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Implications for Interleukin-33 in solid organ transplantation

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Abstract

Interleukin(IL)-33 is a member of the IL-1 cytokine family that has been attributed T helper (Th) type 2 immunity-promoting capacity. However, new studies indicate that IL-33 is a multifunctional protein that acts as transcriptional/signaling repressor, functions as an alarmin alerting the immune system to necrosis, as well as serves as a cytokine that targets cells expressing ST2, the IL-33 receptor. Interestingly, IL-33 is also emerging as a pleiotropic cytokine. Depending on the innate or adaptive immune cells targeted by IL-33, it can not only promote type 2, but also IFN- dominated type 1 immunity. In addition, IL-33 expands regulatory T cells. In this review, we assimilate the current knowledge of IL-33 immunobiology and discuss how IL-33 may mediate such diverse roles in the immune response to pathogens and development of immune-mediated pathologies. The function of IL-33 in shaping alloimmune responses to transplanted organs is poorly explored, but a particularly beneficial role of IL-33 in experimental heart transplant models is summarized. Finally, given the implication of IL-33 in pathologies of the lung and intestine, we discuss how IL-33 may contribute to the comparatively poor outcomes following transplantation of these two organs.

Keywords

Interleukin-33; ST2; alarmin; transplantation

1. Introduction

1.1 Standing out in the IL-1 cytokine superfamily

Presently, there are 11 identified Interleukin-1 (IL-1) cytokine superfamily members (IL-1, -1, IL-1 receptor antagonist (IL-1Ra), IL-18, IL-33, and IL-1F5-IL-1F10) that most likely arose from duplication of a common ancestral gene[1]. As such, IL-1 family members share general structural similarities in their cytokine domain, particularly a 12-stranded -trefoil structure. Yet the IL-1 family has diverged into a group of fascinating molecules with

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pleiotropic, and often disparate, impacts on local and systemic responses to tissue injury and infection[1, 2]. IL-1 superfamily cytokines are expressed or induced in both stromal cells, such as endothelium, epithelium, and keratinocytes, in addition to innate immune cells. Upon their secretion, the agonistic IL-1 family members (IL-1 α , -1 β , -18, -33, IL-1F6, -1F8, and -1F9) signal through receptors closely related to Toll-like receptors (TLR; [3]). Thus, IL-1 family members can induce pro-inflammatory innate cell responses similar to those promoted by TLR ligands. Subsets of T cells also express IL-1 receptors and IL-1 cytokines directly modulate their proliferation, polarization, and immunoregulatory function[1]. In total, IL-1 cytokines are both potent pro-inflammatory mediators of innate immune immunity and important controllers of adaptive immune cell functions.

Given their profound and often feed-forward pro-inflammatory functions, IL-1 cytokines are regulated at several levels to protect against unrestrained inflammation. The importance of this control is exemplified in multiple incapacitating inherited inflammatory diseases that arise due to IL-1 β overproduction or lack of IL-1Ra[1]. Accordingly, most IL-1 cytokines are only expressed at low levels and require local stress or pro-inflammatory stimuli for large-scale transcriptional and translational induction[1]. IL-1 α , -1 β , -18, and -33 are initially generated as signal sequence-lacking pro-cytokines that require processing to bioactive or secreted forms by proteases, whose activity is also be precisely controlled[2]. In addition, the stimuli inducing cytokine production also triggers expression of negative regulators of the IL-1 family cytokines or IL-1R/TLR family members. These include IL-1Ra, that stifles IL-1 activity by blocking IL-1 α / β binding to the IL-1R1, as well as soluble, agonistic decoy receptors (i.e. IL-18-binding protein).

IL-33 was the 11th identified IL-1 family member (IL-1F11) discovered by computational sequence database searches for unidentified IL-1 family members[4]. IL-33 was subsequently identified as the ligand of the orphan receptor, ST2[4], a member of the IL-1R/TIR family possessing a high degree of sequence similarity with the IL-1R[5]. In this review, we describe how IL-33 has emerged, since these early studies, as a unique and multifunctional member of the IL-1 family with proposed cytokine, alarmin, and transcriptional regulatory functions. We also discuss the important immunoregulatory role that we propose IL-33 may play in transplantation. This discussion is based on accumulating clinical and experimental evidence for IL-33 regulation of other disease processes and initial examination of IL-33 in heart transplantation.

2. Current understanding of the immunobiology of IL-33

2.1 Expression and distribution

The human IL-33 gene is located on chromosome 9, while its mouse counterpart is localized on chromosome 19[4]. Thus, like IL-18, found on chromosome 11 in the human and 9 on the mouse, IL-33 has diverged from all other IL-1 family cytokine members, which in both the human and mouse, are located on chromosome 2[1]. Recent studies have characterized two distinct murine mRNA transcripts, *IL33a* and *IL33b*, that are generated from alternative promoters and have different 5' untranslated regions, yet they code for an identical IL-33 proteins following alternative splicing[6, 7]. Although there may be some tissue-specific and stimulus-dependent differences[6], *IL-33a* appears to be the dominate form[6, 7].

Expression of IL-33 RNA and protein has been confirmed in many tissue and cell types[8]. However in humans, the highest constitutive levels of IL-33 protein are observed in fibroblastic reticular, epithelial, and endothelial cells[9, 10]. IL-33 is particularly expressed in the high endothelial venules (HEV), where it was first identified[11]. Using an IL-33- β -galactosidase reporter mouse, it was demonstrated that a quiescent mouse constitutively expresses IL-33 in epithelial cells and α smooth muscle actin⁺ fibroblastic reticular cells,

but not endothelial cells[12]. Thus, some species-specific differences in IL-33 expression may exist. These data are consistent with related examinations of murine tissue that found IL-33 expression typically in the central nervous system, stomach, eye, lymphoid organs, skin, and lung[6, 13, 14]. Other studies have suggested expression in the kidney, pancreas, and heart[4]. Human tissue constitutive expression of IL-33 is widespread, with IL-33 found in the same locations as in mice, as well as being prominent in the epithelial and endothelial cells of most organs and tissues[9].

Pro-inflammatory stimuli, such as TLR ligands[6, 15], cytokines (IL-3 and IL-4[16]; tumor necrosis factor (TNF)- α and IL-1 β [15, 17]), virus and bacterial infections[7, 13] greatly augment IL-33 expression in the above tissues and cells. Relatedly, administration of the TLR4 ligand, bacterial lipopolysaccharide, also induces IL-33 expression in the liver[6, 12] and murine endothelial cells[12]. Tissue pathology is often associated with increases in IL-33. Rheumatoid arthritis (RA) patients display significant synovial IL-33[17]. Likewise, hypertrophic cardiomyopathy is associated with profound increases of IL-33 in cardiac fibroblasts[18]. Atherosclerosis is also associated with augmented IL-33 expression in vascular tissues[19] and allergens drive IL-33 expression in the conjunctiva of the eye[14].

Although IL-33 expression in quiescent cells is confined predominantly to stromal cells, pro-inflammatory stimuli has been reported to induce its expression in murine myeloid cells, particularly macrophages and conventional dendritic cells (DC)[6-8]. Immunoglobulin (Ig) binding to Fc ϵ RI receptors on murine mast cells[20] or DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) on DC, macrophages, and monocytes induce the expression of IL-33[21]. Careful examinations have found that ligation of TLR3 and 4, but not TLR2 or TLR9, can upregulate IL-33 mRNA expression [7]. However, IL-33 expression has not yet been described in human myeloid cells, suggesting that additional species-specific differences in IL-33 expression may exist.

Overall, IL-33 is abundantly expressed and rapidly induced in tissues that are continuously exposed to the external environment or line the vasculature. IL-33 expression therein, as well as possible upregulation of IL-33 in the innate myeloid cells that patrol these tissues, suggest a fundamental role for IL-33 in early immune responses to injury and infection. However, many of these studies, particularly those examining macrophages and DC, have only examined IL-33 message[7, 21] and the functional role of IL-33 protein in these cells and tissues is only emerging, as discussed below.

2.2 Proteolytic processing and release

Database mining for distant IL-1 and fibroblast growth factor proteins lead to the discovery of IL-33 in 2005[4]. Schmitz et al. originally described IL-33 in the human and mouse as 270 and 266 amino acid proteins, with respective calculated molecular weights of 30 and 29.9 kDa[4] (See Figure 1). Human and mouse IL-33 are 55% identical at the amino acid level[4] and, like most IL-1 family members, IL-33 is expressed as a pro-cytokine lacking a classic signaling peptide facilitating secretion in both species (Figure 1). However, unlike IL-1 β and IL-18, which both require protease processing to an active, cytokine-form, IL-33 is similar to IL-1 α , with its full-length, “pro-cytokine” form possessing functional activity[1]. Any mechanisms by which IL-33 may be processed or released regulated, however, are still emerging.

It was proposed initially that, like IL-1 β and IL-18, IL-33 required cleaved by caspase-1 to be secreted as an 18-kDa, “mature” cytokine form, IL-33₁₁₂₋₂₇₀[4]. However, subsequent studies revealed that, where full-length IL-33 is biologically active, caspase-1 does not process IL-33 and cleavage by caspase-3 or caspase-7 diminishes IL-33 activity[22-24]. Consequently, it appears that IL-33 is inactivated by caspases during apoptotic cell death,

but is functional when released from necrotic cells[24]. These observations have led to classification of IL-33 as an alarmin, or an endogenous molecule that alerts the immune system to tissue damage[22]. The alarmin function of IL-33 is particularly implicated in effective CD8⁺ T cell viral control (see below for more details).

Alternative mechanisms of IL-33 processing do appear to also exist. Neutrophil serine proteases cathepsin G and elastase were convincingly shown to cleave full-length human IL-33₁₋₂₇₀ and generate mature forms IL-33₉₅₋₂₇₀, IL-33₉₉₋₂₇₀, and IL-33₁₀₉₋₂₇₀ which display 10-fold increased biological activity compared to full-length IL-33[25]. In vitro-translated murine IL-33₁₋₂₆₆ was similarly cleaved by these neutrophil proteases, generating bands that co-migrated with IL-33₁₀₂₋₂₆₆ and IL-33₁₀₉₋₂₆₆ proteins. In wild type, but not IL-33-deficient mice, bands corresponding to IL-33₁₀₂₋₂₆₆ and full-length IL-33₁₋₂₆₆ were found increased in bronchoalveolar lavage (BAL) fluid following oleic acid-induced acute lung injury[25]. These data suggest when activated neutrophils are recruited to injured lung tissue, they may proteolytically process released IL-33 to more potent forms through the removal of the N-terminal domain.

2.3 Cellular location and non-cytokine functions

Before it was identified as an IL-1 family cytokine, IL-33 was first described by Girard et al. as a N-terminal homeodomain-like Helix-Turn-Helix (HTH) DNA-binding domain containing protein located in the nucleus of lymphoid HEV, and designated nuclear factor from HEV (NF-HEV)[11]. Following recognition of NF-HEV as an IL-1 cytokine by Schmitz et al., further work by Girard's group demonstrated that the N-terminal HTH domain of IL-33 enabled it to interact with heterochromatin and suggested it may act as a transcriptional repressor[26]. Specifically, by fusing green fluorescent protein to the N-terminal (IL-33₁₋₁₁₁) or C-terminal domains (IL-33₁₁₂₋₂₇₀) of human IL-33, Carriere et al. showed that the HTH domain (IL-33₁₋₆₅) within the N-terminal domain of IL-33 was necessary and sufficient for IL-33 targeting to the nucleus, heterochromatin, and mitotic chromosomes in vitro[26]. Their further studies identified a chromatin-binding motif (IL-33₄₀₋₅₈) sufficient for IL-33 to bind the acidic pocket formed by histones H2A and H2B in heterochromatin[27]. In additional in vitro examinations, these authors demonstrated that the N-terminal HTH domain of IL-33, but not the C-terminal cytokine portion, when fused to a Gal4-DNA-binding domain could repress transcription of GAL4-responsive reporter constructs [26, 27]. Although interesting, the capacity of endogenous IL-33 to modulate specific genes is yet to be established. Likewise, as IL-33 deficient mice are developed, it will be critical to establish the role transcription regulation by IL-33 may play in the development and function of tissues expressing this molecule.

In separate a study, the capacity of full-length IL-33 to potentially act as a direct negative regulator of NF- κ B activity has also been reported[28]. Specifically, Ali et al. revealed that following overexpression of IL-33 in human embryonic kidney cells (HEK293) or IL-1 β stimulation of mouse embryonic fibroblasts, IL-33 interacts with active NF- κ B, or p65 and p50 that is free of I κ B α , in the cytoplasm and nucleus. This was mediated through interaction of the N-terminal domain of murine IL-33 (IL-33₆₆₋₁₀₈) with the Rel homology domain/DNA binding domain of p65 and delayed or reduced NF- κ B-regulated gene expression. It will now be important to both substantiate these findings and then define the impact that such regulation of NF- κ B may have in cells with constitutive IL-33 expression. If warranted, it will also be interesting to examine whether IL-33 plays such a role in immune cells, particularly myeloid antigen presenting cells (APC), which increase IL-33 expression following exposure to pro-inflammatory stimuli and are heavily dependent on NF- κ B signaling to promote inflammatory immune responses[29]. In total, IL-33, like high mobility group box 1 and IL-1 α , is a multifunctional protein that performs important roles as a cytokine, an alarmin, and potentially a transcription regulating nuclear factor.

2.4 ST2 and IL-33 signaling

When IL-33 was identified in 2005 as the ligand for ST2 (also known as IL-1 receptor-like 1 (IL-1RL1)), this ended a 16-year period for ST2 as an orphan receptor[4]. ST2 was originally identified in a murine fibroblast cell line as transcript induced by serum with significant similarity to the IL-1R[5]. Subsequently, three isoforms of ST2 were identified: sST2, a soluble molecule; ST2 (also denoted as ST2L), a signaling transmembrane form; and ST2V, a ST2 variant that lacks one of three Ig domains in the extracellular region of ST2[5, 30-32]. Little data on the physiological function of ST2V are available[32], thus only ST2 and sST2 are discussed presently. The ST2 gene is part of the IL-1 gene cluster and ST2 and sST2 are the major products generated from it. Both forms arise through alternative mRNA splicing and differential mRNA expression from two distinct promoters[31, 33, 34]. ST2 is a member of the TLR/IL-1R superfamily, which is characterized by their critical roles in innate immune responses to pathogens and promotion of inflammation[3, 35]. Members of the TLR/IL-1R superfamily share a conserved cytoplasmic Toll-IL-1R (TIR) domain that is necessary for initiating intracellular signaling. ST2 is a classic type I membrane receptor, containing 3 extracellular IgG-like domains, a transmembrane domain, and the distinguishing TIR domain. IL-33 binds to ST2 and recruits the IL-1R accessory protein (IL-1RAcP) to form a heterodimeric receptor[35, 36]. sST2 functions instead as a soluble decoy receptor that binds IL-33 and inhibits its interaction with ST2[37, 38]. IL-33 signaling is dependent on the TIR domain of IL-1RAcP[36] and involves recruitment of myeloid differentiation primary-response protein 88, IL-1R-associated kinases (IRAK)1, IRAK4 and TNF receptor associated factor 6 and subsequent activation of NF- κ B and mitogen-activated protein kinases [4, 39]. Importantly, signaling through homodimerized ST2 without recruitment of IL-1RAcP can activate transcription factor activator protein-1, which is selectively activated in Th2 cells, but not NF- κ B[40]. It is noteworthy that most data concerning IL-33 signaling via ST2 have been generated using recombinant murine IL-33₁₀₉₋₂₂₆. Future studies comparing signaling patterns induced by IL-33₁₀₉₋₂₂₆ to that of full-length IL-33₁₋₂₆₆, or the potent forms generated by elastase and cathepsin G (IL-33₉₅₋₂₇₀, IL-33₉₉₋₂₇₀, and IL-33₁₀₉₋₂₇₀) should be insightful.

3. Role of IL-33 on ST2⁺ cells in health and disease

3.1 Involvement of IL-33 in protective immunity

IL-33 influences many hematopoietic and non-hematopoietic cells due to their expression of ST2. As expected from the diversity of these innate and adaptive immune cells, IL-33, ST2 and sST2 have significant importance in regulation of immune responses to parasitic, fungal, viral and bacterial infections (summarized in Table 1). Likewise, the expression of ST2 on non-immune cells including cardiac fibroblasts[18], cardiomyocytes[41], hepatocytes[42] and lung epithelial cells[43, 44] suggest that local IL-33 may have profound biological impacts on these cells, as well (Table 1).

In brief, IL-33 is primarily associated with the augmentation of Th type 2 immune responses, particularly in augmenting local levels of IL-5 and IL-13[45]. CD4⁺ T cells differentiate into a variety of effector Th subsets and fill unique niches in the immune response. The best understood subsets include IFN- γ ⁺ Th Type 1 (Th1), IL-4, -5, and -13⁺ Th2, and IL-17⁺ Th17 cells[46]. IFN- γ -secreting Th1 cells are crucial to control viral and intracellular bacterial infections, Th2 cells support B lymphocyte-based humoral responses and parasite eradication, whereas Th17 cells are important for removal of extracellular bacteria and fungi. Equally important, is the suppressive capacity of CD4⁺ regulatory T cells (Treg), identified by the expression of the transcription factor forkhead box P3 (Foxp3) or IL-10 (Tr1 cells). Treg are critical to suppression of immune self-reactivity and limit immune responses to prevent tissue damage. In both rodents and humans, the absence of

Foxp3⁺ Treg results in fatal autoimmunity, and these cells are critical for experimental transplant tolerance[47]. The capacity of IL-33 for promotion of type 2 immunity is, in part, mediated through its impact on ST2⁺ CD4⁺ Th2 cells, that secrete IL-5 and IL-13 when stimulated with IL-33[4]. In fact, IL-33 can promote Th2 cytokine production independent of T cell receptor (TCR) signaling[48], and also act as a Th2 chemoattractant[49].

Importantly, IL-33 also promotes Th type 2 immunity through a direct influence on multiple innate immune cells. In study of rodent cells, IL-33 targets ST2⁺ myeloid APC, including DC and macrophages; programming them to polarize naïve CD4⁺ T cells towards IL-5- and IL-13-producing effector Th2 cells [50, 51]. Compelling studies have demonstrated the capacity of IL-33 to expand and induce IL-5 and IL-13 production by newly-identified type 2 innate lymphoid cells (ILC2), which include, in the mouse, nuocytes, natural helper cells and innate helper type 2 cells[8, 52]. IL-33-expanded ILC2 in the small intestine are critical to coordinating immune responses necessary for helminth expulsion[53, 54]. A strong case has been made for the crucial importance of IL-9 production by ILC2 for IL-5- and IL-13 dependent immune responses, although in these studies IL-9 production by ILC2 was not mediated by IL-33[55]. IL-33 is increased in the mouse lung during fungal infection[56] and may support inflammatory, anti-fungal responses of Lin⁻CD25⁺CD44^{hi} ILC in this organ[57]. Eosinophils and basophils are also potent Th2-associated effectors that express ST2 and are directly activated by IL-33[58]. Likewise, mast cells constitutively express high levels of ST2 and respond to IL-33 by production of IL-5, -6, -8, and -13[59, 60]. In total, a wealth of experimental data support mechanisms where IL-33, acting on both innate adaptive and innate immune cells, drives potent type 2 immunity. The importance of IL-33 to type 2 immunity in host immune responses to a range of extracellular pathogens is supported by studies of *St2*-deficient mice, or by the use of IL-33 blocking reagents[45]. Likewise, both experimental and clinical data support a prominent role for IL-33 in Th2-associated allergic and autoimmune diseases [45, 61].

IL-33 may not be the classical Th2 cytokine originally proposed, as under further study, it has exhibited quite pleiotropic properties. Under certain conditions, IL-33 has promoted IFN- γ production and type 1 immunity. This is consistent with the functional activity of a similar IL-1 family cytokine, IL-18. IL-18 can also support both Th1- and Th2-type immunity, depending on additional signaling provided by the environment[62]. One local factor that dictates whether IL-18 promotes Th1 or Th2 responses is the presence of IL-12. With IL-12, IL-18 drives immune responses towards type 1 immunity and drives high levels of T cell IFN- γ production[62]. Yet, in the absence of IL-12, IL-18 can promote Th2 type responses[62]. To date, the study of IL-33 has revealed very similar observations. In addition to elevating production of IL-5 and IL-13, IL-33 can augment the production of IFN- γ by human T cells polarized toward a Th2 phenotype[63]. ST2 is also expressed on natural killer (NK) and invariant NK T (iNKT) cells which are Th1-supporting components of innate immune system. Interestingly, IL-33 augments the production of IFN- γ by both NK and iNKT cells[64], particularly in the presence of IL-12[63]. Following lymphocytic choriomeningitis virus infection in mice, 20% of activated, virus-specific CD8⁺ T cells express ST2, and IL-33 enhances their expansion and production of TNF- α , IL-2, IFN- γ [65]. Interestingly, this study provided evidence that non-hematopoietic cells in the T cell zone of the spleen are the source of IL-33 that is critical for protective CTL responses to replicating RNA and DNA viral infections[65]. Consistent with these observations, Yang et al. found that TCR stimulation of CD8⁺ cells in the presence of IL-12 resulted in T-bet-dependent expression of ST2[66]. In this initial examination[66], as well as a subsequent observation[67], IL-33, acting through IL-12-induced ST2, synergized with TCR activation and IL-12 stimulation to significantly augment CD8⁺ T cell IFN- γ production.

Based on these emerging data, we propose a model (Figure 2) where, during conditions such as helminth infections or tissue damage, IL-33 is released into an environment lacking IL-12 and favors type 2 immunity. Alternatively, during infections that activate APC to secrete IL-12, local IL-33 will support IFN- γ production, particularly that of ST2⁺ CD8⁺ CTLs and NK cells.

3.2 IL-33 involvement in immune-mediated pathologies and potential to shape transplant outcomes

The involvement of IL-33 in development of immune-mediated pathologies, such as inflammatory diseases of the airway (e.g. asthma) or bowel (e.g. Crohn's disease), as well as arthritis, has been the focus of several recent reviews[45, 61, 68]. However, the role of IL-33 in shaping the alloimmune response or physiological processes that dictate solid organ transplant outcomes are relatively unexplored. Transplantation is the procedure of choice to treat end-stage organ failure, providing prolonged survival and improved quality of life. This year in the United States (US) >25,000 lives will be lengthened following late-stage organ failure by transplantation (optn.transplant.hrsa.gov). Alloantigen (Ag)-reactive immune cells, especially T cells, are the primary mediators of transplant rejection. Widespread implementation of clinical allotransplantation only became a reality following development of immunosuppression (IS) that mitigated T cell responses and prevented early transplant rejection. Optimization of IS strategies have led to continued improvements in short-term (<1 year) outcomes, yet long-term outcomes (> 1 year) are suboptimal and have changed little over the past several decades. Specifically, approximately 50% of kidney, liver, and heart transplants will fail within 10 years of their transplantation[69, 70]. Lung and intestine transplants fare much worse, with transplant half-lives of only 4-5 years[70]. Each type of transplant brings with it organ-specific barriers to better outcomes, but there are also common factors leading to graft attrition and patient morbidity and mortality. First, chronic treatment with IS non-specifically restrains the immune system and increases life-threatening infections and certain cancers. Likewise, IS cause numerous toxic side effects, the most pronounced being nephrotoxicity and diabetogenicity. The paramount challenge in organ transplantation is to prevent chronic rejection of the allograft. Chronic rejection (allograft vasculopathy) presents similarly in human heart, kidney, and liver allograft arteries as an immune-mediated, progressive vascular occlusion that results in ischemia and subsequent graft dysfunction[71]. The pathogenesis of chronic allograft vasculopathy (CAV) is poorly understood, but a significant role for allospecific immune responses, particularly IFN- γ -producing T cells and donor-specific antibodies (DSA) is supported by experimental and clinical data[72-74]. In the following sections, we assimilate accumulating knowledge on the role of IL-33 in organ-specific disease pathology with any understood or perceived potential to impact the outcome of several commonly-transplanted solid organs.

3.2.1 Heart Transplantation—As described above, the role of IL-33 in various diseases has been investigated extensively, and the resulting data establish diverse roles for IL-33 in both the pathology of immune-mediated diseases and infection resolution. However, examination of IL-33 as it relates to transplant immunology is a nascent subject area. To date, only 3 published studies have investigated the therapeutic capacity of IL-33 in experimental organ transplantation[75-77]. These studies all utilized the mouse heterotopic heart transplantation (HTx) model and the primary rationale behind administering IL-33 was to take advantage of its established Th2-skewing and direct cardioprotective properties (discussed below). Importantly, studies with this model have also identified a previously unknown capacity of IL-33 for expansion of several populations of regulatory cells (Figure 3).

IFN- γ is the prototypic Th1 cytokine and recognized as a dominant effector molecule in the etiology of both acute and chronic rejection following HTx[71]. Chronic rejection of cardiac allografts manifests as accelerated atherosclerosis resulting in vascular luminal occlusion and progressive ischemic cardiomyopathy[71]. While acute rejection rates have declined over the last several decades, graft atherosclerosis (commonly described as CAV) has remained an unconquered barrier to more acceptable long-term HTx outcomes[78]. In fact, of the > 2,000 heart transplants completed yearly in the US, approximately half will fail due to CAV in a little over 10 years[78]. Activated Th1 cells, but not Th2 cells, are prominent during atherosclerosis development, and IFN- γ facilitates atherosclerotic lesion development through immune cell activation and direction of vascular remodeling[79, 80]. Miller et al. demonstrated that IL-33 prevents atherosclerosis in mice by reducing the formation of plaques via the induction of a Th1-to-Th2 response shift and development of anti-oxidized low-density lipoprotein antibodies in apolipoprotein E-deficient animals on a high-fat diet[19]. There is historic support for the hypothesis that a shift from Th1 to Th2 immunity promotes cardiac allograft survival, facilitates tolerance, and impedes atherosclerosis[79, 81, 82]. Thus, recipient treatment with IL-33 may be beneficial if the alloimmune response is tipped away from the typical Th1 bias following HTx towards a Th2-type immune response. However, both Th1-type and Th2-type cytokines are expressed in acutely rejected grafts[83, 84] and Th2 cytokines promote graft dysfunction and rejection[82]. In particular, IL-5 and eosinophils have been specifically implicated as potent mediators of HTx rejection[85]. Likewise, IFN- γ is anti-proliferative[86], supports activation-induced cell death[86, 87], and is required for tolerance induction in mouse models[86]. Thus, study of how IL-33 precisely regulates Th1 and Th2 responses to shape acute rejection and development of CAV is an area worthy of close examination for both basic discovery and clinical application.

Yin et al. were the first to report on the impact of IL-33 on heterotopic cardiac allograft survival[75]. They found that intraperitoneal (i.p.) administration of 1 μ g/day recombinant murine (mu) IL-33 cytokine (IL-33₁₁₂₋₂₆₆) to C57BL/6 (B6, H-2^b) recipients for 7 days post transplant tripled BALB/c (H-2^d) heart allograft survival time in the absence of any IS[75]. This prolongation in graft survival was associated with increases in splenic IL-4⁺ CD4⁺ T cells and significant increases in splenic and intragraft IL-4 and IL-5 mRNA. A corresponding decrease in CD4⁺ IFN- γ ⁺ splenocytes and IFN- γ message in the graft was also observed. A shift in alloAbs away from IgG2a towards increased IgM and IgG1 further supported IL-33-mediated Th2 skewing in transplanted mice treated with IL-33. Thus, although not directly verified in these studies, it was concluded that the capacity of IL-33 to shift alloreactive immune responses away from type 1 to type 2 immunity facilitates cardiac allograft survival.

Our research has confirmed that i.p. delivery of recombinant muIL-33₁₁₂₋₂₆₆ following heterotopic transplantation of B6 hearts into BALB/c recipients triples graft survival time in the absence of IS (mean survival time of 29 days vs. 9 days for PBS-treated control recipients; [76]). In these studies, we used *St2*^{-/-} mice to demonstrate both that the therapeutic benefit of IL-33 was dependent on recipient expression of ST2, and that delivery of IL-33 to naïve or transplanted mice expanded several potent immunoregulatory populations[76]. These populations included poorly stimulatory myeloid DC, but more importantly, CD11b⁺Gr-1^{int} myeloid-derived suppressor cells (MDSC; [74]). MDSC, are a heterogenous population of immature myeloid cells and myeloid progenitor cells expressing CD11b, moderate levels of Gr-1 (Ly6C/Ly6G) and F4-80, and with potent T cell suppressing capacity[88, 89]. Also in these studies, we revealed that IL-33, in addition to promoting Th2 responses as reported by Yin et al., also supports expansion of Treg. Specifically, administered IL-33 exhibits a potent ability to increase suppressive CD4⁺ Foxp3⁺ T cells, including a ST2⁺ subset in vivo[76]. Heart grafts from IL-33-treated

recipients were less infiltrated by T cells overall, yet had increased numbers of Treg[76]. Treg were fundamental to the therapeutic capacity of IL-33 in promoting cardiac allograft survival, as their depletion before transplantation and IL-33 treatment ablated any therapeutic effect. Brunner et al. also demonstrated that treatment with muIL-33₁₁₂₋₂₆₆ delayed the rejection of MHC class II-mismatched bm12 (H-2^{bm12}) hearts in B6 (H-2^b) recipients. In this model, IL-33 also increased systemic and intra-graft Foxp3⁺ Treg and MDSC[77]. Thus, in addition to supporting Th2 responses, IL-33 possesses distinct immunoregulatory properties, including expansion of Treg and MDSC. Elucidating whether mechanisms by which IL-33 expands Treg and MDSC are distinct from those that promote type 2 immunity will significantly aid efforts to translate these observations in experimental models into beneficial effects for transplant patients.

We also demonstrated that IL-33 monotherapy post heart transplant reduced serum IL-12p40/p70[76], but profoundly increases circulating IL-5 and IL-13, potentially accounting for the lack of IL-33 augmentation of type 1 immunity following heart transplantation. However, IL-33 is not invariably beneficial to heart tissue. Abston et al. have recently reported the administering recombinant muIL-33₁₀₉₋₂₆₆ promoted eosinophilic perimyocarditis in otherwise unmanipulated mice and enhanced disease severity, compromised cardiac function, and exacerbated ventricular dilation in a coxsackievirus-induced eosinophilic perimyocarditis model [90]. Potent stimulation of IL-5 production and associated promotion of eosinophilia were among the first identified characteristics of administered IL-33 [37]. Bearing in mind these new data [90] and the appreciated capacity of IL-5 and eosinophils to mediate HTx rejection [85], targeting IL-5 and eosinophils [91] during IL-33 therapy for HTx should be evaluated as away to both potentiate therapeutic benefits following HTx and also avoid any eosinophilia-associated detrimental effects.

Direct cardioprotective properties of IL-33 and ST2 have been suggested in numerous rodent studies. Two years after its identification, IL-33 was shown to be biomechanically inducible in cardiac fibroblasts and able to block cardiomyocyte hypertrophic signaling[18]. IL-33 administration ameliorated cardiomyocyte hypertrophy and ventricular dysfunction following transverse aortic constriction[18]. Pathology was exacerbated by IL-33 antagonists or in tissues lacking ST2, thus supporting a protective role for local IL-33[18]. Another report has described a capacity for the IL-33/ST2 axis to prevent myocyte apoptosis and improve ventricular function and survival in a myocardial infarction model[41]. Likewise, IL-33 and ST2L expression is increased in vessel endothelial cells and smooth muscle cells during development of atherosclerosis, which is accelerated in the absence of ST2[19]. We demonstrated that the benefits of administered IL-33 following transplantation of MHC-mismatched cardiac allografts were dependent on recipient expression of ST2[76]. Yet, in these studies, we also identified a profound increase in expression of ST2 in acutely rejecting cardiac tissue, particularly in the myocardium. However, the anti-ST2 Ab used could not distinguish between sST2 and ST2; thus, if this ST2 increase is expected to be supportive or antagonistic of any beneficial IL-33 activities on the heart could not be predicted. Also, given the aggressive rejection in this fully MHC-mismatched model, it will be important to define how administered and endogenous IL-33 shapes acute and chronic HTx rejection in less stringent donor/recipient combinations or with IS.

3.2.2 Lung Transplantation—The role of IL-33 in the development and pathology of inflammatory lung diseases has been studied intensively. Both IL-33 and ST2 are elevated in inflammatory lung disease models[45, 92, 93] and genome-wide association studies of large groups of ethnically diverse individuals have found that variants in *IL33* and *IL1RL1* loci are associated with asthma risk[94, 95]. Initial studies examining the role of IL-33 and ST2 using ST2^{-/-} mice were controversial[96-98]. However, IL-33-deficient and IL-33-overexpressing transgenic mice have been valuable models in establishing the importance of

IL-33 in inflammatory lung diseases. Transgenic mice that over-express IL-33 develop spontaneous pulmonary inflammation characterized by eosinophil, neutrophil and monocyte infiltration and augmented BAL IL-5, IL-13 and IL-33[99]. In both ovalbumin/alum-induced or house dust mite-stimulated allergic airway inflammation models, pulmonary inflammation was diminished significantly in mice lacking IL-33. Interestingly, Ag-specific splenocyte proliferation and cytokine production was not changed by the absence of IL-33[100]. These data support a critical role for local IL-33 in the activation of innate cells that facilitate Ag-dependent allergic airway inflammation, but a less pronounced effect in direct support of Ag-specific Th2 cell differentiation and expansion.

Based on the above data, it is easily envisaged that the IL-33/ST2 axis is crucial in shaping short- and long-term transplant outcomes following lung transplantation (LTx). However, there are no clinical or experimental reports on IL-33 in LTx to date. The number of lung transplants performed in adults has been increasing steadily during the past 25 years to >3000 yearly procedures[101]. Median survival of lung transplant recipients remains relatively low compared to other organ transplants, although survival rates are improving, up from 3.9 years during 1988 to 1995 to 5.9 years during 2004 to 2010[101]. Primary graft failure, defined as severe and acute allograft dysfunction developing early (<72 hr) after transplantation remains a significant problem. Pulmonary ischemia/reperfusion (I/R) injury has been considered the major cause of primary graft failure[101-103]. Lung epithelial cells constitutively express some of the highest levels of IL-33, and it will be important to establish how transplant surgery-induced I/R or alloimmune attack of the epithelium modulates IL-33 expression and the release of active forms of IL-33.

Beyond the potential impact of secreted IL-33 on T cells and myeloid APC, IL-33 may also support graft dysfunction in its capacity to stimulate other populations of innate immune cells, particularly neutrophils and ILC2. Pulmonary I/R is typically characterized by massive neutrophilic infiltration[104]. Depletion of neutrophils from donors prior to LTx surgery profoundly reduces transplant injury and improves later pulmonary function[104]. IL-33 was found recently to support neutrophil influx into sites of infection by promoting CXCR2 expression[105]. Thus, neutrophil infiltration and release of highly-active forms of IL-33[25] from the lung allograft epithelium may constitute a particularly detrimental feed forward loop that promotes poor LTx outcomes.

Transplanted lungs are exposed continually to the external environment, and immunosuppressed lung graft patients suffer significant morbidity and mortality from resultant bacterial, viral, and fungal infections[101]. Likewise, chronic lung rejection, or bronchiolitis obliterans (BO), is thought to be propagated by innate immune cell recognition of pathogens via TLRs, adaptive Th1 cellular and humoral alloimmunity, and later autoimmunity[101, 106-108]. It will be important to establish how the alarmin function of IL-33 following the above infections acts on ST2⁺ innate immune cells to mediate protective immunity versus supporting the pathogenesis of BO.

New findings also suggest that local IL-33 may support not only lung inflammation, but also wound healing and repair via its influence on ILC2. In a papain-induced model of asthma-like airway inflammation, IL-33 induced IL-5, -9, and -13 production by ILC2[55]. Through study of *Rag*^{-/-} *IL-2rg*^{-/-} mice, these authors established ILC2 to be the dominant source of IL-9 following papain challenge, but also found that in conditions of low IL-25 or -33, T cell produced-IL-2 can support ILC2 activities[109]. Further studies in this model support Lin⁻ Sca-1⁺ c-kit^{+/lo} CD25⁺ CD127⁺ RORγ⁺ lung natural helper (NH) cells as direct targets of IL-33 released from the lung stroma and IL-33-activated NH cells playing a key role in the development of T cell-independent eosinophilia and type 2 cytokine secretion[110]. Yet, a recent study revealed that NH cells are also central to the maintenance of lung function

and tissue repair following influenza infection through their production of amphiregulin[111]. These protective effects could be antagonized by administration of anti-ST2, thus it appears IL-33 may be crucial in the balance between inflammation and homeostasis in the lung[111]. The recent development of orthotopic lung transplant models in the mouse[104, 112] should allow precise establishment of the role of both IL-33 and ILC2 in lung transplantation, with further insights into the endogenous function of IL-33 and ILC. In particular, it will be important to establish how current IS impacts ILC2 production of IL-5 and IL-13, particularly potent mediators of tissue fibrosis. Failure of IS to suppress ILC2 cytokine function may be an important mechanism leading to the fibrous obliteration of the airways typical of LTx and detrimental to clinical outcomes.

3.2.3 Intestinal transplantation—The use of intestinal transplantation (ITx) for treatment of intractable gastrointestinal failure or as an alternative to parenteral feeding, has increased slowly, but steadily, over the last several decades[113]. Similar to LTx, ITx is characterized by significant morbidity, mortality, and high rates of allograft loss after transplantation[113, 114]. Also like the lung, high constitutive levels of IL-33 are found in both the mouse and human gastrointestinal (GI) tract[12, 115]. Although there is a lack of formal studies investigating IL-33 in the poor clinical outcomes associated with ITx, data from other models examining parasite infection of the GI system suggest IL-33 may be an important consideration in ITx. IL-33 is elevated during nematode infections of the gastrointestinal system, and IL-33 administration supports worm expulsion[116]. These data support local IL-33 as an indicator of parasite infection and mediator of the subsequent inflammatory responses required for their removal. Thus, IL-33 released due to I/R, transplant surgery-induced inflammation, or the subsequent alloimmune responses, may trigger or exacerbate ITx rejection. Ulcerative colitis is a well-known form of idiopathic inflammatory bowel disease that affects the entire gastrointestinal tract. In patients, as well as in experimental rodent models, IL-33 and ST2 are increased in both inflamed bowel tissue and serum[115, 117-121]. Ulcerative colitis patients exhibit IL-33 expression in myofibroblasts[115] in addition to afflicted epithelium and lamina propria mononuclear cells[121]. IL-33 levels decrease with anti-TNF therapy[121] and Oboki et al. found that IL-33^{-/-} mice exhibit delayed development of dextran sodium sulfate-induced colitis[100]. Yet, a beneficial role for IL-33 administration has also been demonstrated in a mouse trinitrobenzene sulfonic acid-induced colitis model[122]. Specifically, delivery of recombinant IL-33₁₁₂₋₂₆₆ reduced experimental colitis and was associated with induction of Th2 cytokines and significant increase in Treg responses. Depletion of Tregs abrogated the protective effect of IL-33 in this model, again signifying the important influence that IL-33 may have on Treg for resolution of inflammation.

3.2.4 Involvement of IL-33 in broader issues related to transplantation—Hypoxic conditions induce inflammation[123] and the superior survival exhibited by recipients of allografts from living, unrelated donors over those from cadaveric donors supports the concept that I/R injury to allografts promotes later graft failure and rejection[124]. Necrosis is commonplace after I/R and given our current understanding of IL-33 immunobiology, IL-33 released from necrotic cells may provide an alarmin signal that augments the inflammatory processes involved in I/R injury and allograft rejection. The potential impact of graft-derived or administered IL-33 in I/R injury in the context of transplantation has yet to be examined. However, data examining the influence of IL-33 on I/R organ injury in the non-transplant setting have been intriguing, but complicated by conflicted findings. Sakai et al. recently demonstrated that recombinant muIL-33₁₀₉₋₂₆₆ reduced hepatocellular injury and liver neutrophil accumulation after I/R[42]. Their data provide mechanistic insights supporting a direct protective effect of IL-33 on ST2⁺ hepatocytes. This is in direct contrast to an earlier study that supported a detrimental impact

of endogenous IL-33, which was suppressed by delivery of sST2, in warm liver I/R injury[125]. Treatments with sST2 were also beneficial following intestinal I/R injury[126]. Yet, others have found a significant protective capacity of exogenous IL-33 when the hearts of diabetic animals were subjected to I/R injury[127]. In these cases, IL-33 was particularly effective in preventing myocyte apoptosis. Understanding how endogenous vs. exogenous IL-33 functions in I/R injury will benefit from further study using IL-33- and ST2-deficient mice to resolve these conflicting findings. This knowledge is expected to be particularly beneficial and lead to methods that focus on IL-33 activity during organ harvest and subsequent transplantation to lessen I/R and improve allograft outcomes.

Intravenous immunoglobulin (IVIG) products are generated from pooled human plasma and have been used for treatment of inflammatory disorders, particularly those mediated by antibodies for at least 20 years. The use of IVIG is traditional therapy for treatment of antibody-mediated rejection of solid organs. Likewise, IVIG is gaining favor as a means to “desensitize” transplant patients with existing DSA antibodies generated through prior transplants, blood transfusions, or pregnancy[128]. Yet the precise immunological mechanism by which IVIG provides any therapeutic benefit for antibody-mediated ailments is poorly understood. Recently, Anthony et al. used a model of serum-induced arthritis to demonstrate that IVIG targeted DC-SIGN on myeloid APC, including mDC, macrophages, and monocytes, and induced their expression of IL-33[21]. IL-33 facilitated increases in Th2-type cytokines, particularly IL-4, which induced expression of the inhibitory Fc receptors on macrophages, thus suppressing their activation[21]. Interestingly, IL-33 delivery alone could limit serum-induced arthritis, but not in IL-4- or IL-4R α -deficient mice. Nevertheless, administered recombinant IL-33 and endogenous IL-33 have both been found to exacerbate disease in collagen-induced arthritis models [17, 129]. These studies in arthritis models underscore the complex nature of IL-33 Immunobiology that is emerging when IL-33 alone, or combined with other local stimuli, shapes the function of non-hematopoietic and hematopoietic cells expressing ST2. Focused study in diverse immunological models is needed to establish exactly how endogenous and administered IL-33 contributes to the regulatory and inflammatory pathways culminating in disease pathology and control. A better understanding of the mechanisms leading to the pleiotropic function of IL-33, especially those mediating the involvement of IL-33 in regulatory pathways, could be translated into benefits for transplant recipients.

4. Conclusion and future directions

The relatively few studies conducted to date into the specific role of IL-33 in alloimmunity/experimental solid organ transplant models limit any definitive conclusions about the role that IL-33 and ST2 may play in transplant outcome. However, functional analysis of IL-33 in other models of immunity and immunopathology fully support the capacity of this cytokine to influence both innate and adaptive immune cells that form the basis of recipient alloreactivity. The observation that administered IL-33 increases Treg and MDSC, both proposed to be central to experimental tolerance[47], supports the potential to commandeer the regulatory capacity of IL-33 for the good of transplant recipients. Advancing our understanding of the mechanisms by which this cytokine can also support Th1 and Th2 type responses should allow the development of methods to counter the pro-inflammatory capacities of IL-33 and accelerate this translation. Likewise, how donor, or “passenger” ILC2 shape LTX and ITx outcomes, especially in the presence of IL-33, will be important to consider.

While it is apparent that pathological conditions and pro-inflammatory stimuli can increase IL-33 expression in affected tissues and murine myeloid cells, the physiological and immunological functions of endogenous IL-33 in these localities are only emerging.

Transplant models utilizing IL-33- or ST2-deficient donors and recipients will provide an excellent means to clarify how pathophysiological conditions shape IL-33 function in immune cells versus organ tissues. Hopefully these examinations, combined with studies in other disease models, will provide needed clarity to the persisting questions regarding how endogenous IL-33 is processed and the functional capacity of “matured” forms of IL-33 in vivo. Likewise, such transplant studies can develop a better appreciation for the physiological functions of endogenous IL-33, both inside and outside the cell, versus what results from administration of “IL-33-like” recombinant peptides. Yet, administration of recombinant IL-33 cytokine promoted HTx survival in the absence IS in three independent studies, and thus suggests that IL-33-like, ST2-binding peptides warrant further exploration as potential biologicals for use following heart transplantation. Also, although repeated systemic administration of recombinant IL-33 may not be necessarily reflective of the endogenous role this cytokine plays in immune responses, that such treatments prevent CAV and support IS-free HTx survival, suggest that application of new knowledge of IL-33 immunobiology may contribute to overcoming these two primary obstacles to long-term survival in clinical heart transplantation. In the three studies testing the therapeutic capacity of short-term IL-33 following HTx, all studies reported success in extending transplant survival in the absence of IS, but not tolerance. Testing whether IL-33 synergizes with low dose IS or aides tolerance-inducing regimens may be especially promising for HTx. Overall, IL-33 is has emerged prominently and broadly in our immune response to pathogens, as well as in development of immune-mediated pathology. It can be expected that future examinations will demonstrate that the IL-33/ST2 axis is an influential immune pathway that fundamentally shapes short- and long-term transplant survival.

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Abbreviations

APC	antigen presenting cell
BAL	bronchoalveolar lavage
CBM	chromatin-binding motif
CAV	chronic allograft vasculopathy
DC	dendritic cells
DC-SIGN	DC-specific intercellular adhesion molecule-3-grabbing non-integrin
DSA	donor specific antibodies
Foxp3	forkhead box P3
HEV	high endothelial venules
HTH	helix-turn-helix
HTx	heart transplantation
i.p.	intraperitoneal
I/R	ischemia/reperfusion
IFN	interferon

Ig	immunoglobulin
IL	interleukin
IL-1Ra	IL-1 receptor antagonist
IL-1RAcP	IL-1R accessory protein
IL1RL1	IL-1 receptor-like 1
ILC2	type 2 innate lymphoid cell
IRAK	IL-1 receptor-associated kinase
IS	immunosuppression
ITx	intestinal transplantation
IVIG	intravenous immunoglobulin
LTx	lung transplantation
MDSC	myeloid-derived suppressor cells
mu	murine
NH	natural helper cell
NF-HEV	nuclear factor from HEV
NLS	nuclear localization signal
RA	rheumatoid arthritis
sST2	soluble ST2
TCR	T cell receptor
Th	helper T cell
TIR	Toll-IL-1R
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T cell

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Highlights

IL-33 is expressed in the epithelium and vasculature of transplanted solid organs.
Distinct domains allow IL-33 to act as a cytokine and a regulatory nuclear factor.
IL-33 is a pleiotropic cytokine that promotes Type-1, -2 and regulatory responses.
Data support a protective role for IL-33 following heart transplantation.
IL-33 may contribute to poor lung or intestine transplant outcomes.

Figure 1. Schematic representation of human and mouse IL-33 proteins

IL-33 is a conserved protein with a high level of amino acid (aa) sequence identity shared between the (A.) human and (B.) mouse homologs. The IL-33 protein consists primarily of two domains, a N-terminal homeodomain-like helix-turn-helix (HTH, yellow region) and an IL-1-like cytokine domain (green region). The N-terminal domain of both human and mouse IL-33 is highly similar (57% sequence identity) and contains a chromatin-binding motif (CBM; orange region) and nuclear localization signal (NLS; red region). The CBM and NLS mediate nuclear translocation and association with histones in heterochromatin. The cytokine domain is also well-conserved between mouse and human (57% identity) and contains 12 β -sheets that form the β -trefoil structure characteristic of IL-1 cytokines. Identified protease cleave sites are indicated by $\square$. Proteolytic processing resulting in bioactive forms of IL-33 are indicated by + and cleavage resulting in inactivation is signified by X. The respective commonly utilized form of both human and mouse IL-33 are indicated.

Figure 2. Potential mechanisms underlying the contradictory functions of IL-33 in type 1 and type 2 immunity

IL-33 is released from damaged endothelial or epithelial cells and acts on cells expressing the IL-33 receptor, ST2, to support potent type 2 immunity as indicated. ST2⁺ eosinophils, basophils, and mast cells, as well as type 2 innate lymphoid cells (ILC2), and CD4⁺ T helper type 2 (Th2) cells. Myeloid antigen presenting cells (APC), such as dendritic cells (DC) and macrophages also express ST2, and IL-33 imparts these APC with the capacity to polarize naïve CD4⁺ T cells towards Th2 effectors secreting IL-5 and IL-13. However, activation of APC through ligation of their Toll-like receptor (TLR) by pathogen-associated molecular patterns (PAMPs) found in viral or bacterial products stimulates APC secretion of IL-12. IL-12 induces expression of ST2 on CD8⁺ T cells and NK/NK T cells, enabling IL-33 to augment the IFN- γ production of these cells and promoting type 1 immunity. Caspases 3 and 7 are activated during apoptosis and cleave IL-33 in the cytokine domain, limiting its capacity to promote either a type 1 or 2 immune responses.

Figure 3. Therapeutic function of administered IL-33 in heart transplantation

Initiation of intraperitoneal (i.p.) delivery of recombinant IL-33₁₀₉₋₂₆₆ at the time of heart transplantation promotes survival of MHC-mismatched allografts in the absence of immunosuppression. This phenomenon is associated with expanded regulatory immune populations, particularly Foxp3⁺ regulatory T cells (Treg), but also myeloid-derived suppressor cells (MDSC). Both Treg and MDSC exhibit a potent capacity to suppress alloreactive T cell proliferation and function. Thus, IL-33-expanded regulatory cells may block the typical IFN- γ -dominated acute rejection of cardiac allografts by CD4⁺ Th1 and CD8⁺ cytotoxic T lymphocytes (CTL). IL-33 administration also promotes Th2 immunity, particularly increasing levels of IL-5 and IL-13. Th2 immunity is also ascribed beneficial properties following experimental heart transplantation in some studies.

Table 1

Influence of IL-33 on ST2 expressing cells. GM-CSF: granulocyte macrophage colony-stimulating factor; MCP-1: monocyte chemotactic protein-1; NO: nitric oxide; XIAP: X-linked inhibitor of apoptosis protein; cIAP1: cellular inhibitor of apoptosis 1; Bcl-2: B-cell lymphoma 2.

Cell type	Influence of IL-33/ST2 binding	Reference
Hematopoietic cells		
Th2 cells	Increases production of IL-5 and -13	[58]
CD8⁺ T cells	With IL-12 promotes cell expansion and IFN- γ production	[66, 67]
B1 cells	Cell activation and proliferation Increases in IgM, IL-5, and IL-13 production	[130]
Neutrophils	Facilitates migration and chemotaxis	[105, 131]
Basophils	Induces superoxide anion generation Induces IL-4 and -13 and CXCL8 production	[58]
Eosinophils	Induces differentiation and degranulation Promotes survival and eosinophilia Induce superoxide anion and CXCL8 production	[100, 132, 133]
Mast cells	Promotes IL-5, -6, -10 and -13 production Promotes TNF, CXCL8, CCL1 and GM-CSF production	[59, 60]
Dendritic cells	Upregulate MHC class II and CD86 Promote IL-6 production Facilitate capacity for Th2 polarization	[51]
Macrophages	Macrophage polarization (M2)	[50]
NK/iNKT cells	Augment IFN- γ production	[63, 64]
ILC2	Produce IL-13 and help eliminate helminth infections Promote lung-tissue homeostasis after influenza virus infection Mediate influenza-induced airway hyper-reactivity	[53, 111, 134]
Foxp3⁺ Treg	Increased systemic incidence	[76, 77]
Non-hematopoietic cells		
Endothelial cells	Induce IL-6 and CXCL8 and MCP-1 production Stimulate endothelial NO production Promote angiogenesis and vascular leakage	[43, 135, 136]
Epithelial cells	Induce CXCL8 production	[43]
Fibroblasts	Induce MCP-1 and 3 and IL-6 Antagonize angiotensin II- and phenylephrine-induced cardiomyocyte hypertrophy	[18, 137]
Myocardial cells	Induce expression of XIAP, cIAP1 and survivin Prevents cardiomyocyte apoptosis	[41]
Hepatocytes	Activation of NF- κ B and increased Bcl-2 expression Reduce hepatocyte death following I/R	[42]