast growth factor 2 (FGF-2)[167] displaying the β-trefoil fold that is common to members of the IL-1 family and putatively part of the structure of IL-33. IL-33 additionally contains an evolutionarily conserved homeodomain-like helix–turn–helix (HTH) DNA binding domain that is necessary and sufficient for nuclear targeting[38].



Isoforms

The gene for ST2 spans ~40 kb on human chromosome 2q12, and is part of the larger human IL-1 gene cluster of ~200 kb (Genbank accession number AC007248). ST2 is conserved across species, with homologues in the genomes of mouse (*Mus musculus* chromosome 1), rat (*Rattus norvegicus* chromosome 9) and fruitfly (homologues to the *Drosophila melanogaster* Toll protein). The initial discovery of ST2 in 1989 by two independent laboratories working with growth-stimulated mouse 3T3 fibroblasts[2,19,20] described a 2.7 kb transcript encoding a ~37 kD unglycosylated secreted protein corresponding to a 60–70 kD glycosylated product, which in hindsight represented the soluble form of ST2, sST2 (REFS 21,22). In 1993, a 5 kb transcript was identified with a putative transmembrane motif. The protein product of this transcript proved to be the transmembrane receptor ST2L[23]. Four isoforms of ST2 exist — sST2, ST2L, ST2V and ST2LV. The soluble (sST2, also known as IL1RL1-a) and the transmembrane (ST2L, also known as IL1RL1-b) forms arise from a dual promoter system to drive differential mRNA expression[24–26]. sST2 lacks the transmembrane and cytoplasmic domains contained within the structure of ST2L and includes a unique nine amino-acid C-terminal sequence[27]. The overall structure of ST2L is similar to the structure of the type I IL-1 receptors, which are comprised of an extracellular domain of three linked immunoglobulin-like motifs, a transmembrane segment and a TIR cytoplasmic domain. ST2V and ST2LV are two splice variants of ST2. Loss of the third immunoglobulin motif and alternative splicing in the C-terminal portion of ST2, resulting in a unique hydrophobic tail, produces ST2V[28], whereas alternative splicing, leading to deletion of the transmembrane domain of ST2L, produces ST2LV[29].

Expression and tissue localization

The earliest expression of ST2 in mice is in fetal liver tissue with restricted expression in haematopoietic organs in the adult. More detailed investigation revealed that ST2L is restricted to the surface of Th2 and mast cells, but that it is not expressed by Th1 or other immune cells[30–32]. ST2L may therefore serve as a marker for effector Th2 cells[4].

Whereas ST2L is constitutively expressed primarily in haematopoietic cells, sST2 expression is largely inducible and initially seemed to be restricted to integument (including fibroblasts), retinal, mammary and osteogenic tissue[25,33]. Mouse dermal tissue can be stimulated to express sST2 after exposure to ultraviolet radiation or the proinflammatory cytokines tumour necrosis factor (TNF), IL-1α and IL-1β. In contrast to murine tissues, constitutive sST2 expression might be more ubiquitous in humans[34].

Experiments with human tissue revealed that the splice variant ST2V is expressed predominantly in gastrointestinal organs including stomach, large and small intestine, and spleen. Its expression is notably absent from liver and cardiac tissues, and confocal microscopy in cells transfected with ST2V suggests restricted localization at the plasma membrane[35].

ST2LV was recently discovered in a search for a chick (*Gallus gallus)* ST2 homologue. This isoform seems to be expressed during the latter half of embryogenesis in cerebral, ocular, cardiac and pulmonary tissue, and its expression declines relative to the robust expression in ocular tissue in the adult[29]. The cellular localization, as well as the expression and tissue localization, of ST2LV in other species is yet to be explored.

ST2 ligand identification: IL-33

A major roadblock in understanding the function of ST2 was the lack of a functional endogenous ligand. However, in 2005, a β-trefoil fold protein sequence derived by superposition of IL-1 and fibroblast growth factor (FGF) protein structures was used to mine the public genomic database, which lead to the discovery of a novel member of the IL-1 family from a dog cDNA library. The mouse and human sequences of this candidate gene were deduced by expressed sequence tag alignment, which mapped to human chromosome 9p24.1 and mouse chromosome 19qC1. By sequence analysis, the protein was found to contain a pro-domain with a full-length mass calculated at 30 kD. *In vitro* translation of this protein and treatment with caspase-1 yielded a processed protein of 18 kD that activated the ST2 receptor[10]. This protein, which was named IL-33 (also known as IL-1F11), has now been classified as a member of the IL-1 interleukin family, whose members are characterized by an array of 12 β-strands (the IL-1/FGF β-trefoil fold) and the absence of a classical secretory N-terminus peptide sequence (BOX 1).

Interestingly, the IL-33 protein had previously been isolated in a search for the ST2 ligand, in which Kumar and colleagues identified two proteins produced by quiescent 3T3 cells that were precipitated by an ST2-Fc fusion protein. These molecules were ~32 kD and 18 kD in mass, which probably represented the uncleaved and mature IL-33 proteins, respectively[36]. In addition, in 2003, as part of a search for transcripts unique to high endothelial venules, a gene was identified with a sequence that localized to human chromosome 9p24.1. Antibodies raised against this peptide sequence precipitated a 30 kD protein[37]. The sequence of this protein was later confirmed to be identical to that identified by computational methods in 2005 by Schmitz and colleagues[38].

IL-33 contains a putative DNA-binding domain[37] and is localized in the nucleus, most notably within heterochromatin subdomains and mitotic chromosomes. The N-terminus of

IL-33 contains an evolutionarily conserved homeodomain-like helix–turn–helix (HTH) DNA binding domain, which is necessary and sufficient for nuclear targeting and is involved in the repression of transcription that has been ascribed to IL-33 (REF. 38). Precursor IL-33 may require caspase cleavage to yield a mature protein capable of nuclear targeting[39]. The existence of a cytokine as an extracellular ligand as well as an intranuclear effector is not unprecedented; a similar function has previously been noted for IL-1α and HMBG1 (REF. 40). Thus, IL-33 might have intracellular functions that are independent of binding to the ST2 receptor.

IL-33/ST2 signalling

The mode by which IL-33 exerts its effect has not been fully established but it probably acts similarly to other members of the IL-1 family, specifically IL-1β and IL-18. It has been proposed that upon synthesis, precursor IL-33 enters specialized secretory lysosomes. Caspase-1-dependent cleavage of pro-IL-33, lysosomal navigation and fusion with the cell plasma membrane may result in the release of IL-33 into the interstitium as an active cytokine[10,11]. IL-33 could then interact with its receptor on a target cell membrane to affect downstream signalling and/or be transported to the target cell nucleus, where it could act as a DNA binding factor (FIG. 1). However, although *in vitro* evidence of caspase-1 cleavage has been published[10,39], *in vivo* data has been less convincing[38]. A caspase-1 cleavage sequence within the primary IL-33 protein structure is not conserved across all species[38] and definitive localization of pro-IL-33 within lysosomal structures has not yet been reported. The heterochromatin-binding of IL-33 resembles the biology of IL-1α more closely than other members of the IL-1 family and, as has recently been suggested for pro-IL-1α, it is possible that caspase-1 acts as a secretory targeting factor for pro-IL-33 (REF. 41).

IL-33 might also act in an autocrine fashion as well as a secreted paracrine or endocrine effector, but active secretion of IL-33 from cells has also not yet been documented.

In general, upon activation of a Toll-like receptor/IL-1-receptor superfamily member, the transmembrane receptor's TIR domain dimerizes with the TIR domain of cytosolic adaptor molecules. The adaptor proteins MyD88 and the associated protein IL-1R-associated kinase (IRAK) activate downstream mitogen-activated protein kinase (MAPK)-kinases through TNF receptor-associated factor 6 (TRAF6) signalling, which in turn activates activator protein 1 (AP-1) through c-Jun N-terminal kinases (JNKs). TRAF6 also activates the inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) complex, leading to downstream liberation of active NF-κB from the complex (reviewed in REF. 42).

Features of the Toll-like receptor/IL-1-receptor system that are specific to IL-33 signalling have recently been clarified. IL-33 appears to bind a receptor complex composed of ST2L and IL-1RAcP. The affinity of IL-33 for ST2L is enhanced in the presence of IL-1RAcP, and mast cells from IL-1RAcP-deficient mice failed to release IL-6 upon IL-33 exposure[43]. In agreement with this finding, *IL-1RAcP*-null mice exposed to IL-33 failed to mount the typical cellular hyperplasia and inflammatory-cell infiltrate that is seen in wild type counterparts[44]. Additionally, the inability of D6/76 E4 cells to respond to IL-33, can be rescued by IL-1RAcP transfection, which leads to downstream NF-κB activation[45].

Events downstream of IL-33 stimulation may include phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, JNKs and activation of NF-κB[10]. Interestingly, although TRAF6 appears to be required for IL-33-mediated NF-κB activation and downstream induction of Th2 cytokines, IL-33-mediated ERK activation might be TRAF6 independent[46].

However, the relationship between ST2L and NF-κB activation has been a matter of some debate. In multiple models, activation of ST2L results in AP-1 activation independent of NF-κB[47], and activation of NF-κB in HEK293 cells can be inhibited by prior ST2L transfection. Thus, ST2L may exert an anti-NF-κB effect by sequestering MyD88 and MAL[48]. In basophils, cytokine release upon exposure to IL-33 requires ST2L and MyD88 (REF. 49). IL-33 activates NF-κB but attenuates the activation of NF-κB by angiotensin II or phenylephrine in cardiomyocytes, and to a lesser degree in cardiac fibroblasts[12]. These data indicate that IL-33 may function as a modulator of NF-κB and canonical Toll-like receptor/IL-1-receptor signalling (FIG. 1).

sST2 as a decoy receptor

Whereas ST2L mediates the effect of IL-33 on Th2-dependent inflammatory processes, sST2 has been implicated in the attenuation of Th2 inflammatory responses. Moreover, a role for sST2 as a decoy receptor, similar to that of IL1-R2 in IL-1 signalling, is emerging.

Administration of an antibody raised against a fusion protein of human Fc and the ST2 extracellular domain, reduced pulmonary eosinophilia in mice exposed to aerosolized ovalbumin compared with controls. Administration of the fusion protein itself produced similar results[30]. In another set of experiments, exposure of murine splenocytes to sST2 inhibited the production of Th2 cytokines such as IL-4 and IL-5, but not the Th1 cytokine, INFγ, when exposed to ovalbumin *in vitro*. In a gene transfer model, intravenous administration of sST2 cDNA to mice before exposure to aerosolized ovalbumin reduced the concentration of these cytokines, as well as the cell count of eosinophils, in bronchoalveolar lavage fluid compared with mice exposed to non-coding DNA or vehicle alone[50]. These data suggest that the ST2 gene may function not only as a mediator of IL-33 function in its ST2L transmembrane form, but also as an inhibitor of IL-33 and Th2 function, potentially via its soluble sST2 form acting as a decoy receptor (reviewed in REF. 3). To directly investigate this possibility, a murine thymoma cell line was stably transfected to express ST2L. Exposure to sST2 inhibited the binding of IL-33 to ST2L on the cell surface, and pre-treatment with sST2 suppressed the induction of NF-κB normally observed following IL-33 stimulation[51].

This inhibitory effect of sST2 on IL-33 signalling through ST2L is also apparent in the cardiovascular system. Administration of recombinant IL-33 to cultured rat neonatal cardiomyocytes blocked the pro-hypertrophic effects of angiotensin II or phenylephrine. However, administration of sST2 reversed this anti-hypertrophic effect of IL-33 (REF. 12). Furthermore, growth stimulation of cultured rat neonatal cardiomyocytes with phorbol 12-myristate 13-acetate resulted in production of total IL-33 and sST2 protein. However, a fall in free IL-33 protein was noted after pre-incubation with sST2-Fc fusion protein[12]. These

data suggest that the soluble form of ST2 may sequester IL-33 and, thereby, modulate IL-33/ST2L signalling. Further study is required to ascertain if these *in vitro* findings correspond with physiologically meaningful effects of sST2 *in vivo*.

Role of IL-33/ST2 in disease

Inflammatory conditions

IL-33 was originally described as a modulator of inflammation, tipping the balance towards CD4+ T helper-cell type 2 mediated immune responses. IL-33 may serve as a chemotactic factor for Th2 cells[52] and induce the production of the Th2-associated interleukins IL-4, IL-5 and IL-13 (REF. 10). Mice lacking the gene for IL-4, the prototypical Th2 cytokine, still express ST2L on the surface of mature Th2 cells[53], albeit the ability to mount a Th2-cytokine response to an antigen is reduced compared with wild-type counterparts[54]. Mice with targeted deletion of ST2 develop normally and exhibit normal Th2-cell maturation, but display altered Th2-mediated responses in an antigen-specific fashion. They are able to mount an IL-4 and IgE response to infection with the helminthic parasite *Nippostrongylus brasiliensis* that is equivalent to their wild-type counterparts[55] but do not form pulmonary granulomas in response to intravenous injection of *Schistosoma mansoni* eggs[56]. Taken together, these data suggest that ST2L and IL-4 production could serve as markers for distinct subpopulations of Th2 cells[57] or as parallel complimentary systems, each sufficient but not necessary for the initiation of Th2 immune responses to antigen (FIG. 2).

The observation that ST2L is a cell surface marker as well as an effector molecule in the regulation of Th2 cell function is consistent with subsequent studies demonstrating a role for IL-33/ST2 in diseases associated with a Th2 response such as asthma, rheumatoid arthritis, collagen vascular diseases and pleural malignancy.

Asthma

It has long been held that T-cells have a central role in the pulmonary response to allergen[57]. Specifically, evidence points to a primary contribution from Th2 cells[58]. Mice lacking both IL-4 and IL-5 exhibit airway hyper-responsiveness following exposure to aerosolized ovalbumin, implicating an IL-4/IL-5 independent process[59]. This process may involve the IL-33/ST2 system as most[50,55,60,61], though not all[55], studies examining ST2 suggest that it is required for antigen-induced airway inflammation.

Specifically, Hoshino and colleagues generated an *ST2*-null mouse by replacing the first ~1300 base pairs of the ST2 gene on mouse chromosome 1 (corresponding with the first 96 amino acids of the mature protein) with a neomycin resistance gene cassette via homologous recombination[55]. At 20 weeks of age, the mice appeared healthy and fertile. Both mast cells and splenic CD4+ cells were skewed towards the Th2 lineage by *in vitro* exposure to IL-4 and anti-INFγ antibody. The resulting Th2-developing cells produced equivalent amounts of IL-4 as cells from their wild-type counterparts. Furthermore, pre-immunized *ST2*-null and wild-type animals exposed to aerosolized ovalbumin displayed a quantitatively equivalent IgE and eosinophil response. Pulmonary histology from *ST2*-null mice displayed wide-spread inflammation. By contrast, using similar models of pulmonary inflammation, several studies have suggested a critical role of sST2 in Th2 cell function. Intravenous gene transfer

of murine sST2 (REF. 38) or administration of an immunoglobulin against IL-33 (REF. 37) resulted in marked attenuation of airway inflammation in response to aerosolized ovalbumin challenge. The reason for the discrepancies between studies utilizing antibody-based IL-33 inhibition versus genetic deletion of ST2 is unclear. Genetic deletion of ST2 should result in the loss of all ST2 isoforms unlike selective loss of sST2 function with an antibody-based approach. It is possible that genetic deletion of ST2 resulted in compensatory induction of other Th2 activating pathways. Furthermore, the use of an antibody against IL-33 may exert non-specific effects that might not have been initially appreciated. However, the totality of data suggests that the IL-33/ST2 system is involved in Th2-mediated immune responses in the lung.

It seems that IL-33 might also participate in mast-cell activation during the response to allergen. Exposure of human or murine mast cells to IL-33 results in the secretion of various interleukins and chemokines[62,63]. Exposure of mice to exogenous IL-33 results in airway hyperresponsiveness and airway goblet-cell hyperplasia in a lymphocyte-independent process[49]. Direct exposure to IL-33 results in epithelial hypertrophy and mucus accumulation in bronchial structures[10]. In keeping with the proposed decoy function ascribed to sST2, pre-exposure to sST2 results in reduced production of Th2 cytokines in a mouse model of allergen-induced pulmonary inflammation[50]. As a clinical correlate, serum levels of sST2 are elevated in patients who suffer from an acute exacerbation of bronchial asthma compared with healthy controls[5]. Additionally, serum sST2 levels are increased in both serum and bronchoalveolar lavage samples from patients with acute eosinophilic pneumonia[64].

Fibroproliferative diseases

The IL-33/ST2 system might also participate in the fibrotic response to tissue injury[65]. ST2 expression gradually increases concurrently with other Th2-associated cytokines in lung tissue as well as in cultured alveolar epithelia after exposure to bleomycin, a pulmonary toxin[66]. In agreement with this finding, patients with an acute exacerbation of idiopathic pulmonary fibrosis, but not those with stable disease, have elevated serum levels of sST2 (REF. 67).

Mice exposed to the hepatotoxin carbon tetrachloride exhibited an accelerated post-injury fibrotic response when treated with an sST2-Fc fusion protein. The Th2 cytokines IL-4, IL-5, IL-10 and IL-13 were elevated in isolates of the intrahepatic lymphocytes. In this case, the effect seemed to be mediated by the ability of the fusion protein to block TLR-4 mediated signalling[68]. Involvement of sST2 in TLR-4 signalling had been suggested previously, in the model of LPS-induced sepsis as mentioned below[69,70].

The fibrotic response to injury is a feature of most tissues. The involvement of the IL-33/ST2 system in other organs and modes of injury, such as lacerative cutaneous lesions and radiation fibrosis, is yet to be investigated.

Rheumatoid arthritis and autoimmune diseases

Rheumatoid arthritis is characterized by Th1-dominant cellular inflammation[71,72]. Administration of an Fc-sSt2 fusion protein resulted in a reduction in both the severity of

collagen-induced arthritis in DBA/1 mice and of serum levels of INFγ and TNFα[6]. How the fusion protein is functioning with respect to IL-33/ST2L in this experiment is unclear; however, Th2 cytokines were not upregulated.

IL-33 might also participate in antigen-induced cutaneous and articular pain processing. Mice administered an intra-articular irritant in the form of bovine serum albumin exhibited dose-dependent hypernociception. This response is IL-33-mediated and inhibited by administration of sST2 (REF. 73). Curiously, in this model of antigen-induced integumental and articular injury, IL33 appears to be a proximal mediator in a cascade of cytokine production that involves TNFα, IL-1β, INFγ, endothelins and prostaglandins. This suggests that IL-33 may well have a more promiscuous role in T-cell-mediated inflammatory processes beyond that of Th2-dominated responses.

In patients, IL-33 is abundantly expressed in rheumatic synovium as measured by *in situ* hybridization[38]. Elevated sST2 levels have been found in the sera of patients with systemic lupus erythematosis, progressive systemic sclerosis and in patients with Wegener's granulomatosis or Behcet's disease[7], suggesting a role for the IL-33/ST2 system in a wider array of autoimmune diseases.

Sepsis and trauma

As has been recently reviewed, patients with severe trauma or systemic inflammatory syndromes demonstrate elevated serum levels of IL-4 and IL-10, and decreased levels of Th-1 cytokines; these biomarkers generally portend a worse prognosis[74]. Similarly, serum levels of sST2 were elevated in patients admitted to an intensive care unit with a diagnosis of sepsis or after sustaining significant trauma[8].

It is possible that these elevations of serum sST2 are pathogenic, rather than simply a biomarker. Exogenous administration of sST2 to mice exposed to LPS, as a model of sepsis, resulted in reduced serum levels of IL-6, IL-12 and TNFα and increased survival[69]. Specifically, as pertains to the pulmonary effects of LPS challenge, pretreatment with sST2 reduced the expression of proinflammatory cytokines from murine alveolar macrophages *in vitro*[75]. Mice lacking the ST2 gene lack the ability to develop tolerance to repeated exposure to endotoxin[48]. Furthermore, the known protective effects of macrophage-activating lipopeptide 2 (MALP2) might operate through ST2L signalling[76].

Malignancy

A possible association between ST2 and tumorigenesis was suggested by the induction of ST2 expression in growth-stimulated cells[19,20,77]. ST2 can also be stimulated by transgenic expression of HA-Ras in a mouse model of mammary adenocarcinoma, an apparent recapitulation of the mammary epithelial cell ontogeny that is induced by the HA-Ras oncoprotein[78]. In addition, levels of sST2 and the Th2 cytokines IL-4 and IL-10 were higher in pleural fluid obtained from patients with carcinomatous pleurisy compared with those with pulmonary effusions with a tuberculous or cardiac aetiology[79]. Although these data are suggestive, formal investigation of activation of the IL-33/ST2 system in human malignancies is lacking.

Cardiovascular disease

sST2 as a biomarker

In 2002, using microarrays, our laboratory noted that the transcript for ST2 was markedly upregulated in mechanically-stimulated cardiomyocytes. Both the transmembrane and soluble forms of ST2 were induced, with the soluble form displaying more robust expression[80]. *In vivo*, the cardiac ST2 transcript and serum ST2 protein were increased following the induction of myocardial infarction[80]. These experiments suggested that ST2 had the potential to be a biological marker for mechanical overload in the heart. Initial evidence for this was found in patients who had suffered an acute myocardial infarction, as serum ST2 levels were elevated one day post-event and declined thereafter; these ST2 levels correlated with serum creatine kinase, a standard marker of myocardial injury, and inversely correlated with left ventricular function[80]. As a result of this, sST2 levels were analysed in over 800 patients presenting to hospital with an acute ST-elevation myocardial infarction (STEMI) at several time points over the first day after presentation. sST2 levels at the time of presentation correlated with the incidence of in-hospital and 30-day mortality, even though these levels were much lower than subsequently measured values during hospitalization. Additionally, multivariate analysis demonstrated that the initial serum ST2 level was independently associated with the incidence of 30-day mortality after controlling for established clinical risk indicators in STEMI such as age, heart rate, blood pressure, infarct territory, Killip class and time from symptom onset to treatment[81].

Mechanical overload of the myocardium is a feature of many types of heart failure, not only the loss of myocardium due to infarction. Based on this, a subsequent study analysed serum levels of ST2 in patients with non-ischaemic congestive heart failure (CHF) — defined as a reduced left ventricular ejection fraction, and severe symptoms with minimal exertion or at rest (New York Heart Association class III–IV). Serum levels of sST2 at the time of study entry correlated with serum noradrenaline levels, a marker of systemic neurohormonal activation, as well as serum brain natriuretic peptide (BNP) levels. BNP is a useful biomarker in patients suffering from a myocardial infarction or heart failure with both prognostic and diagnostic utility. BNP is used routinely in clinical practice[82]. A rise in serum ST2 levels was found to independently predict the risk for reaching a subsequent endpoint of mortality or cardiac transplantation in a multivariate model that included measurements of the natriuretic peptides BNP and ANP[83].

sST2 may also be an important biomarker in the hospital emergency room. The PRIDE study (or Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department), a prospective, blinded study of patients presenting to an emergency department with dyspnea, was initially conducted to validate the use of N-terminal pro-brain natriuretic peptide (NT-proBNP) testing in differentiating acute CHF from other causes of shortness of breath[84]. However, in an analysis of blood samples from nearly 600 patients, serum concentrations of sST2 were significantly higher in those presenting with acute systolic heart failure compared with patients presenting with other causes of dyspnea. Patients with a serum sST2 level above the median of 0.23 ng per ml had an 11-fold increased risk for death at one year compared with patients with lower serum sST2

concentrations. When stratified by decile, patients in the lowest decile had a 1 year mortality of less than 5%, whereas those in the highest decile had a 1 year mortality of nearly 45%[14].

Further studies have suggested that sST2 provides prognostic information that is independent of BNP. Analysis of serum samples from 1,200 patients enrolled in the Clopidogrel as Adjunctive Reperfusion Therapy — Thrombolysis in Myocardial Infarction (CLARITY-TIMI) 28 study, which studied patients presenting with an acute STEMI, showed that serum sST2 levels correlated with impaired epicardial coronary flow and the subsequent risk of cardiovascular death or CHF[85]. In a multivariate analysis, sST2 was independently predictive of cardiovascular death or CHF within 30 days of presentation. Patients presenting with STEMI with a baseline NT-pro-BNP in the highest quartile displayed an odds ratio of 2.4 for the risk of cardiovascular death or heart failure at 30 days, whereas those with a baseline sST2 level in the highest quartile had an odds ratio of 3.6. Moreover, the sST2 quartile level was complementary in predicting the risk of downstream cardiovascular death or heart failure when added to the traditional model of TIMI-risk score and NT-pro-BNP quartile. The TIMI-risk score is a well-validated, clinical score which allows the clinician to predict the risk of mortality, recurrent infarction or ischaemia in patients presenting with an acute coronary syndrome[86,87]. In patients presenting with STEMI, but with a low TIMI-risk score, those with the highest quartile sST2 and NT-pro-BNP levels demonstrated a 6.6-fold increased risk of cardiovascular death or heart failure at 30 days; this is equivalent to the risk afforded by a high TIMI-risk score. Patients with a high TIMI-risk score and highest quartile baseline sST2 and NT-pro-BNP levels had an ~25-fold increased risk of cardiovascular death or heart failure[88].

Although the above data suggest that sST2 has a role in ascertaining the prognosis of patients presenting with an acute coronary syndrome, whether sST2 contributes to cardiovascular risk prediction in patients without active coronary disease remains unstudied. However, multi-marker prognostication is an area of active investigation. Recently, a study using data from the Uppsala Longitudinal Study of Adult Men (ULSAM) registry, evaluated a cohort of more than 1,000 Swedish men over a follow-up period of 10 years[89]. When adding Troponin I, NT-pro-BNP, cystatin C and C-reactive protein to the standard cadre of prognostic indicators (including age, systolic blood pressure, total cholesterol, presence of diabetes mellitus, smoking status and body mass index), the Cox regression model C statistic increased significantly. Furthermore, the fold increase in the risk of cardiovascular death rose in concert with the number of biomarkers elevated on baseline analysis, from a 3-fold increase with elevation in a single biomarker to a 16-fold increase with an elevation in all four biomarkers. How sST2 may contribute to the cardiovascular prognosis of patients in the general population remains to be investigated.

In summary, sST2 levels have repeatedly proven to be of potential value as a biomarker. Measurement of serum sST2 in patients presenting to a hospital emergency ward with acute dyspnea or myocardial infarction might provide useful prognostic information for stratifying care. In this sense, serum levels of sST2 might be used in conjunction with existing biomarkers such as BNP, which is routinely measured when patients present with these conditions.

IL-33/ST2 and heart disease

Most current pharmacotherapeutic strategies for heart failure treatment target systemic neurohormonal responses, including excessive compensatory renin–angiotensin and aldosterone signalling. Many of the available medications were initially approved for the treatment of hypertension but were later found to have beneficial effects on cardiovascular outcome following myocardial infarction or in heart failure. Indeed, it is surprising that relatively few heart failure therapies have arisen from an understanding of myocardial biology.

Pathological examination of human myocardial tissue after infarction documents a progressive inflammatory reaction. As classically described, a robust polymorphonuclear leukocyte response is observed within the first day after infarction, which is eventually replaced by lymphocytes, followed by macrophages over the ensuing week[90,91]. In animal models, manipulation of this inflammatory-cell infiltration results in attenuation of the severity of experimental infarction (reviewed in REF. 92). Early approaches to attenuate experimental infarction used corticosteroids in a non-selective anti-inflammatory maneuver[93–96], but this failed to benefit patients[97]. More recent attempts to manipulate the inflammatory response to myocardial injury in humans have also been met with limited clinical success (reviewed in REF. 98). It is intriguing to consider the role that fibroblast-derived sST2 might have in leukocyte recruitment and leukocyte–fibroblast–myocyte crosstalk. There is evidence for CD4+ T-cell involvement in post-infarct inflammation[99] as well as precedent evidence for TLR signalling in post-infarct ventricular remodelling[100]. The specific role that the IL-33/ST2 system might have in these inflammatory signalling cascades is yet to be determined.

Fibrosis and scar formation in the heart is a component of the maladaptive ventricular responses to injury[101,102]. It is thought that the interaction of myocytes and fibroblasts in the context of biomechanical overload leads to fibroblast proliferation and deposition of extracellular matrix, resulting in a reduction in ventricular compliance[103–106]. This stiffening of the myocardium increases pulmonary vascular and systemic venous pressures resulting in shortness of breath and peripheral oedema. Furthermore, a common cause of death in heart failure is a lethal arrhythmia, often the result of fibrosis-induced changes in ventricular myocardium or in the conduction system. Some of the most successful pharmacologic therapies for heart failure reduce ventricular fibrosis. For example, hyperactivity of the renin–angiotensin–aldosterone neurohormonal axis results in end-organ fibrosis, and inhibition of this axis reduces cardiac fibrosis and improves outcome after myocardial infarction and in congestive heart failure[107–110].

Although fibrosis and scar formation implies an interaction between cardiomyocytes and fibroblasts within the injured myocardium, the cellular and paracrine signalling mechanisms remain poorly defined. Evidence that sST2 is a biomarker for cardiac biomechanical strain suggests that the IL-33/ST2 system could be a potential pathophysiological mediator of this fibrosis.

Early experimental animal data suggested that the ST2 gene is expressed in osteogenic tissue *in vitro*[111] and that sST2 resides in the extracellular matrix of integumental tissue[33]. These

findings raise the possibility that ST2 is involved in the growth or homeostasis of matrix components. In terms of cardiovascular disease, possible involvement of sST2 in ventricular matrix remodelling has been suggested. Both cardiac fibroblasts and cardiomyocytes express IL-33 and sST2, and expression levels are increased by two well-characterized stimuli of cardiac fibrosis: biomechanical strain and angiotensin II[80]. In an *in vivo* model of ventricular pressure overload, partial aortic constriction enhances IL-33 protein expression in fibroblasts of the left ventricle. When these experiments were carried out in mice with germline deletion of ST2, increased ventricular fibrosis as well as cardiomyocyte hypertrophy ensued. Furthermore, enhanced chamber hypertrophy and reduced ejection fraction (a marker of ventricular function) were noted in *ST2*-null mice compared with wild-type mice. Treatment of wild-type mice with exogenous IL-33 reduced cardiac hypertrophy, reduced gene expression of BNP and increased survival after aortic constriction compared with untreated controls (FIG. 3). These data are indicative of a previously unrecognized cardioprotective role for IL-33/ST2 signalling in fibroblast–cardiomyocyte crosstalk during biomechanical overload[12].

IL-33/ST2 and atherosclerosis

CD4+ cells have been implicated in the pathogenesis of atherosclerotic vascular disease (reviewed in REFS 112–114), and the presence of activated T-cells in human atherosclerotic lesions was documented over two decades ago[115,116]. It has been suggested that the clonal expansion of a subset of T-cells from an initially polyclonal T-cell population[117] might underlie the transition from a stable to an unstable vascular plaque[118]. In both humans and mouse models of accelerated atherogenesis, CD4+ T-cells appear to be central to lesion progression[119], and immunodeficiency reduces the atherosclerotic burden in most[120–122] but not all[123] murine models of atherosclerotic vascular disease. Macrophages, dendritic cells and smooth muscle cells in atherosclerotic plaques express MHC class II receptors and may serve as antigen-presenting cells to effector T-cells in vascular lesions[124]. Numerous antigens have been suggested, with oxidized low density lipoprotein (ox-LDL) attracting much attention[125–127]. These CD4-dependent, antigen-associated mechanisms of atherogenesis seem to act in parallel with antigen and CD4-independent mechanisms[128–130].

Of the CD4+ subtypes, Th1 cells seem to dominate the atherosclerotic plaque, which may be decisive for lesion progression[131,132]. This is in agreement with the over-representation of the prototypical Th1 cytokines INFγ and IL-2 in atherosclerotic plaques[133,134], the observation that enhanced INFγ exposure potentiates atherogenesis[135–137], and that genetic inhibition of Th1 differentiation abrogates it[138]. Furthermore, levels of IL-18, a Th1-associated cytokine, correlate with plaque instability[139]. As immune responses are thought to result from a balance between Th1 and Th2 effects, with predilection of one response occurring at the expense of the other, it could be hypothesized that promoting a Th2 response might be protective. Some studies of the effects of IL-4 and IL-5 in atherogenesis appear to validate this[140,141]. However, experiments with Apolipoprotein E[142] and LDL-receptor[143] deficient mice that are also rendered IL-4 deficient have indicated a more complicated role for this cytokine, as in both instances lesion formation was reduced when compared with control mice. This might be due to the effects of IL-4 on extracellular matrix composition, specifically protease and elastase production and putative destabilization of

vascular lesions[144,145]. As IL-33/ST2 could represent a parallel or augmentative path to Th2-cell differentiation and activation, the role of this system in atherogenesis was investigated. In mice with germline deletion of ApoE, leading to accelerated atherosclerosis[146–148], IL-33 reduced aortic atherosclerotic plaque development and induced serum levels of antibodies to ox-LDL (FIG. 4). Conversely, administration of the decoy receptor, sST2, resulted in a significantly higher aortic plaque burden compared with control mice, theoretically due to inhibition of IL-33 signalling. Interestingly, treatment with a neutralizing antibody against IL-5 reversed the protective effect of IL-33 and blunted the induction of ox-LDL antibodies without affecting the overall lipid profile of the animals. Plaque content of macrophages, T-cells, smooth muscle cell and collagen was similar between the treated and untreated groups[13]. Previously published data suggest that IL-5 might induce athero-protective ox-LDL antibodies[141]. Collectively, these data suggest that IL-33 may be exerting its anti-atherosclerotic effect by inducing IL-5-stimulated ox-LDL antibody production.

Thus, in both heart failure and atherosclerosis, IL-33 signalling appears beneficial, and sST2 might act as a decoy receptor to reduce IL-33 signalling through the ST2L receptor. This idea is consistent with sST2 representing a biomarker for worse prognosis in patients with cardiovascular disease.

Manipulation of the IL-33/ST2 system

ST2 has long been regarded as a novel therapeutic target in the development of pharmacotherapy for inflammatory diseases such as asthma and rheumatoid arthritis. Although approaches to regulate the IL-33/ST2 system have not yet entered the clinical arena, examples of the potential benefits of ST2 administration in the treatment of arthritis and sepsis have been reported. However, given the involvement of the IL-33/ST2 system in the wide variety of processes discussed above, strategies aimed to inhibit the IL-33/ST2 pathway to treat common inflammatory diseases must be followed with caution, as they could have unintended consequences. The finding of a cardioprotective role for IL-33/ST2 signalling exemplifies this. It is unlikely that inhibition of the IL-33/ST2 system would have a major impact on the unstressed cardiovascular system, but in patients with increased cardiac biomechanical overload, such as in those suffering from myocardial infarction or heart failure, or perhaps even as subtle as that in patients with mild hypertension, the risk for cardiovascular morbidity could potentially be increased (FIG. 5). Recently, therapeutics such as rofecoxib (Vioxx) and rosiglitazone (Avandia) have come under scrutiny for their ability to increase cardiac morbidity; an effect that was theorized but never fully explored until post-marketing studies were conducted[149–152]. If history is to be a lesson, the potential untoward cardiac effects of IL-33-based anti-inflammatory therapeutics should be thoroughly investigated as part of drug development.

As indicated, increasing IL-33/ST2 signalling might invoke a cardioprotective effect. Based on current understanding of the system, this could be accomplished by a number of ways. These include, direct administration of IL-33 or strategies to enhance cardiac fibroblast IL-33 release, administration of an ST2L agonist or sST2 antagonist, or administration of a molecule designed to sequester adaptor proteins such as MyD88 or MAL[100,153–156]. Brint

and colleagues have demonstrated that ST2L negatively regulates TLR-4 signalling by sequestering the adaptor protein MyD88 upon endotoxin challenge[48]. Abrogation of myocardial ischaemic damage, post-infarct ventricular maladaptation and pressure-overload-induced cardiac hypertrophy have been noted in most, though not all, animal models deficient in TLR-4[100,153–155]. This process also appears to involve sequestering of MyD88 (REF. 156). Thus, it is possible that MyD88 represents a common point in the cardioprotective signalling of ST2L and TLR-4. These data raise the hypothesis that selective sequestration of MyD88 with a small molecule might exert a cardioprotective effect. This approach has yet to be experimentally validated. Elucidation of the precise cytosolic and intranuclear effects of ST2L signalling might uncover unique cardiovascular targets for drug development. Again, caution must be taken when pursuing stimulation of IL-33/ST2 signalling, as a therapeutic strategy: it is possible that supraphysiologic IL-33/ST2 activation may yield undesirable results such as the exacerbation of inflammatory and autoimmune conditions such as asthma, rheumatoid arthritis and certain collagen vascular diseases. These limitations might require the development of site-specific delivery systems. However, the degree of inflammation that is induced by IL-33 therapy may depend upon the dose and the duration of therapy[12], thus it is possible that transient IL-33 therapy could improve cardiovascular outcomes with minimal instigation of undesirable inflammatory reactions. Our preliminary data indicate that a brief course of IL-33 can in fact prevent cardiomyocyte apoptosis and improve outcome after experimental myocardial infarction without pulmonary inflammation (unpublished observations).

Concluding remarks

IL-33/ST2 has emerged as an intercellular signalling system that participates in processes as varied as the antigen/allergen response, autoimmunity, organ fibrosis and cardiac injury. Exploration of the effects of this pathway is underway. An effort to clarify the role of IL-33/ST2 in the crosstalk between end-organ effector cells and their environment, specifically the surrounding matrix and inflammatory cells, may lend itself to the discovery of novel therapeutic targets for the treatment of diseases such as asthma, rheumatoid arthritis, atherosclerosis and heart failure.

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Glossary

Th2 cells	A subset of the T-cell pool hypothesized to drive an immune response that is characterized by production of interleukin-4, -5,-6 and -10 (among others) in response to extracellular pathogens
Cytokines	Small proteins released by cells of the immune system for the purpose of intercellular crosstalk. Interleukins, derived specifically from leukocytes, are a subset of these proteins

Th1 cells	A subset of the T-cell pool hypothesized to drive an immune response that is characterized by the production of interleukin-2 and interferon-γ (among others) in response to intracellular pathogens
Fibrosis	Process by which normal tissue is replaced with scar tissue, mostly consisting of extracellular proteins produced by fibroblasts
Atherosclerotic vascular disease	A disease that is pathologically defined by the formation of lipid-rich lesions within the artery wall and which results in luminal narrowing and loss of vascular elasticity. It is characterized by a significant T-cell and macrophage inflammatory response to oxidized low-density lipoprotein
The Toll-like/IL-1-receptor superfamily	A superfamily of related cytokine receptors. They are similar in that they contain a common intracellular domain, the Toll/Interleukin-1 receptor (TIR) domain
Expressed Sequence Tag	(EST). Short, unique sequence of DNA that can be used to identify the larger gene transcript of which it is a part of. It is created by sequencing mRNA that represents a portion of the expressed sequence of a gene. ESTs have been used extensively to identify new genes within the genome
High endothelial venules	Post-capillary tissue involved in leukocyte extravasation from lymphoid tissue
Autocrine, paracrine and endocrine	Describe the type of interaction between a cell, its secreted compound and the affected target cell. Autocrine effects are those in which the effector cell is of the same type as the target cell. Paracrine effects are those in which the secreted protein exerts its effect on cells within the local vicinity of the effector cell. Endocrine effects are those which occur at a distance (the effector cell secretes its proteins into the blood stream)
Angiotensin II	A protein that circulates in the bloodstream and exerts a myriad of physiological effects. Effects of angiotensin II include arterial vasoconstriction, renal blood filtration and sodium absorption, cardiac myocyte hypertrophy and ventricular fibrosis, platelet aggregation, adrenal aldosterone secretion and increased thirst sensation in the brain
Cardiomyocytes	Specialized, striated muscle cells of the heart. These cells are contiguous with one another, allowing the rapid transmission of chemical and electrical signals between them. An extracellular matrix of proteins, secreted by resident fibroblasts, serves to both mechanically bind them and transduce information about the extracellular environment

Decoy receptors	Proteins that can bind the ligand of functional cellular receptors, effectively reducing the concentration of ligand that is available to the active receptor
Antigen	Substance which can induce an immune response. Generally it is a fragment of a protein or polysaccharide that is derived from a structural component of a pathogen, such as a component of the bacterial cell wall
Sepsis	A pattern of body-wide responses to overwhelming infection. It is characterized by alteration in core body temperature, vasodilation with attendant drop in blood pressure and rise in heart rate, and leukocyte response. These responses are thought to be mediated by the release of inflammatory cytokines
Endotoxin	A lipopolysaccaride within the gram-negative bacterial cell wall that upon infection may instigate sepsis, septic shock and its associated complications
Myocardial infarction	Term used to describe the death of heart tissue due to a loss of blood supply
STEMI	Term used to describe the most severe type of heart attack. Defined by elevation of the ‘ST-segment’ on the standard electrocardiogram, this entity is typified by complete occlusion of a coronary artery and subsequent death of downstream cardiac tissue
The Killip classification	A risk stratification system developed by Killip and colleagues in 1967 after a two-year observation of an unselected group of 250 patients presenting to hospital with myocardial infarction. It employs physical exam findings consistent with heart failure or cardiogenic shock to categorize patients into one of four classes. The class assigned correlates with mortality at 30 days after the infarction
Brain natriuretic peptide (BNP)	Protein that is released from ventricular myocardial cells under stress or strain. It is cleaved from its precursor pro-BNP along with N-terminal-pro-BNP. Its biological effects include systemic vasoconstriction and renal sodium loss
Odds ratio	Ratio of the odds of an outcome among exposed individuals compared with the odds of the outcome among unexposed individuals
C statistic	A quantitative measure of the ability of a test to discriminate between two cohorts, typically those with and without a disease. The C statistic varies between 0.5 and 1.0, with a higher value denoting better discriminatory power. For binary outcomes, C is identical to the area under the receiver operating characteristic

	(ROC) curve, or a plot of sensitivity (SN) versus one minus specificity (1-SP) of the test in question
Antigen-presenting cell	Cell which processes and presents antigen on its cell surface to effector immune cells, for example, T-cells. The antigen is displayed within a specialized protein receptor, known as the major histocompatibility complex, along with other co-receptors that are necessary for effector immune cell activation
Apolipoprotein E	(ApoE). A protein component of some lipoproteins. Lipoproteins are conglomerates of proteins and lipids that serve to shuttle fat and cholesterol through the bloodstream. ApoE allows its lipoproteins to be taken up by the liver as part of the normal process of lipid clearance from the blood. *ApoE*-null mice have high blood levels of cholesterol and display spontaneous atherosclerosis

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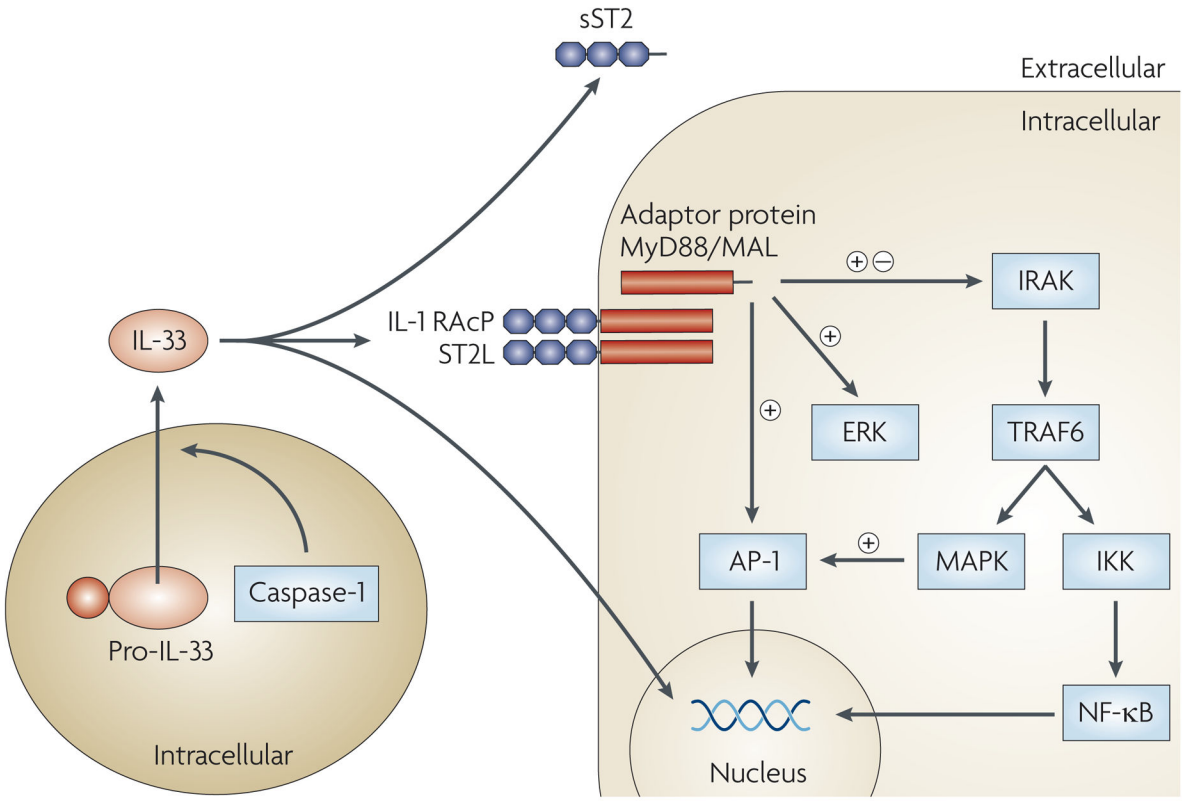

Figure 1. A model for IL-33/ST2 signalling

The myeloid differentiation factor 88 (MyD88)-dependent pathway of Toll-like receptor signalling involves Toll/Interleukin-1 receptor (TIR) dimerization between the receptor and the MyD88-adaptor-like protein (MAL). Recruitment of MyD88 and downstream activation of TNF receptor-associated factor 6 (TRAF6) via Interleukin-1 receptor-associated kinase (IRAK) proteins results in TRAF6-mediated activation of the inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) complex and liberation of NF-κB from the complex. Free NF-κB is then able to bind DNA and act as a gene transcription regulator (reviewed in REF. 158). IL-33 signalling appears to share many of these properties and events downstream of IL-33 stimulation may include phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, JNKs as well as activation of NF-κB[10]. It has been proposed that caspase-1-dependent cleavage of pro-IL-33, subsequent lysosomal navigation and fusion with the cell plasma membrane results in release of IL-33 into the interstitium as an active cytokine[10,11]. IL-33 binds to its receptor complex composed of ST2L (the transmembrane isoform of ST2) and the IL-1 receptor accessory protein IL-1RAcP[44]. Subsequent sequestering of the adaptor proteins MyD88 and MAL results in modulation of IRAK mediated TRAF6 activation and subsequent mitogen-activated protein kinase (MAPK) and IKK/NF-κB activation[42,48]. The nature of this modulation of NF-κB activity by IL-33 is complex. In unstimulated cardiac myocytes and fibroblasts *in vitro*, exposure to IL-33 activates NF-κB. However, NF-κB activation via hypertrophic stimuli is attenuated by exposure to IL-33 (REF. 12). Interestingly, although TRAF6 appears to be required for IL-33-mediated NF-κB activation and downstream induction of Th2 cytokines, IL-33-mediated ERK activation might be TRAF6 independent[46]. Furthermore, IL-33 might activate the transcription factor AP-1

independently of its effects on NF-κB[47]. Exactly where the pivotal points of IL-33 signal regulation reside along this pathway from IL-33 receptor activation to NF-κB activity modulation is still unclear. Even before IL-33 binds to its receptor, its action could be altered by the decoy receptor soluble ST2 (sST2). sST2 is a variant of the full-length ST2 gene lacking the transmembrane and cytoplasmic domains contained within the structure of the transmembrane isoform of the gene[27]. sST2 in the extracellular environment might bind free IL-33, thereby effectively decreasing the concentration of IL-33 that is available for ST2L binding and reducing the biological effect of IL-33 (REF. 12).

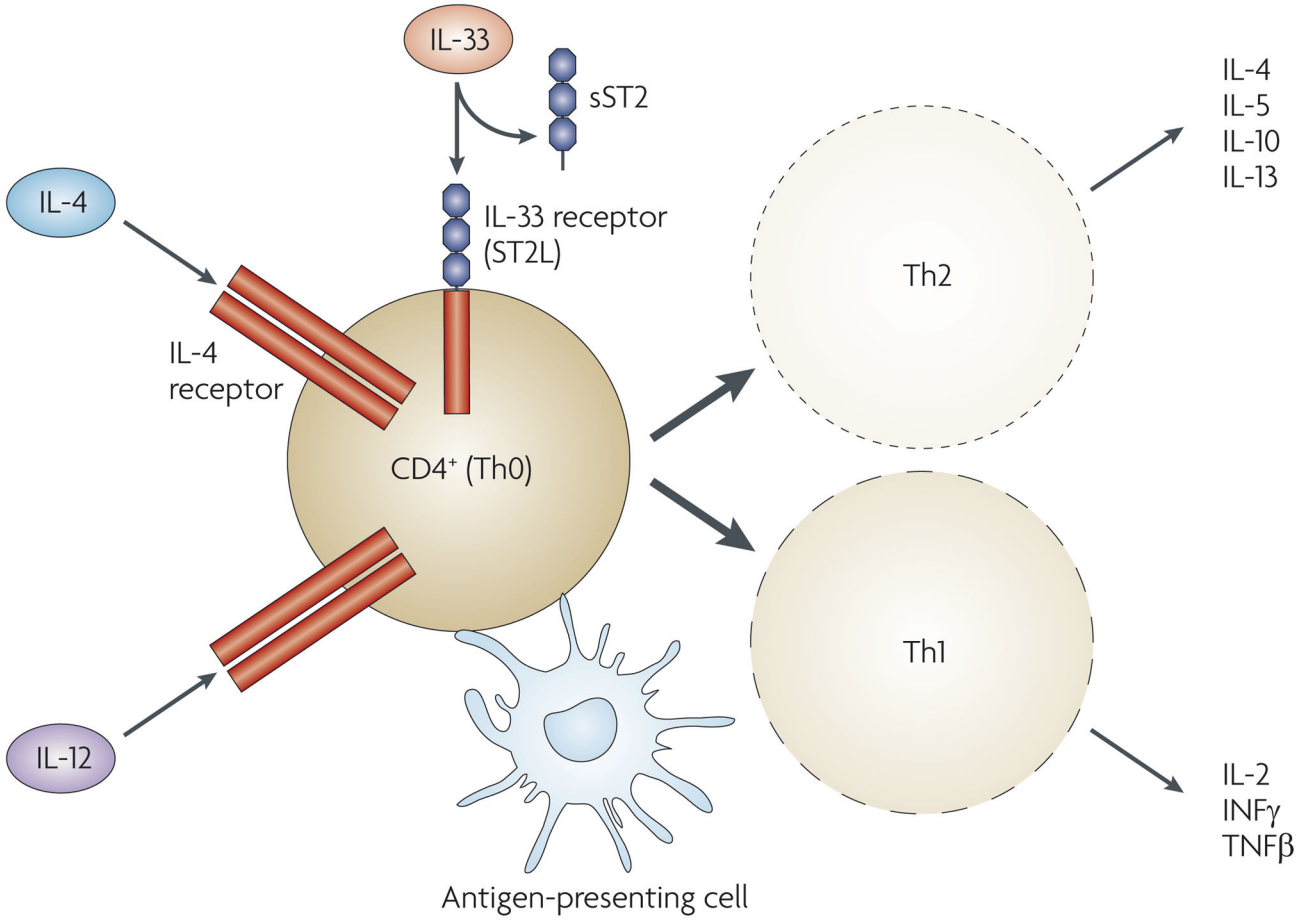

Figure 2. IL-33 in the type 2 immune response

Upon exposure to antigen and the proper interleukin milieu, CD4+ Th0 cells commit to either the Th1 or the Th2 lineage. As classically described, 'type 1' immune responses are typified by proliferation and activation of Th1 cells via exposure to certain interleukins including IL-12 and IL-18. Activated Th1 cells release characteristic cytokines such as IL-2 and interferon γ (INFγ) (among others) in response to pathogens. The 'type 2' response is typified by proliferation and activation of Th2 cells and release of their characteristic cytokines IL-4, IL-5 and IL-13 (among others) in response to extracellular, for example, parasitic, pathogens. How the appropriate immune response is chosen upon a particular threat has been the focus of much research and debate. Cells that first encounter invading pathogens (antigen-presenting cells) present foreign antigens to Th0 cells. Antigen presentation in combination with secretion of specific cytokines promotes the commitment of Th0 cells towards one lineage over another, and the initiation of a counter immune response to the infection (reviewed in REF. 165). In the presence of an antigen, direct stimulation of ST2L or exposure to IL-4 appears to be sufficient for the activation of Th2 cells and the release of Th2-associated cytokines[10]. Exposure to IL-33 results in chemotaxis of Th2 cells[52] and the release of Th2-associated cytokines[10]. IL-33 can coax the release of Th2-associated cytokines from mast cells[63] as well as basophils[166]. IL-33 might also promote superoxide production and degranulation of eosinophils[167]. Interestingly, recent

evidence hints at a more promiscuous role for IL-33 as it has been found to induce INFγ release from antigen-exposed Th2 cells, natural killer (NK) cells and invariant NK T cells[166]. To regulate this IL-33 mediated type 2 response, soluble ST2 (sST2) in the extracellular environment might act as a decoy, binding free IL-33 available to ST2L. Recently, it has been shown that a soluble form of the IL-1 receptor accessory protein (IL-1RAcP) might serve as a co-decoy, enhancing the ability of sST2 to inhibit IL-33 signalling[168].

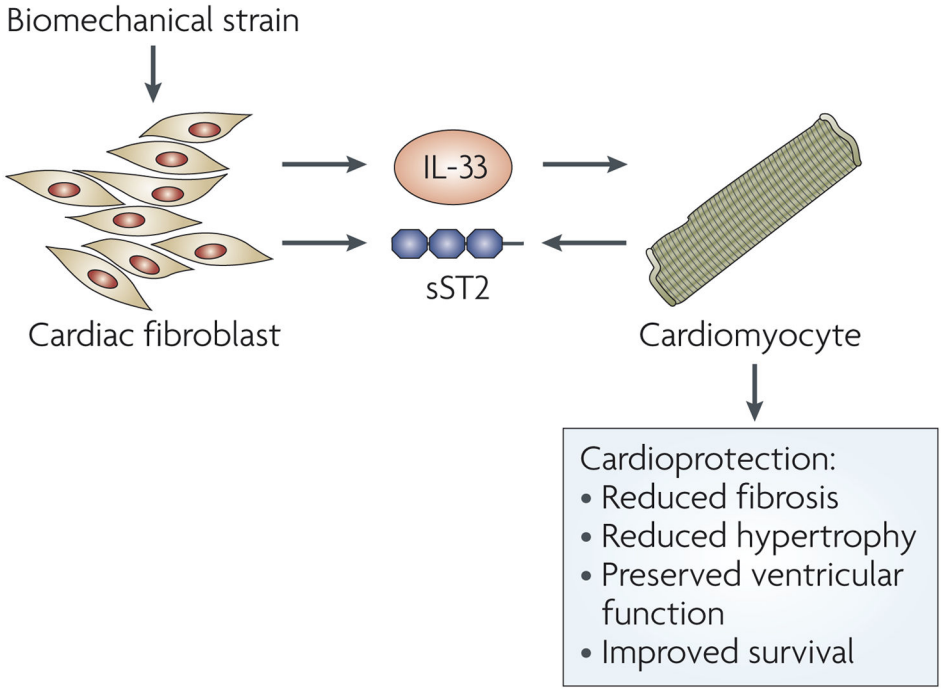

Figure 3. IL-33/ST2 signalling is a novel cardioprotective fibroblast–cardiomyocyte paracrine system

Any condition that alters the geometry or loading conditions of the left ventricle of the heart might alter the mechanical strain exerted on each individual cardiomyocyte. Myocytes are able to sense these changes in biomechanical strain and respond to them[101,105,169]. Disease conditions that increase the stresses and strains on the ventricle, such as myocardial infarction, hypertension and valvular disease, result in hypertrophy of the myocytes and enhanced deposition of extracellular proteins (ventricular fibrosis), which, at least in the early adaptive phase of response, tends to normalize ventricular wall stress[170]. These responses ultimately prove maladaptive, leading towards clinical heart failure[171–173]. The IL-33/ST2 system is emerging as a novel fibroblast–cardiomyocyte communication system that might abrogate these maladaptive processes. In response to biomechanical strain both cardiac myocytes and cardiac fibroblasts produce mature IL-33, although fibroblasts appear to be the dominant source. When *in vitro* cardiomyocytes subjected to hypertrophic signals were exposed to IL-33, the hypertrophic response was reduced. Addition of soluble ST2 (sST2) reversed this inhibition of hypertrophy, suggesting that it might serve as a decoy receptor. sST2 can be produced by both cardiac fibroblasts and cardiomyocytes. The ventricles of mice can be subjected to overt pressure overload by surgically constricting their aortae. In such a model, exposure to IL-33 reduced the normal ventricular hypertrophy and fibrosis that is seen as a consequence of the increased ventricular strain. Furthermore, the inevitable decrement in ventricular function and premature mortality noted in the mice subjected to ventricular pressure overload was reduced with IL-33 treatment[12].

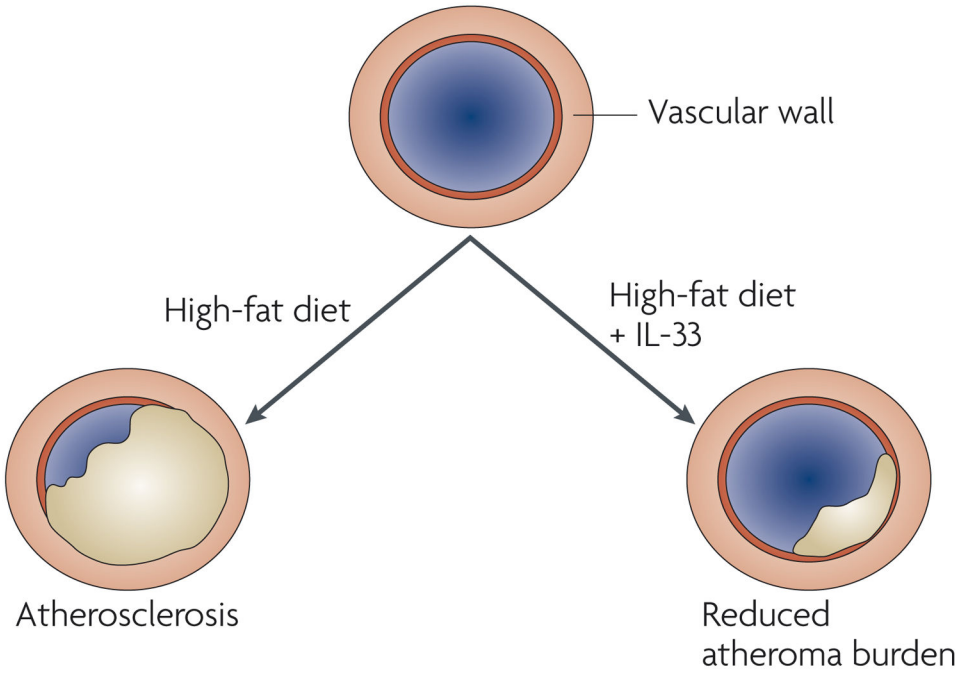

Figure 4. IL-33/ST2 reduces atheroma formation

Atherosclerosis has been described as a chronic inflammatory disease of the vascular wall, characterized by a type I T-cell response to oxidized low density lipoprotein (LDL), as well as other antigens. It is this active inflammation that is thought to underlie the instability of some atherosclerotic lesions, leading to plaque rupture, subsequent clot formation, vessel lumen occlusion and the resultant downstream tissue infarction[113,114]. One strategy to inhibit the vascular inflammation of atherosclerosis might be to shift the balance towards Th2 immune-cell activation. The IL-33/ST2 system could be one pathway towards inducing this shift in balance. Mice lacking the gene for apolipoprotein E fed a high-fat diet have high serum cholesterol levels and develop atherosclerosis. When these mice are treated with IL-33, they display reduced aortic atherosclerotic plaque burden and lower levels of serum antibodies to oxidized LDL compared with control mice. Furthermore, when pre-treated with sST2 before IL-33 exposure, these mice display increased atherosclerosis compared with those not treated with soluble ST2; this is consistent with the anti-IL-33 effect of the soluble isoform of ST2.

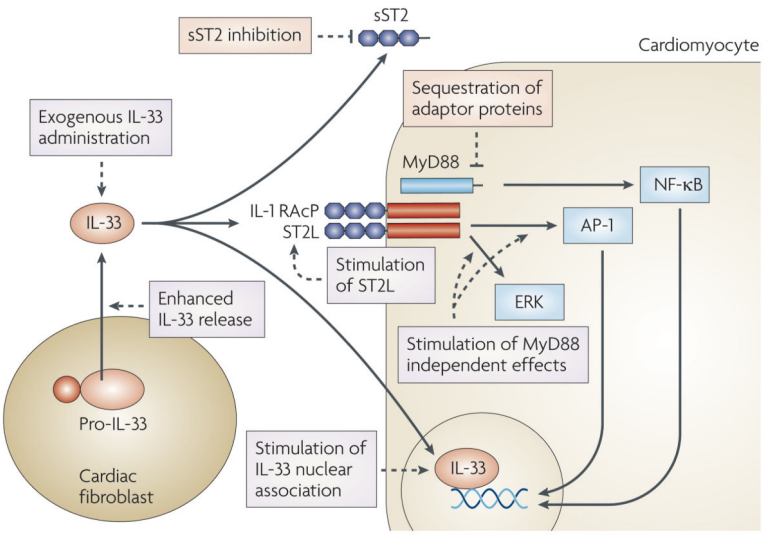

Figure 5. Strategies and consequences of IL-33/ST2 modulation

Stimulation of the IL-33/ST2 system might activate a cardioprotective programme in the context of ventricular biomechanical stress[12,80] and a number of approaches could be taken to modulate IL-33/ST2 signalling to capitalize on this cardioprotective activity. The IL-33 system may be activated by exogenous administration of IL-33 or by promoting IL-33 release from resident cardiac fibroblasts. Alternatively, availability of IL-33 to its receptor complex could be increased by inhibiting the IL-33 decoy receptor soluble ST2 (sST2). The system could also be activated by pharmacotherapeutics designed to directly stimulate the IL-33 receptor. Intracellular targeting may also be possible: Sequestration of the myeloid differentiation factor 88 (MyD88) by exogenous compounds might mimic the cardioprotective effects of IL-33 administration. If further clarity could be obtained regarding the nature and means by which IL-33 modulates nuclear factor-κB (NF-κB) activity, or with regard to the contribution of NF-κB-independent effects (such as direct stimulation of adaptor protein 1 (AP-1) or extracellular signal-regulated kinase (ERK)) to overall cardioprotection, these may also prove to be points along the IL-33 signalling cascade that are amenable to manipulation. Precisely how the nuclear and non-nuclear effects of IL-33 result in downstream cardioprotection is unclear. Further study into these processes could help identify novel methods of cardioprotection. Due to the involvement of the IL-33/ST2 system in a variety of processes, activation of this pathway may have unintended consequences. The involvement of IL-33 in Th2-mediated inflammation suggests that such a strategy might result in exacerbation of arthritic, asthmatic, rheumatologic and gastrointestinal inflammatory conditions. Conversely, inhibition of the IL-33/ST2 system to modulate these inflammatory conditions could result in increased cardiovascular injury in the face of ventricular strain. Care must be taken if the IL-33/ST2 system is to be manipulated for potential clinical gain.