The Reparative Roles of IL-33

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Abstract. When discovered in the early 2000s, interleukin-33 (IL-33) was characterized as a potent driver of type 2 immunity and implicated in parasite clearance, as well as asthma, allergy, and lung fibrosis. Yet research in other models has since revealed that IL-33 is a highly pleiotropic molecule with diverse functions. These activities are supported by elusive release mechanisms and diverse expression of the IL-33 receptor, STimulation 2 (ST2), on both immune and stromal cells. Interestingly, IL-33 also supports type 1 immune responses during viral and tumor immunity and after allogeneic hematopoietic stem cell transplantation. Yet the IL-33–ST2 axis is also critical to the establishment of systemic homeostasis and tissue repair and regeneration. Despite these recent findings, the mechanisms by which IL-33 governs the balance between immunity and homeostasis or can support both effective repair and pathogenic fibrosis are poorly understood. As such, ongoing research is trying to understand the potential reparative and regulatory versus pro-inflammatory and pro-fibrotic roles for IL-33 in transplantation. This review provides an overview of the emerging regenerative role of IL-33 in organ homeostasis and tissue repair as it relates to transplantation immunology. It also outlines the known impacts of IL-33 in commonly transplanted solid organs and covers the envisioned roles for IL-33 in ischemia-reperfusion injury, rejection, and tolerance. Finally, we give a comprehensive summary of its effects on different cell populations involved in these processes, including $ST2^+$ regulatory T cells, innate lymphoid cell type 2, as well as significant myeloid cell populations.

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INTRODUCTION

Our knowledge of interleukin-33 (IL-33) immunobiology has evolved significantly over the last 2 decades. Although its early role as a driver of potent type 2 immune responses has been concretely established, roles for this novel interleukin-1 (IL-1) superfamily member in homeostasis, tissue repair, and regeneration have also emerged. Several previous reviews have covered the molecular biology of IL-33, its genetic and protein characterization, 3D structure, and intra- and extracellular regulation and expression.[1-3] Therefore, we have kept the review of these areas brief and only summarized key points needed for comprehension of subsequent sections on the reparative function of IL-33. We also opted to divide repair and regeneration into 3 different categories. First, we will discuss the known role of IL-33 in establishing tissue homeostasis during development. Then we will describe its function during tissue repair and the mechanisms involved postinjury. Finally, we will focus on its potential role in organ transplantation, specifically, its contributions to the resolution of ischemia-reperfusion injury (IRI), and its role in promoting early repair and graft tolerance.

BIOLOGY OF IL-33

The *IL-33* gene is located on chromosome 9 in humans and 19 in mice and generates a 270-amino acid protein (Figure 1A) formed by 2 conserved domains. These include an N-terminal domain mediating nuclear localization and chromatin binding, and a C-terminal IL-1-like cytokine domain responsible for its activities.[1,2,4,5] These 2 regions are connected by a central linker region that can be cleaved by endogenous and exogenous serine/threonine proteases.[6,7] IL-33 is constitutively expressed in various cell types under homeostatic conditions: in epithelial cells of the barrier tissues, endothelial cells and adventitial stromal cells of the vascular system, lymphoid tissue fibroblast reticular cells, as well as cells of the nervous system such as the glial cells, neurons, and astrocytes.[1,2,4,5] Cellular stress caused

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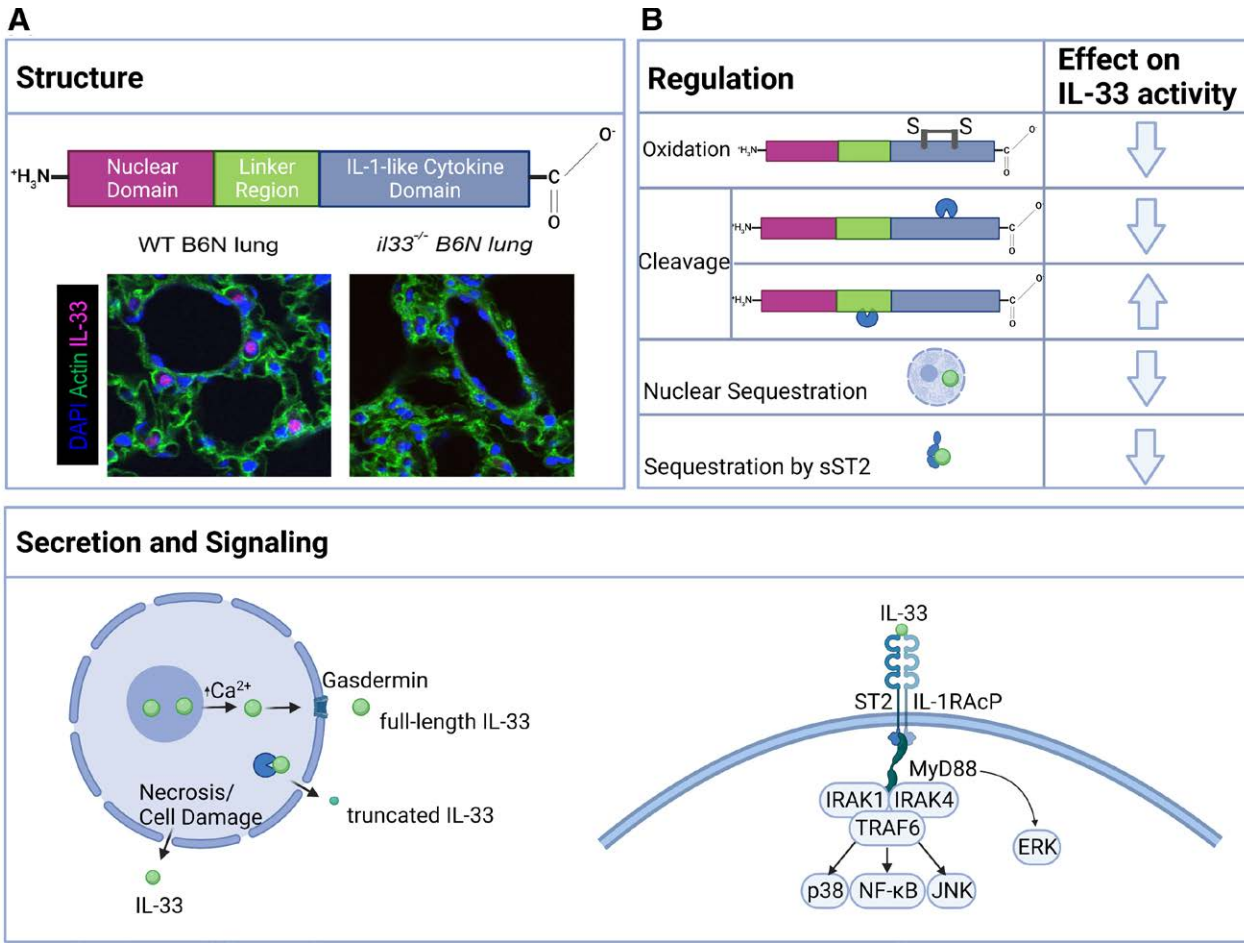

FIGURE 1. IL-33 structure, release, and regulation. (A) IL-33 is a protein formed by 2 conserved domains, an N-terminal nuclear localization domain and a C-terminal domain mediating cytokine activities. A central linker domain connects the 2. Confocal images of IF staining for DAPI (blue), actin (green), and IL-33 (purple) on wild-type (WT) and IL-33-deficient (*il33v*$^{-/-}$) C57BL/6N (B6N) mouse lung tissue. (B) IL-33 activity is regulated by several different mechanisms. Oxidation and formation of disulfide bridges at the C-terminus decrease its activity. Cleavage in the IL-1 cytokine domain by caspases induced during apoptosis leads to inactive IL-33, while cleavage at the linker domain generates a more potent truncated form of the IL-33 protein. Finally, free IL-33 is regulated by either nuclear sequestration or the soluble ST2 (sST2) decoy receptor. (C) The release of IL-33 is less understood and appears to involve multiple mechanisms. Necrosis and cell damage leading to the release of full-length IL-33. Increasing calcium concentration in the cytoplasm drives IL-33 translocation into the cytoplasm and secretion as a full-length protein via Gasdermin-based pores. Cleavage at the linker domain generates a truncated, more active IL-33 protein. The binding of IL-33 to its receptor ST2 promotes a conformational change and recruitment of IL-1 receptor accessory protein (IL-1RAcP). This receptor complex binds to intracellular MyD88, IRAK1 and IRAK4, and TRAF6, leading to downstream activation of several MAP kinases and nuclear factor kappa B (NF-κB), supporting proliferation and secretion of cytokines. Figure created with BioRender.com. IL-33, interleukin-33; DAPI: 4',6-diamidino-2-phenylindole; IRAK, interleukin-1 receptor-associated kinase; MyD88, myeloid differentiation primary response 88; TRAF-6; tumor necrosis factor receptor-associated factor 6.

by tissue injury or inflammation amplifies and induces de novo IL-33 expression. For example, IL-33 expression is increased in the intestinal epithelial cells of patients diagnosed with graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (AlloHCT), and in mucosal tissues after radiation or chemotherapy.[8] In atopic dermatitis, keratinocytes exhibit increased nuclear IL-33 expression,[9] which becomes cytoplasmic upon skin barrier disruption.[10] Some fibroblasts express IL-33 constitutively, while others, such as activated fibroblasts and myofibroblasts, have inflammation-induced IL-33.[1,11]

Because of its nuclear localization domain, IL-33 is predominantly concealed in the nucleus under homeostatic conditions but freed to the cytoplasm and extracellular space during stress and cellular injury and death. As such, IL-33 is classified as a damage-associated molecular pattern (DAMP) or alarmin. These are self-derived molecules that are typically sequestered but released during cellular stress, injury, or necrotic breakdown to signal tissue damage to the immune system.[1-3,12] The exact mechanisms of IL-33 release during injury are not fully understood, and recent studies have identified potential release pathways that do not involve cellular death and necrosis.[13-15] Released IL-33 is functional as a full-length protein, but can be cleaved in the linker domain by proteases to generate a shorter, N-terminally truncated form that is more potent (Figure 1B, C). However, cleavage in the IL-1 cytokine domain by caspases induced during apoptosis leads to the inactivation of IL-33.[1,2,5,6] Once released, IL-33 binds to its membrane-bound receptor, STimulation 2 (ST2) (Figure 1C), which is part of the Toll-like receptor (TLR)/IL-1 receptor-like family.[2] This binding promotes a conformational change in the receptor and

facilitates recruitment of the IL-1 receptor accessory protein co-receptor (IL1RAcP), similar to IL-1β.[7,16] Complete formation of the IL-33R complex results in the activation of a MyD88-IRAK-TRAF6-based signaling pathway that terminates in the activation of mitogen-activated protein (MAP) kinases and nuclear factor kappa B (NF-κB) (Figure 1C).[5,17]

Following the discovery of IL-33, the delivery of recombinant forms of the IL-33 protein has revealed that this new IL-1 superfamily member was a potent driver of type 2 immunity.[4] Administered IL-33 caused ample production of IL-5, IL-13, IgA, and IgE, and stimulated eosinophilia and pathological changes in the mucosal epithelium.[4] These identified dominant type 2 activities led to the rapid establishment of a role for IL-33 in ST2$^+$ Th2$^-$ and Mast cell-mediated parasite clearance,[2,18] as well as involvement in asthma, allergy, and lung fibrosis.[2] However, early studies hinted at reparative functions for IL-33 after cardiac injury.[18] These examinations also revealed the capacity of IL-33 to stimulate ST2$^+$ immune cells, including regulatory T cells (Treg), innate lymphoid cell type 2 (ILC2s), and important myeloid cell populations that support homeostasis and resolve the injury.[19-24] Our work and that of others also began to establish how IL-33 actions on ST2$^+$ Treg were relevant to transplantation, as they supported heart transplant survival[24,82] and were needed to prevent GVHD after AlloHCT.[25] Yet, other studies found that IL-33 can also initiate potent type 1 immune responses by CD4 and CD8 T cells during anti-viral and tumor responses.[26-28] This type 1 driving capacity of IL-33 was also observed following AlloHCT due to the capacity of IL-33 to act as a potent co-stimulatory signal that causes alloreactive T cell Th1 differentiation.[8,26] Given these potent and pleiotropic functions, nuclear localization and compartmentalization of IL-33 are necessary to maintain homeostasis. This was demonstrated by experiments using a mouse model in which a deleted nuclear localization domain led to lethal systemic inflammation.[29] As previously mentioned, cleavage in the cytokine domain by caspases (Figure 1B) is one of the mechanisms of negative regulation of IL-33.[1] Once in the extracellular space, rapid oxidation and formation of disulfide bridges can also inactivate IL-33 or disrupt its binding to ST2 (Figure 1B).[30] Likewise, IL-33 can be sequestered by soluble ST2 (sST2), a product of alternative splicing of the transcript for ST2, which also prevents IL-33 binding to transmembrane ST2 and IL-33/ST2 signaling (Figure 1B).[31,32] Thus, a picture of IL-33 being a potent, yet highly regulated, pleiotropic immune signal able to stimulate type 1, type 2, and regulatory responses have crystallized. Yet data are emerging that IL-33, at least at the low local level, is released during the establishment and maintenance of homeostasis of tissues and organ systems. In this review, we provide an overview of the regenerative role of IL-33 in organ homeostasis and tissue repair and describe, or at least speculate, how these mechanisms relate to transplantation immunology. We also outline the known and proposed impacts of IL-33 in commonly transplanted solid organs during IRI, rejection, and tolerance.

IL-33 AND HOMEOSTASIS

Homeostasis is the self-regulating process by which biological systems maintain stability in response to changing external conditions such as nutritional availability, energy expenditure, environmental conditions, traumas, stresses, and microbial insults.[33] The expression of IL-33 in most tissues and apparent IL-33 release during cell turnover seems to lead to important roles for IL-33$^+$ and ST2$^+$ immune cells in local and systemic homeostasis. Likewise, IL-33 supports the early development of tissues and organs and establishes homeostatic baselines (Summarized in Figure 2A). Throughout organogenesis and later development, tissues develop unique regulatory mechanisms for the protection and establishment of homeostasis through interactions between the various local cell subtypes, as well as the restoration of homeostasis after an insult. For example, IL-33 is expressed by different cell populations in the intestinal mucosa, including pericryptal fibroblasts and small intestinal epithelial cells, and its expression there is regulated based on the environmental signals present.[4,34] Upon release, it was shown to promote the differentiation and hyperplasia of progenitor cells into specific epithelial secretory cells that are important for innate immunity and crucial for the maintenance of gut homeostasis.[35] IL-33 also promotes intestinal peristalsis and improves gut motility through serotonin release by enterochromaffin cells, suggesting a role in maintaining the neuroendocrine interactions needed for intestinal homeostasis and function.[36] IL-33 is also spontaneously produced during the alveolar phase of lung development, causing the accumulation of ILC2s. Exposure of mice to house dust mite antigen during this period can lead to a skewed IL-33-mediated immune response with a strong Th2 cell bias.[37] However, IL-33 also boosts ILC2 function in tissue remodeling and homeostasis through the production of amphiregulin (Areg), an epidermal growth factor receptor ligand that stimulates cell growth and survival.[37] Yet overactivation or sustained action of this pathway can contribute to allergic airway disease.[37] IL-33 expands lung-resident ILC2s that produce IL-5 and IL-13, and a loss of this population after H1N1 viral infection was associated with poor lung function and epithelial integrity.[38] Such type 2 immune responses after injury, particularly the type 2 cytokine IL-13, drive the polarization of macrophages into an anti-inflammatory/reparative subset often labeled as M2 or alternatively activated macrophages. IL-13 signaling is important in shifting the early appearing alveolar macrophages into M2-like anti-inflammatory reparative macrophages, an effect that lasts throughout development and is tightly linked to ILC2s.[39] These findings suggest a primordial function of IL-33 in establishing and restoring homeostasis during neonatal and adult life in the respiratory and gastrointestinal tract.

Visceral adipose tissue (VAT) is primarily composed of white adipocytes that serve as energy storage depots. As such, the VAT is highly metabolically active, but also serves as an endocrine organ that releases cytokines and other bioactive molecules, such as adiponectin. These signals control body weight, insulin sensitivity, inflammation, and lipid metabolism.[40] Under normal homeostatic conditions, the VAT has a high proportion of ST2$^+$ Klrg1$^+$ Treg, as opposed to the low ST2$^+$ Treg in the spleen, which inferred a potential role of IL-33/ST2 in adipose tissue homeostasis and metabolism.[41] Accordingly, VAT ST2$^+$ Treg were shown to control inflammation resolution using IL-10 secretion and protect against diabetes through their differentiation and expansion via the IL-33/ST2 axis and the proliferation-activation receptor gamma (PPARγ) transcription factor (TF).[42] PPARγ is also upregulated by IL-33

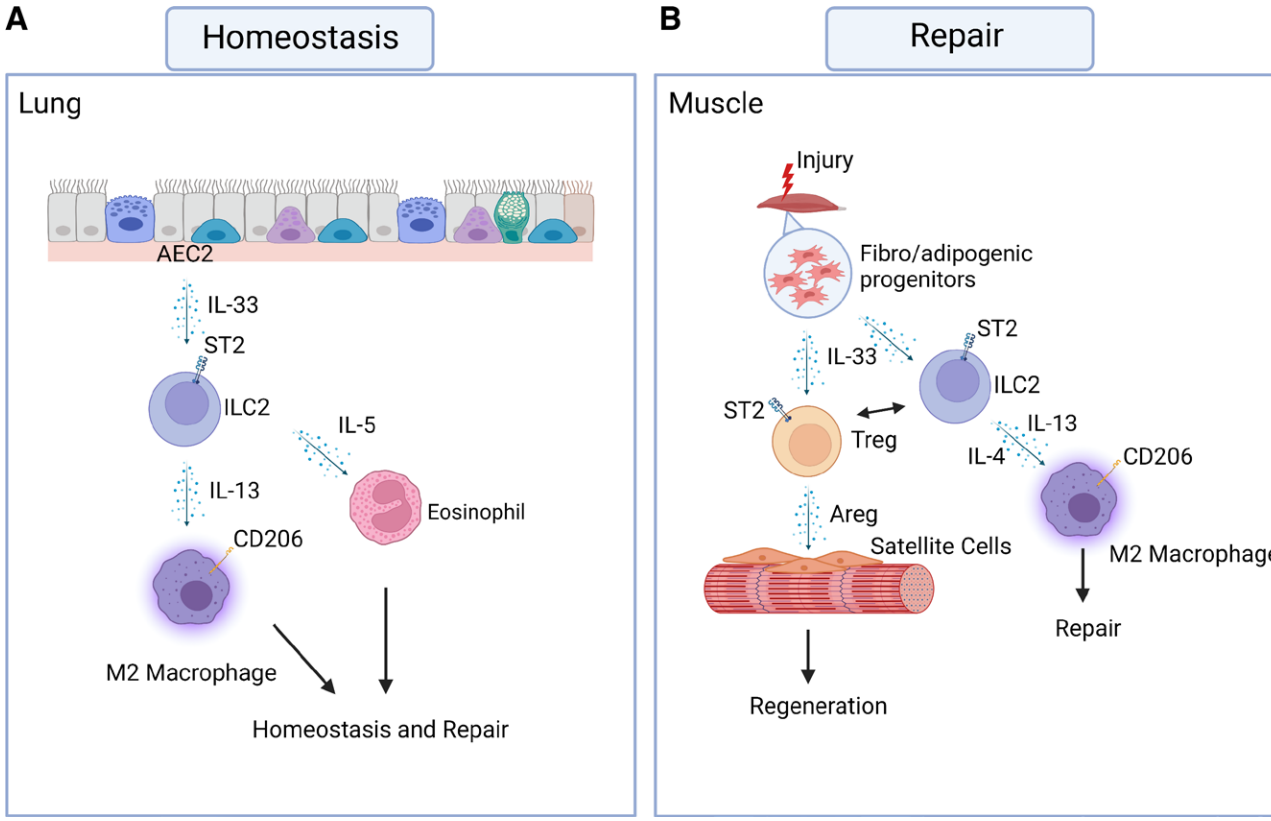

FIGURE 2. Examples of IL-33 functions in homeostasis and repair: (A) in the establishment of lung homeostasis, type 2 alveolar cells (AECs) of the lung epithelium release IL-33, which targets abundantly present ST2$^+$ ILC2s, leading to secretion of IL-13 and IL-5 to promote the formation of reparative M2 macrophages and eosinophils that support homeostasis and repair. (B) After muscle injury, fibro-adipogenic progenitors are the primary source of IL-33 and stimulate ST2$^+$ Treg and ILC2s. Treg secrete Areg, a growth factor that acts on satellite cells and promotes muscle regeneration. Secretion of IL-4 and IL-13 by ILC2s leads to the generation of reparative M2 macrophages. Figure created with BioRender.com. IL-33, interleukin-33; ILC2, innate lymphoid cell type 2; ST2, stimulation 2.

in adipose tissue ILC2s and increases IL-33 responsiveness in ILC2s.[43] IL-33 in the VAT helps maintain homeostasis by stimulating ILC2s to produce type 2 cytokines, such as IL-5 and IL-13, that stimulate local eosinophils and generate alternatively activated macrophages.[44] Yet, these effects of IL-33 are lost in obesity as these cell populations decrease in number, despite the VAT being enriched for IL-33.[45] ILC2s are responsible for the beneficial process of adipocyte beiging in the VAT, which is an IL-33-dependent process.[46] During beiging, ILC2s act on adipocytes to increase their expression of uncoupling protein 1, which regulates their energy expenditure and helps to restore metabolic homeostasis.[47] ILC2s also contribute to glucose homeostasis by limiting the uptake and storage of saturated fatty acid, which is dependent on ST2.[48] During aging, however, IL-33 secretion becomes primarily mediated by mesothelial cells, as opposed to mesenchymal cells in young mice. This appears to decrease IL-33 availability in adipose tissue. This change, along with the simultaneous upregulation of sST2, might explain the dysregulated signaling pathway that prevents the maintenance of healthy ILC2s, limits body temperature control, and disrupts energy expenditure homeostasis.[49] On another note, levels of sST2 increase in obesity and are associated with the development of insulin resistance.[50] In total, the ST2–IL-33 axis is central to the regulation of systemic energy homeostasis. The uterus is the site of implantation of a semi-allogenic fetus, with which the mother has to maintain a state of tolerance, while also providing protection from pathogens. IL-33 is widely expressed in placental endothelial cells, macrophages,[51] fibroblasts,[52] and trophoblasts, and IL-33 deficiency is associated with decreased trophoblast migration and development of preeclampsia.[53] Stromal cells from the decidual layer of the placenta secrete IL-33, shifting the immune phenotype toward a Th2 subtype, inhibiting natural killer (NK) cells' cytotoxicity, and promoting fetal tolerance.[54] The interaction between NK cells and myeloid cells is at the center of local Treg induction,[55] and induced Treg are needed to establish immune tolerance to the fetus at the maternal – fetal interface.[56,57] The uterus is also rich in ILC2s that respond to IL-33 but are also dependent on the female sex hormone estrogen for survival and expansion. This makes ILC2s subject to changing IL-33 and hormonal physiologic changes during pregnancy.[58] Dysregulated ILC2 expression has also been found to restrict fetal growth and results in adverse pregnancy outcomes in LPS-treated rats compared to controls.[59] Other cell types responding to IL-33 released as a DAMP under stress conditions are the ST2$^+$ B cells. When IL-33 is increased and released as a DAMP during pregnancy, ST2$^+$ B1 B cells expand and play a significant role in maintaining a type 2 anti-inflammatory immune response. This prevents preterm birth,[60] potentially through secretion of the anti-inflammatory

molecule active progesterone-induced blocking factor 1 (PIBF1).[61] These studies suggest that IL-33 targets ST2$^+$ immune cell populations during pregnancy to promote tolerance and establish and maintain local homeostasis between the mother and fetus. Clearly, better characterization and understanding of these homeostatic pathways may lead to novel IL-33-based therapeutics that can support solid organ transplant functions, especially in the case of lungs and intestines. The role of IL-33 to aid normal metabolism and controlling energy homeostasis could be useful to counter metabolic dysregulation caused by commonly utilized immunosuppressants.[62,63]

IL-33 IN TISSUE REPAIR

The ability of IL-33 to support homeostasis resides, at least in part, in its ability to expand and promote cytokine secretion of ILC2s and Treg, which secrete type 2 cytokines and molecules involved in tissue repair and regeneration in response to IL-33 (Figure 2B). Following skin injury, ILC2s are activated by IL-25 and IL-33 released from damaged cells, which under the regulation of GATA3, secrete IL-4, IL-5, and IL-13.[64] Both IL-33 and ILC2s are needed for re-epithelization following skin injury in both mice and humans.[65] Mice lacking IL-33 have an inadequate ILC2s response that leads to impaired wound healing, which can be reversed with exogenous IL-33 administration.[65] As introduced above, IL-13 stimulates M2 macrophage differentiation and this macrophage population mediates several steps during wound healing, promotes angiogenesis, and drives the formation of granulation tissue during the early phases of wound repair. They also are important for promoting wound closure and scar formation.[66] Intraperitoneal administration of recombinant IL-33 increases the population of macrophages expressing CD206 and arginase 1, both markers of alternatively activated macrophages, and accelerates wound closure and re-epithelialization.[67] Moreover, a deficiency in ST2 leads to an increase in pro-inflammatory macrophages and myeloid cells in the wound, resulting in impaired wound closure.[68] Other skin resident cells that are important for repair are the intraepithelial lymphocytes (IELs) found among the basal epithelial cells in the skin. IELs are similar to the ILC2s residing in the dermis and constitutively express IL-13. By producing IL-13, they potentiate an epithelial cell stress response leading to the release of IL-25, IL-33, and thymic stromal lymphopoietin, which promotes the type 2 immune response needed for epidermal integrity.[69] In this case IL-13-induced stress and injury response in the skin leads to the release of IL-33, which in turn can act as a positive feedback regulator and amplifier of IL-13 production to support wound repair. However, a pro-fibrotic effect of IL-33 and type 2 immune response can promote scar tissue in the skin and other organs. Early gestational wounds are usually scarless, yet the administration of IL-33 to early gestational murine wounds led to the formation of scars due to the activation of dermal fibroblasts and their overexpression of collagen.[70] This was also shown following repetitive subcutaneous injections of IL-33 in mice, which led to increased inflammation and fibrosis at the injection sites, mediated by the IL-13 produced by eosinophils.[71] Again more evidence that the functions of IL-33 in skin repair must be tightly regulated.

IL-33 also appears to play an important role in the regeneration of skeletal muscle after injury. Here, IL-33 is constitutively expressed in fibroadipogenic progenitor cells (FAPs) of the muscle and their numbers increase after injury. However, FAPs decrease with age, and as such, older mice have a smaller population of FAPs and lower levels of IL-33. This correlates with reduced Treg migration and proliferation in injured tissues, which is associated with limited muscle regeneration.[72,73] A local type 2 immune response, particularly involving IL-4 and IL-13 signaling, recruits more IL-33-expressing FAPs to play a vital role in skeletal muscle regeneration.[74] Satellite cells are multipotent stem cells that are able to generate more satellite cells or differentiated skeletal muscle cells. ST2$^+$ Treg are responsive to IL-33 in injured muscles and contribute to regeneration by secreting Areg, which acts on satellite cells to support their differentiation.[19] Experiments with ST2-deficient Treg revealed similarly impaired accumulation of Treg in injured muscle, resulting in delayed muscle repair and regeneration.[72] Thus these data indicate IL-33 is necessary for effective muscle repair and regeneration due to its direct action on ST2$^+$ Treg and processes that affect IL-33 availability or ST2 expressions such as aging or genetic factors might lead to delayed or dysregulated repair responses.

The regenerative functions of IL-33 have also been demonstrated in the intestinal epithelium using the chronic dextran sulfate sodium (DSS)-induced[75] and trinitrobenzene sulfonic acid-induced colitis.[76] These IL-33-driven regenerative mechanisms were attributed to the stimulation of a robust type 2 immune response, increased Treg infiltration, and the suppression of Th1/Th17 responses.[77] Other mechanisms involve IL-33-induced ILC2s Areg secretion that protects against tissue injury, promotes mucin secretion, causes goblet cell hyperplasia, and restores gastrointestinal tract homeostasis.[78] However, one study observed IL-33-induced intestinal inflammation in DSS-colitis attributed to an aggravated Th2 response, and targeting the IL-33/ST2 axis resolved the aggravating effects.[79] These differences might be due to IL-33 concentration, the timing of IL-33 treatment, or mediated by other confounders that may work with IL-33 to skew the immune response towards a reparative versus inflammatory phenotype. In a model of *Helicobacter pylori* infection, the early upregulation of IL-33 in gastric epithelial cells and fibroblasts directed the immune response to generate alternatively activated macrophages, an effect absent after chronic and prolonged infection.[80] IL-33 in gastric tissue postinfection is also associated with increased gastric epithelial cell migratory potential, proliferation, and decreased apoptosis. However, it has been suggested that the pro-proliferative and anti-apoptotic functions of IL-33 may also contribute to a pro-carcinogenic effect.[81] It is apparent that IL-33 has potent reparative functions in regenerative tissues, such as skeletal muscle or mucosal barrier tissues. This is due, at least in part, to IL-33-induced type 2 responses involving IL-13 and the stem cell growth factor Areg by ST2$^+$ Treg and ILC2s. However, it is also clear that these functions of the IL-33/ST2$^+$ immune cell pathway need to be tightly regulated as it can lead to fibrosis and type 2-associated pathology, as well as tumorigenesis.

IL-33 IN TRANSPLANTATION

The clear role of IL-33 in tissue repair and regeneration makes it a molecule of interest in transplantation, in which injury and repair are inherent in the associated surgical procedures and anti-allograft immune responses. As such, there is a growing body of literature on how IL-33 shapes outcomes after solid organ transplantation (Figure 3). We recently showed that higher levels of graft IL-33 were maintained in patients having minimal coronary artery vasculopathy post-HTx, which might be attributed to a protective role of IL-33.[24] To test if IL-33 was beneficial after transplantation, we utilized a mouse MHCII-mismatched model and compared the outcomes of IL-33-deficient and IL-33-expressing grafts. In these studies, we showed that local fibroblasts upregulate IL-33 rapidly post-HTx, which acts on infiltrating monocytes and macrophages to keep them from becoming pro-inflammatory and supporting chronic rejection.[24] Using recipients with ST2-deficient monocytes and macrophages, we revealed that IL-33 signaling to macrophages limits inducible nitric oxide synthase expression and augments fatty acid uptake. Fatty acids are needed for type 2 cytokine-induced expression of CD206, CD301, and RELMα, which are functional phenotypic markers of reparative macrophages. The loss of IL-33 signaling to macrophages or the absence of IL-33 in the heart grafts results in accelerated graft loss. Our work and that of others have shown that injections of IL-33 posttransplant expand ST2$^+$ Treg that prolong allograft survival across MHC barriers in rodents.[82-84] IL-33 expression is decreased in cardiomyocytes of diabetic mice subjected to IRI. This was associated with increased apoptosis and activation of protein kinase C BII, and these effects were reversed by exogenous IL-33.[85] One of the protective benefits of IL-33 in IRI might be due to activation of the p38/MAPK signaling pathway and inhibition of HMGB1 expression and release.[86] In total, IL-33 is a clinically relevant and seemingly beneficial tissue injury signal that coordinates a Treg and reparative macrophage response early after cardiac tissue injury and heart transplantation.

Our past analysis of transplant recipients also found that tumor-necrosis factor gamma, IL-1β, or interferon-gamma (IFNγ) may elevate the IL-33 antagonist sST2 in the graft and serum during rejection.[87] Importantly, serum levels of sST2 can distinguish recipients undergoing rejection (acute cellular and antibody-mediated) from those with apparent stable grafts at similar time points posttransplantation.[87-89] Measures of this molecule in the serum of recipients, in combination with other markers of cardiac function or injury, may have the opportunity to reduce the need for invasive biopsies in heart transplant recipients. This would be similar to the current use of serum sST2 as an indicator and prognostic marker for GVHD.[90]

IL-33 function after kidney transplantation has also been the subject of focused study. In a study by Thierry et al, a correlation was found between serum levels of IL-33 post-kidney transplantation and cold ischemia time. Levels of sST2 were also found to increase posttransplantation.[91] By comparing the serum of renal transplant recipients divided into stable and chronic allograft dysfunction (CAD), it was found that levels of IL-33, IL-2, IL-4, and IL-10 were significantly higher in CAD patients than in stable recipients.[92] IL-33 and ST2 expression are

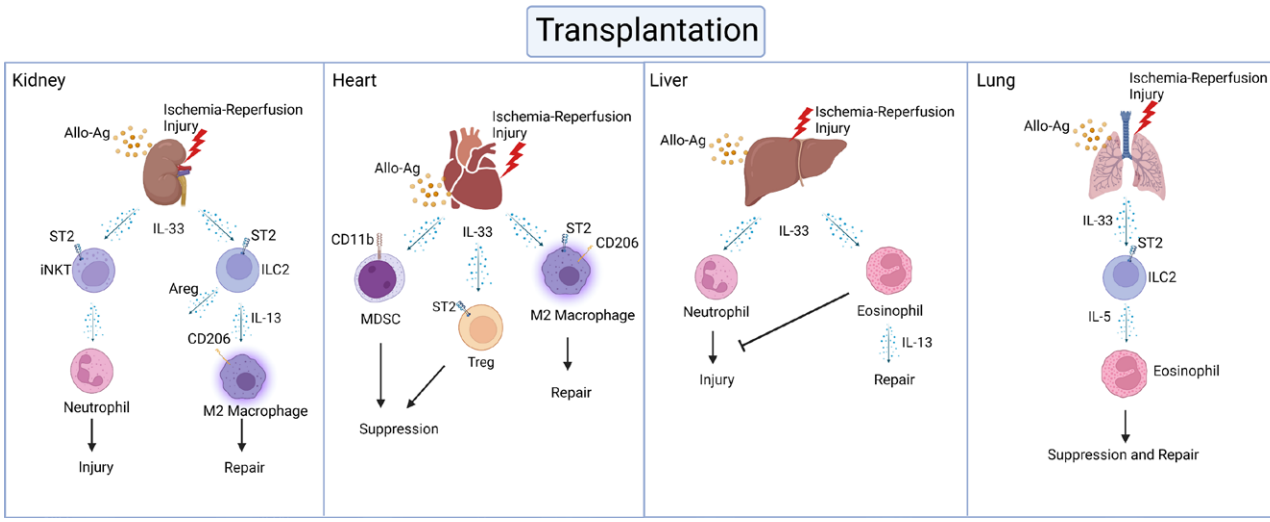

FIGURE 3. IL-33 functions in transplantation. The roles of IL-33 in transplantation outcomes are still being elucidated in experimental models, but the functions of IL-33 after transplant are very organ-specific. IRI and alloimmune response after kidney transplantation can cause IL-33 release to target different cell populations. IL-33 stimulates iNKT cells through their ST2 receptor, which promotes neutrophil infiltration in the graft and subsequent IFNγ release and injury. On the other hand, IL-33 also targets ST2$^+$ ILC2s to encourage the secretion of Areg and the release of IL-13 to aid the recruitment and generation of reparative M2 macrophages. After heart transplantation, released IL-33 acts early on macrophages preventing the generation of pro-inflammatory macrophages and aiding the generation of reparative M2 macrophages. IL-33 can also promote graft survival by recruiting and expanding ST2$^+$ Treg and regulatory myeloid cells, including myeloid-derived suppressor cells (MDSCs). In liver transplantation, IL-33 release causes neutrophil accumulation and tissue injury. Yet, IL-33 targeting of eosinophils leads to their increased IL-13 secretion and repair to counter neutrophil accumulation in the graft. Similarly, IRI in lung transplantation causes ample secretion of IL-33, causing IL-5 secretion by ILC2s to target eosinophils and promote experimental tolerance. Figure created with BioRender.com. IL-33, interleukin-33; IRI, ischemia-reperfusion injury; iNKT cells, invariant natural killer T cells; ILC2, innate lymphoid cell type 2; IFNγ, interferon-gamma; ST2, stimulation 2.

upregulated during renal allograft rejection compared with no rejection and are higher in acute rejection compared to chronic.[92] Using a mouse model of IRI, Cao et al showed that IL-33 is protective against renal injury and fibrogenesis. This role is mediated by ILC2s mounting a type 2 immune response, involving the release of IL-4 and IL-13. This causes local macrophages to differentiate into a reparative and anti-inflammatory subset secreting Areg and expressing Heme oxygenase-1, an anti-inflammatory enzyme believed to control renal IRI.[93] These findings were replicated in a humanized mouse model and raised the possibility of using IL-33 and ex vivo expanded ILC2s as renoprotective agents to fight renal IRI.[93]

Yet, ST2$^+$ innate-like cells iNKT can infiltrate the kidney early after renal IRI in mice. They are postulated to recruit neutrophils, which lead to the production of IFNγ and early tissue injury. These cells can be expanded in vivo and in vitro by IL-33, indicating that an early release of IL-33 after IRI might also contribute to the subsequent tissue injury through its effect on iNKT cells, supporting the emerging influential role of innate (and innate-like) cells in transplantation.[94-96] These concepts were supported in rodent IRI models where an IL-33 or NKT deficiency led to less myeloid cell recruitment after transplantation and protected against IRI.[97] DAMPs released from necrotic and damaged renal tubular epithelial cells (RTECs) act on healthy RTECs as well as immune cells, and induce TLRs expression and activation of inflammatory signaling pathways.[98] In vitro studies where anti-ST2 antibodies or recombinant IL-33 were added to mixtures containing human proximal epithelial cells, and sera from patients diagnosed with kidney rejection showed a decreased inflammatory response, thus supporting a potential therapeutic function for anti-ST2.[99] Another study conducted by the same group showed that IL-33 staining was positive in the renal tubules of patients with CAD, and this was associated with epithelial to mesenchymal transition (EMT).[100] EMT is suggested to be responsible for renal fibrosis posttransplant, which is mediated by myofibroblasts derived from the tubular epithelia.[101] Significantly, EMT was inhibited by antagonizing the p38/MAPK pathway.[100] Because renal fibrosis is a leading cause of allograft failure, efforts are being made to optimize diagnostic and therapeutic tools to limit and reverse kidney fibrosis posttransplantation.[102] Thus, as opposed to its cardioprotective role in heart transplantation, a beneficial reparative or regulatory role of IL-33 in kidney transplantation is not as clear. As such, efforts to harness the reparative or regulatory functions of IL-33 to help these recipients may be complicated.

Human liver transplant samples also show increased IL-33 release early during ischemic injury. High IL-33 levels peak after reperfusion, which correlates with graft dysfunction, renal failure, and postreperfusion syndrome.[103] DAMPS released during IRI can be pro-inflammatory, or reparative, depending on the receptors and signaling pathways they activate.[104] Rodent liver IRI causes the release of IL-33 by liver sinusoidal endothelial cells and aids the formation of neutrophil extracellular traps and activation of the inflammatory cascade.[105] In a mouse model of warm hepatic IRI, IL-33 was found to be immediately released from hepatic endothelial cells and was involved in tissue injury through the recruitment of neutrophils.[103] Blockage of ST2 at the time of reperfusion leads to an increase in neutrophil recruitment to the liver and subsequent tissue injury. In contrast, recombinant IL-33 injection before ischemia is associated with reduced liver injury and neutrophil accumulation.[106] However, IL-33 did not affect inflammatory cytokine production in this study. Hence, the authors concluded that the IL-33/ST2 axis is hepatoprotective through direct actions on hepatocytes that activate NF-κB and increase the expression of Bcl-2.[106] Yet iNKT cells play a role in liver IRI, and IL-33 may promote their pro-inflammatory function as identified in the described kidney model.[107] However, a recent study has suggested that IL-33 stimulation of eosinophils can be hepatoprotective and promote healthy liver function after IRI by stimulating IL-13 production.[108] The functions of IL-33 after liver transplantation seem to be predominantly pro-inflammatory. Yet, IRI studies suggest that potent reparative pathways involving eosinophils may be targetable if done precisely to avoid stimulating pro-inflammatory cells such as neutrophils and iNKT cells.

Given the ample expression of IL-33 in the lung, it is surprising how little information exists regarding the impact of IL-33 on lung transplantation outcomes. IL-33 is induced by the first breaths of life in type II epithelial cells (AEC2s) to generate a type 2 environment rich in ILC2s, eosinophils, basophils, and mast cells secreting IL-5, IL-9, and IL-13.[39] These type 2 immune cells support a large group of alternatively activated macrophages that support tissue regeneration and repair, thus protecting against a loss of function during infection.[109] However, a recent study by Guo et al found that the IRI upregulated IL-33, which promoted IL-5 production by graft-resident ILC2s to increase local eosinophils supporting tolerance after lung transplant.[109] Interestingly, this IL-33/IL-5/eosinophil-mediated mechanism relied on immunosuppression to preserve donor ILC2s. These exciting data support the concept that IRI not only triggers pro-inflammatory pathways but also initiates reparative and regenerative mechanisms.

Several recent studies have suggested that the regulatory and reparative properties of IL-33 may be exploited to improve outcomes after vascular composite allograft (VCA) transplantation. In a mouse skin transplantation model, IL-33-expanded Treg were able to prolong allograft survival. Moreover, enhanced Treg infiltration was shown in the IL-33-treated Treg group potentially due to the upregulation of chemokine receptors necessary for graft-homing (eg, *CCR2*, *CCR4* genes) and downregulation of others needed for lymph node homing (eg, CCR7).[110] Studying Treg migration is complex, yet might prove beneficial to fully understand their function. For example, migration to the graft is essential to promote repair and prevent rejection, but egression from the graft into the draining lymph nodes might be needed for prolonged allograft survival, through suppression of conventional T cells.[111,112] Moreover, attempts have been made to engineer ST2$^+$ human Treg for potential use in cell therapy. These IL-33 expanded ST2$^+$ Treg exhibited enhanced proliferation, production of Areg, and downregulation of lymph-node homing genes, an observation that matches their preferential tissue-protective function.[113] In a different study, daily IL-33 injection after skin transplantation increased regulatory myeloid cells and Treg, which was associated with improved graft survival.[114] In a hindlimb

ischemia model, IL-33 administration facilitated blood flow restoration, whereas ST2-deficient mice had impeded tissue repair.[115] Additionally, IL-33 stimulated revascularization and the pro-angiogenic functions of endothelial cells. Together, these studies suggest that delivering IL-33 or IL-33-expanded Treg may be a particularly effective way to support VCA survival and function early after transplantation.

CONCLUSIONS

It has become evident that the IL-33/ST2 axis has essential roles in the initial establishment of homeostasis and its restoration after injury. Attempts to define how these roles shape short- and long-term outcomes after transplantation are revealing distinct organ-specific differences due to yet unclear mechanisms. Fairly robust evidence suggests that early after heart and lung transplantation, IL-33 supports positive effects through actions on Treg, ILC2s, and macrophages. Delivery of local IL-33 via perfusion, hydrogels (as in[116]), or other controlled delivery mechanisms should be continued to be developed to support IRI resolution, tissue repair, and regeneration, as well as the regulation of early alloimmunity. In the case of liver and kidney transplantation, where the presence of IL-33 predominantly supports early inflammation, cell therapy approaches using IL-33-expanded and activated Treg or ILCs may be required to avoid detrimental IL-33 stimulated responses by neutrophils or iNKTs. In total, IL-33 represents one of the first DAMPs to exhibit potent reparative capabilities and is central to homeostasis at both the local and systemic levels. Like all potent immunomodulatory molecules, there are detrimental effects of having too little, too much, or persistent IL-33 available. However, new rodent tools allowing cell-specific deletion of IL-33 or ST2 at different points after IRI or transplantation will allow us to pinpoint the times and cell types to target to amplify IL-33 reparative properties effectively. Such studies will also elucidate when targeting IL-33 or IL-33 responsive cells may be necessary to avoid IL-33-mediated inflammation or dysregulated repair leading to fibrosis.

REFERENCES

1. Cayrol C, Girard JP. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. *Immunol Rev.* 2018;281:154–168.
2. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* 2016;16:676–689.
3. Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol.* 2016;17:122–131.
4. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479–490.
5. Dwyer GK, D'Cruz LM, Turnquist HR. Emerging functions of IL-33 in homeostasis and immunity. *Annu Rev Immunol.* 2022;40:15-43.
6. Lefrancais E, Duval A, Mirey E, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc Natl Acad Sci USA.* 2014;111:15502–15507.
7. Cayrol C. IL-33, an alarmin of the IL-1 family involved in allergic and non allergic inflammation: focus on the mechanisms of regulation of its activity. *Cells* 2021;11:107.
8. Reichenbach DK, Schwarze V, Matta BM, et al. The IL-33/ST2 axis augments effector T-cell responses during acute GVHD. *Blood* 2015;125:3183–3192.
9. Savinko T, Matikainen S, Saarialho-Kere U, et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J Invest Dermatol.* 2012;132:1392–1400.
10. Toyama S, Moniaga CS, Nakae S, et al. Regulatory T cells exhibit interleukin-33-dependent migratory behavior during skin barrier disruption. *Int J Mol Sci.* 2021;22:7443.
11. Sponheim J, Pollheimer J, Olsen T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am J Pathol.* 2010;177:2804–2815.
12. Dwyer GK, Turnquist HR. Untangling local pro-inflammatory, reparative, and regulatory damage-associated molecular-patterns (DAMPs) pathways to improve transplant outcomes. *Front Immunol.* 2021;12:611910.
13. Hung LY, Tanaka Y, Herbine K, et al. Cellular context of IL-33 expression dictates impact on anti-helminth immunity. *Sci Immunol.* 2020;5:eabc6259.
14. Katz-Kiriakos E, Steinberg DF, Kluender CE, et al. Epithelial IL-33 appropriates exosome trafficking for secretion in chronic airway disease. *JCI Insight* 2021;6:e136166.
15. Momota M, Nagayama M, Okude H, et al. The Ca(2+)-dependent pathway contributes to changes in the subcellular localization and extracellular release of interleukin-33. *Biochem Biophys Res Commun.* 2020;530:699–705.
16. Gunther S, Deredge D, Bowers AL, et al. IL-1 family cytokines use distinct molecular mechanisms to signal through their shared co-receptor. *Immunity* 2017;47:510–523.e4.
17. Martin MU. Special aspects of interleukin-33 and the IL-33 receptor complex. *Semin Immunol.* 2013;25:449–457.
18. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity* 2015;42:1005–1019.
19. Burzyn D, Kuswanto W, Kolodin D, et al. A special population of regulatory T cells potentiates muscle repair. *Cell* 2013;155:1282–1295.
20. Liu Q, Dwyer GK, Zhao Y, et al. IL-33-mediated IL-13 secretion by ST2+ Tregs controls inflammation after lung injury. *JCI Insight* 2019;4:e123919.
21. Schiering C, Krausgruber T, Chomka A, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* 2014;513:564–568.
22. Oboki K, Ohno T, Kajiwara N, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci USA.* 2010;107:18581–18586.
23. Dagher R, Copenhaver AM, Besnard V, et al. IL-33-ST2 axis regulates myeloid cell differentiation and activation enabling effective club cell regeneration. *Nat Commun.* 2020;11:4786.
24. Li T, Zhang Z, Bartolacci JG, et al. Graft IL-33 regulates infiltrating macrophages to protect against chronic rejection. *J Clin Invest.* 2020;130:5397–5412.
25. Matta BM, Reichenbach DK, Zhang X, et al. Peri-alloHCT IL-33 administration expands recipient T-regulatory cells that protect mice against acute GVHD. *Blood* 2016;128:427–439.
26. Dwyer GK, Mathews LR, Villegas JA, et al. IL-33 acts as a costimulatory signal to generate alloreactive Th1 cells in graft-versus-host disease. *J Clin Invest.* 2022;132.
27. McLaren JE, Clement M, Marsden M, et al. IL-33 augments virus-specific memory T cell inflation and potentiates the efficacy of an attenuated cytomegalovirus-based vaccine. *J Immunol.* 2019;202:943–955.
28. Afferni C, Buccione C, Andreone S, et al. The pleiotropic immunomodulatory functions of IL-33 and its implications in tumor immunity. *Front Immunol.* 2018;9:2601.
29. Bessa J, Meyer CA, de Vera Mudry MC, et al. Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. *J Autoimmun.* 2014;55:33–41.
30. Cohen ES, Scott IC, Majithiya JB, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun.* 2015;6:8327.
31. Hayakawa H, Hayakawa M, Kume A, et al. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem.* 2007;282:26369–26380.
32. Lipsky BP, Toy DY, Swart DA, et al. Deletion of the ST2 proximal promoter disrupts fibroblast-specific expression but does not reduce the amount of soluble ST2 in circulation. *Eur J Immunol.* 2012;42:1863–1869.
33. Billman GE. Homeostasis: the underappreciated and far too often ignored central organizing principle of physiology. *Front Physiol.* 2020;11:200.
34. Guabiraba R, Besnard AG, Menezes GB, et al. IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice. *Mucosal Immunol* 2014;7:1079–1093.
35. Mahapatro M, Foersch S, Hefele M, et al. Programming of intestinal epithelial differentiation by IL-33 derived from pericryptal fibroblasts in response to systemic infection. *Cell Rep* 2016;15:1743–1756.

36. Chen Z, Luo J, Li J, et al. Interleukin-33 promotes serotonin release from enterochromaffin cells for intestinal homeostasis. *Immunity* 2021;54:151–163.e6.
37. de Kleer IM, Kool M, de Bruijn MJ, et al. Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung. *Immunity* 2016;45:1285–1298.
38. Monticelli LA, Sonnenberg GF, Abt MC, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol.* 2011;12:1045–1054.
39. Saluzzo S, Gorki AD, Rana BMJ, et al. First-breath-induced type 2 pathways shape the lung immune environment. *Cell Rep* 2017;18:1893–1905.
40. Li C, Spallanzani RG, Mathis D. Visceral adipose tissue Tregs and the cells that nurture them. *Immunol Rev.* 2020;295:114–125.
41. Delacher M, Imbusch CD, Hotz-Wagenblatt A, et al. Precursors for nonlymphoid-tissue Treg cells reside in secondary lymphoid organs and are programmed by the transcription factor BATF. *Immunity* 2020;52:295–312.e11.
42. Vasanthakumar A, Moro K, Xin A, et al. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. *Nat Immunol.* 2015;16:276–285.
43. Fali T, Aychek T, Ferhat M, et al. Metabolic regulation by PPAR γ is required for IL-33-mediated activation of ILC2s in lung and adipose tissue. *Mucosal Immunol* 2021;14:585–593.
44. Molofsky AB, Nussbaum JC, Liang HE, et al. Innate lymphoid type 2 cells sustain isceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med.* 2013;210:535–549.
45. de Oliveira MFA, Talvani A, Rocha-Vieira E. IL-33 in obesity: where do we go from here? *Inflamm Res.* 2019;68:185–194.
46. Lee MW, Odegaard JI, Mukundan L, et al. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* 2015;160:74–87.
47. Brestoff JR, Kim BS, Saenz SA, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 2015;519:242–246.
48. Okamura T, Hashimoto Y, Mori J, et al. ILC2s improve glucose metabolism through the control of saturated fatty acid absorption within visceral fat. *Front Immunol.* 2021;12:669629.
49. Goldberg EL, Shchukina I, Youm YH, et al. IL-33 causes thermogenic failure in aging by expanding dysfunctional adipose ILC2. *Cell Metab.* 2021;33:2277–2287.e5.
50. Zhao XY, Zhou L, Chen Z, et al. The obesity-induced adipokine sST2 exacerbates adipose Treg and ILC2 depletion and promotes insulin resistance. *Sci Adv.* 2020;6:eaay6191.
51. Fock V, Mairhofer M, Otti GR, et al. Macrophage-derived IL-33 is a critical factor for placental growth. *J Immunol.* 2013;191:3734–3743.
52. Topping V, Romero R, Than NG, et al. Interleukin-33 in the human placenta. *J Matern Fetal Neonatal Med.* 2013;26:327–338.
53. Chen H, Zhou X, Han TL, et al. Decreased IL-33 production contributes to trophoblast cell dysfunction in pregnancies with preeclampsia. *Mediators Inflamm.* 2018;2018:9787239.
54. Hu WT, Huang LL, Li MQ, et al. Decidual stromal cell-derived IL-33 contributes to Th2 bias and inhibits decidual NK cell cytotoxicity through NF-κB signaling in human early pregnancy. *J Reprod Immunol.* 2015;109:52–65.
55. Vacca P, Cantoni C, Vitale M, et al. Crosstalk between decidual NK and CD14+ myelomonocytic cells results in induction of Tregs and immunosuppression. *Proc Natl Acad Sci USA.* 2010;107:11918–11923.
56. Sharma A, Rudra D. Emerging functions of regulatory T cells in tissue homeostasis. *Front Immunol.* 2018;9:883.
57. Samstein RM, Josefowicz SZ, Arvey A, et al. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* 2012;150:29–38.
58. Bartemes K, Chen CC, Iijima K, et al. IL-33-responsive group 2 innate lymphoid cells are regulated by female sex hormones in the uterus. *J Immunol.* 2018;200:229–236.
59. Kozai K, Iqbal K, Moreno-Irusta A, et al. Protective role of IL33 signaling in negative pregnancy outcomes associated with lipopolysaccharide exposure. *FASEB J.* 2021;35:e21272.
60. Valeff N, Juriol L, Quadrana F, et al. Expression of IL-33 receptor Is Significantly Up-Regulated In B cells during pregnancy and in the acute phase of preterm birth in mice. *Front Immunol.* 2020;11:446.
61. Huang B, Faucette AN, Pawlitz MD, et al. Interleukin-33-induced expression of PIBF1 by decidual B cells protects against preterm labor. *Nat Med.* 2017;23:128–135.
62. Bhat M, Pasini E, Das A, et al. Diabetogenic effects of immunosuppression: An integrative analysis. *Transplantation* 2020;104:211–221.
63. Bayer ND, Cochetti PT, Anil Kumar MS, et al. Association of metabolic syndrome with development of new-onset diabetes after transplantation. *Transplantation* 2010;90:861–866.
64. Jim G. Castellanos, Randy S. LongmanThe balance of power: innate lymphoid cells in tissue inflammation and repair. *J Clin Invest*. 2019;129:2640–2650.
65. Rak GD, Osborne LC, Siracusa MC, et al. IL-33-dependent group 2 innate lymphoid cells promote cutaneous wound healing. *J Invest Dermatol.* 2016;136:487–496.
66. Lucas T, Waisman A, Ranjan R, et al. Differential roles of macrophages in diverse phases of skin repair. *J Immunol.* 2010;184:3964–3977.
67. Yin H, Li X, Hu S, et al. IL-33 accelerates cutaneous wound healing involved in upregulation of alternatively activated macrophages. *Mol Immunol.* 2013;56:347–353.
68. Lee JS, Seppanen E, Patel J, et al. ST2 receptor invalidation maintains wound inflammation, delays healing and increases fibrosis. *Exp Dermatol.* 2016;25:71–74.
69. Dalessandri T, Crawford G, Hayes M, et al. IL-13 from intraepithelial lymphocytes regulates tissue homeostasis and protects against carcinogenesis in the skin. *Nat Commun.* 2016;7:12080.
70. Wulff BC, Pappa NK, Wilgus TA. Interleukin-33 encourages scar formation in murine fetal skin wounds. *Wound Repair Regen.* 2019;27:19–28.
71. Rankin AL, Mumm JB, Murphy E, et al. IL-33 induces IL-13-dependent cutaneous fibrosis. *J Immunol.* 2010;184:1526–1535.
72. Kuswanto W, Burzyn D, Panduro M, et al. Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells. *Immunity* 2016;44:355–367.
73. Tidball JG. Regulation of muscle growth and regeneration by the immune system. *Nat Rev Immunol.* 2017;17:165–178.
74. Heredia JE, Mukundan L, Chen FM, et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* 2013;153:376–388.
75. Grobeta P, Doser K, Falk W, et al. IL-33 attenuates development and perpetuation of chronic intestinal inflammation. *Inflamm Bowel Dis.* 2012;18:1900–1909.
76. Duan L, Chen J, Zhang H, et al. Interleukin-33 ameliorates experimental colitis through promoting Th2/Foxp3(+) regulatory T-cell responses in mice. *Mol Med.* 2012;18:753–761.
77. Zhu J, Wang Y, Yang F, et al. IL-33 alleviates DSS-induced chronic colitis in C57BL/6 mice colon lamina propria by suppressing Th17 cell response as well as Th1 cell response. *Int Immunopharmacol.* 2015;29:846–853.
78. Monticelli LA, Osborne LC, Noti M, et al. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci USA.* 2015;112:10762–10767.
79. Zhu J, Yang F, Sang L, et al. IL-33 aggravates DSS-induced acute colitis in mouse colon lamina propria by enhancing Th2 cell responses. *Mediators Inflamm.* 2015;2015:913041.
80. Buzzelli JN, Chalinor HV, Pavlic DI, et al. IL33 is a stomach alarmin that initiates a skewed Th2 response to injury and infection. *Cell Mol Gastroenterol Hepatol* 2015;1:203–221.e3.
81. Gonciarz W, Krupa A, Chmiela M. Proregenerative activity of IL-33 in gastric tissue cells undergoing helicobacter pylori-induced apoptosis. *Int J Mol Sci.* 2020;21:1801.
82. Turnquist HR, Zhao Z, Rosborough BR, et al. IL-33 expands suppressive CD11b+ Gr-1(int) and regulatory T cells, including ST2L+ Foxp3+ cells, and mediates regulatory T cell-dependent promotion of cardiac allograft survival. *J Immunol.* 2011;187:4598–4610.
83. Yin H, Li XY, Jin XB, et al. IL-33 prolongs murine cardiac allograft survival through induction of TH2-type immune deviation. *Transplantation* 2010;89:1189–1197.
84. Brunner SM, Schiechl G, Falk W, et al. Interleukin-33 prolongs allograft survival during chronic cardiac rejection. *Transpl Int.* 2011;24:1027–1039.
85. Rui T, Zhang J, Xu X, et al. Reduction in IL-33 expression exaggerates ischaemia/reperfusion-induced myocardial injury in mice with diabetes mellitus. *Cardiovasc Res.* 2012;94:370–378.
86. Ruisong M, Xiaorong H, Gangying H, et al. The protective role of interleukin-33 in myocardial ischemia and reperfusion is associated with decreased HMGB1 expression and up-regulation of the P38 MAPK signaling pathway. *PLoS One.* 2015;10:e0143064e0143064.
87. Mathews LR, Lott JM, Isse K, et al. Elevated ST2 distinguishes incidences of pediatric heart and small bowel transplant rejection. *Am J Transplant.* 2016;16:938–950.

88. Pascual-Figal DA, Garrido IP, Blanco R, et al. Soluble ST2 is a marker for acute cardiac allograft rejection. *Ann Thorac Surg.* 2011;92:2118–2124.
89. Grupper A, AbouEzzeddine OF, Maleszewski JJ, et al. Elevated ST2 levels are associated with antibody-mediated rejection in heart transplant recipients. *Clin Transplant.* 2018;32:e13349.
90. Hill GR, Koyama M. Cytokines and costimulation in acute graft-versus-host disease. *Blood* 2020;136:418–428.
91. Thierry A, Giraud S, Robin A, et al. The alarmin concept applied to human renal transplantation: evidence for a differential implication of HMGB1 and IL-33. *PLoS One.* 2014;9:e88742.
92. Zhang J, Wang Z, Xu Z, et al. The potential role of IL-33 in renal transplant recipients with chronic allograft dysfunction. *Ann Transplant.* 2016;21:611–618.
93. Cao Q, Wang Y, Niu Z, et al. Potentiating tissue-resident type 2 innate lymphoid cells by IL-33 to prevent renal ischemia-reperfusion injury. *J Am Soc Nephrol.* 2018;29:961–976.
94. Bourgeois E, Van LP, Samson M, et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol.* 2009;39:1046–1055.
95. Li L, Huang L, Sung SS, et al. NKT cell activation mediates neutrophil IFN-γ production and renal ischemia-reperfusion injury. *J Immunol.* 2007;178:5899–5911.
96. Charmetant X, Bachelet T, Dechanet-Merville J, et al. Innate (and innate-like) lymphoid cells: emerging immune subsets with multiple roles along transplant life. *Transplantation* 2021;105:e322–e336.
97. Ferhat M, Robin A, Giraud S, et al. Endogenous IL-33 contributes to kidney ischemia-reperfusion injury as an alarmin. *J Am Soc Nephrol.* 2018;29:1272–1288.
98. DeWolf SE, Kasimsetty SG, Hawkes AA, et al. DAMPs released from injured renal tubular epithelial cells activate innate immune signals in healthy renal tubular epithelial cells. *Transplantation* 2022;106:1589–1599.
99. Yu MY, Kwon S, Moon JJ, et al. Role of the IL-33/ST2 pathway in renal allograft rejection. *Exp Cell Res.* 2021;405:112705.
100. Xu Z, Zhao C, Wang Z, et al. Interleukin-33 levels are elevated in chronic allograft dysfunction of kidney transplant recipients and promotes epithelial to mesenchymal transition of human kidney (HK-2) cells. *Gene* 2018;644:113–121.
101. Stone RC, Pastar I, Ojeh N, et al. Epithelial-mesenchymal transition in tissue repair and fibrosis. *Cell Tissue Res.* 2016;365:495–506.
102. Saritas T, Kramann R. Kidney allograft fibrosis: diagnostic and therapeutic strategies. *Transplantation* 2021;105:e114–e130.
103. Barbier L, Robin A, Sindayigaya R, et al. Endogenous interleukin-33 acts as an alarmin in liver ischemia-reperfusion and is associated with injury after human liver transplantation. *Front Immunol.* 2021;12:744927.
104. Lu L, Zhou H, Ni M, et al. Innate immune regulations and liver ischemia-reperfusion injury. *Transplantation* 2016;100:2601–2610.
105. Yazdani HO, Chen HW, Tohme S, et al. IL-33 exacerbates liver sterile inflammation by amplifying neutrophil extracellular trap formation. *J Hepatol.* 2017;68:130-139.
106. Sakai N, Van Sweringen HL, Quillin RC, et al. Interleukin-33 is hepatoprotective during liver ischemia/reperfusion in mice. *Hepatology* 2012;56:1468–1478.
107. Ferhat MH, Robin A, Barbier L, et al. The impact of invariant NKT cells in sterile inflammation: The possible contribution of the alarmin/cytokine IL-33. *Front Immunol.* 2018;9:2308.
108. Wang Y, Yang Y, Wang M, et al. Eosinophils attenuate hepatic ischemia-reperfusion injury in mice through ST2-dependent IL-13 production. *Sci Transl Med.* 2021;13:eabb6576.
109. Guo Y, Mei Z, Li D, et al. Ischemia reperfusion injury facilitates lung allograft acceptance through IL-33-mediated activation of donor-derived IL-5 producing group 2 innate lymphoid cells. *Am J Transplant.* 2022;22:1963-1975.
110. Kawai K, Uchiyama M, Hester J, et al. IL-33 drives the production of mouse regulatory T cells with enhanced in vivo suppressive activity in skin transplantation. *Am J Transplant.* 2021;21:978–992.
111. Xiong Y, Ahmad S, Iwami D, et al. T-bet regulates natural regulatory T cell afferent lymphatic migration and suppressive function. *J Immunol.* 2016;196:2526–2540.
112. Lam AJ, Hoeppli RE, Levings MK. Harnessing advances in T regulatory cell biology for cellular therapy in transplantation. *Transplantation* 2017;101:2277–2287.
113. Lam AJ, MacDonald KN, Pesenacker AM, et al; iGenoMed Consortium. Innate control of tissue-reparative human regulatory T cells. *J Immunol.* 2019;202:2195–2209.
114. Gajardo T, Morales RA, Campos-Mora M, et al. Exogenous interleukin-33 targets myeloid-derived suppressor cells and generates periphery-induced Foxp3(+) regulatory T cells in skin-transplanted mice. *Immunology* 2015;146:81–88.
115. Dong Q, Tian J, Zheng W, et al. Interleukin-33 protects mice against hindlimb ischemic injury by enhancing endothelial angiogenesis. *Int Immunopharmacol.* 2022;109:108850.
116. Griffin DR, Archang MM, Kuan CH, et al. Activating an adaptive immune response from a hydrogel scaffold imparts regenerative wound healing. *Nat Mater.* 2021;20:560–569.