THE ROLE OF INNATE LYMPHOID CELLS IN THE HEART AND CARDIAC INFLAMMATION

by
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Abstract

Innate lymphoid cells (ILCs) are an innate immune cell population that is known to play an important role in infection and inflammation at diverse tissues. ILCs are recognized as an important source of type 2 cytokines and an innate counterpart of T effector cells. Over the past decade, ILCs have been shown to contribute to mounting immune responses against pathogens at mucosal barriers, regulating tissue inflammation, promoting tissue repair and remodeling and maintaining metabolic homeostasis. Although ILCs have been investigated intensively at many mucosal sites including the intestine and lungs, our understanding of the roles of ILCs in the heart is limited. Here we used an IL-33-induced pericarditis mouse model to determine the role of ILCs in the heart and the underlying mechanism on how ILCs contribute to cardiac inflammation. We identified an accumulation of group 2 innate lymphoid cells (ILC2s) in IL-33-induced pericarditis with a marked increase of eosinophils infiltrating to the heart. Using ST2-deficient and IL-33-deficient mice, we found that IL-33 signaling through its receptor ST2 is essential to induce the expansion of ILC2s and pericarditis. We showed the existence of IL-33 feedback loop containing cardiac fibroblasts as a main source of IL-33 in the heart where endogenous IL-33 expression is upregulated upon exogeneous IL-33 administration. *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were resistant to pericarditis, whereas *Rag2*$^{-/-}$ mice develop inflammation comparable to WT mice, suggesting that ILC2s, not T cells and B cells, are required for pericarditis development. ILC2s transferred to the heart of ILC-deficient *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice restored their susceptibility to eosinophil infiltration. Moreover, ILC2s directed cardiac fibroblasts to produce eotaxin-1 *in vitro* which might potentially promote eosinophil trafficking to the

heart. We also found that eosinophils reside in the mediastinal cavity of naïve and IL-33-treated mice, which implies a possibility that the mediastinal cavity might serve as a reservoir of eosinophils for non-vascular trafficking to the heart. Eosinophils transferred to the mediastinal cavity of eosinophil-deficient ΔdblGATA1 mice after IL-33 treatment migrated more effectively to the heart than intravenously transferred eosinophils. In conclusion, our results demonstrate a pathogenic role of ILC2s in driving pericarditis development with implications for therapeutic strategies.

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Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

A. Innate lymphoid cells

Innate lymphoid cells (ILCs) are innate immune cells that do not express antigen-specific receptors expressed by adaptive lymphocytes, T cells and B cells [1]. ILCs develop from common lymphoid progenitors (CLPs), the same progenitors as T cells and B cells do [2, 3]. Therefore, they are characterized by a lymphoid morphology and the absence of rearranged antigen-specific receptors [1-4]. The nomenclature proposed previously classified ILCs into three groups, ILC1s, ILC2s and ILC3s with subsets based on their function and transcription factor expression [1]. However, with improvement in understanding of the development of ILCs, classification of ILCs into five groups was recently proposed – natural killer (NK) cells, ILC1s, ILC2s, ILC3s and lymphoid tissue-inducer (LTi) cells – based on development [4]. ILC1s, ILC2s and ILC3s mirror $CD4^+$ T helper (Th)1, Th2 and Th17 cells, respectively, in terms of effector function, while NK cells mirror $CD8^+$ cytotoxic T cells [1]. LTi cells induce the development of secondary lymphoid organs during fetal development [5]. ILCs develop from common innate lymphoid progenitors (CILPs) that are derived from CLPs (***Figure 1***) [3, 6, 7].

In an early stage of ILC development, Id2 is required for the development of all ILCs and NK cells as shown in the study using Id2-deficient mice lack of ILCs and NK cells [8-12]. Depending on the expression of transcription factors during development, ILCs are differentiated into ILC1s, ILC2s and ILC3s [13]. Transcription factors that govern the differentiation of ILCs into their subsets are characterized by many studies (***Figure 1***). NK

cells and ILC1s are dependent on T-box transcription factor (T-bet) for their development [6]. Whereas ILC1s are strictly dependent on T-bet, NK cells are present in T-bet-deficient mice [14]. ILC2s rely on transcription factors including GATA3 and RORα [11, 15-18]. GATA3 is one of key transcription factors driving the development of all $CD127^+$ ILCs [19]. GATA3 is especially important for the maintenance and survival of ILC2s as the absence of GATA3 inhibits the development and function of ILC2s [11, 15, 16, 19]. ILC3s and LTi cells are dependent on the transcription factor RORγt [20, 21]. Although the development of ILC3s is dependent on RORγt in mice, IL-17-producing subset, not IL-22-producing subset, is absent in RORC-deficient patients [22].

ILC1s are characterized by interferon gamma (IFNγ) production and ILC2s produce Th2-associated cytokines, mainly IL-5 and IL-13 [6, 8, 23-26]. ILC3s comprise natural cytotoxicity receptor $(NCR)^+$ ILC3s and NCR^- ILC3s and produce IL-17 and/or IL-22 [21, 27-29]. Since ILCs produce cytokines as T cells do, they are regarded an innate counterpart of T effector cells. However, ILCs act early in immune response, whereas it takes several days for T cells to react and function as an effector because they have to clonally expand and develop antigen-specificity.

ILCs are found in both lymphoid organs and non-lymphoid organs. ILCs in non-lymphoid organs are derived from ILC progenitors recruited from the blood. ILCs possess slightly different phenotypes in different tissues in terms of marker expression despite redundancy in common markers. Most ILCs are tissue-resident cells and maintain their tissue residency in homeostasis and during acute inflammation [30]. However, ILC3s can migrate from the lamina propria of the intestine to the draining mesenteric lymph nodes and NK cells and inflammatory ILC2s are found in the circulation [30, 31].

ILC subsets are important for immunity against diverse infections. ILC1s are critical for defense against viruses, certain bacteria such as enteric bacteria, *Clostridium difficile*, and intracellular parasites such as *Toxoplasma gondii* [6, 32]. ILC2s are known to be involved in innate immunity against parasites such as *Nippostrongylus brasiliensis* [8, 25]. ILC3s are involved in the innate immune response to extracellular bacteria [21, 29]. ILCs can also play critical roles in regulation of inflammation at mucosal and barrier surfaces. Intra-epithelial ILC1s and ILC3s producing IFNγ induce inflammation in some mouse models of colitis [24, 29]. IFNγ-producing ILCs may be involved in inflammatory bowel disease such as Crohn's disease [23, 24]. IL-17-producing ILC3s have also been shown to play a role in inflammatory bowel disease in T-cell-independent models [29, 33]. ILC2s have been shown to play a detrimental role in various type 2 inflammatory disorders in animal models including allergic lung inflammation [34-36]. ILC2s are associated with asthma and chronic rhinosinusitis in humans [37, 38]. ILC2s might be also involved in the pathogenesis of atopic dermatitis [39, 40]. ILC3s producing IL-17 and IL-22 have been associated with the inflammatory skin disease psoriasis vulgaris [41, 42]. Besides functions as effectors in innate immune response, ILCs are also involved in tissue homeostasis including metabolism, regeneration and tissue repair in response to tissue damage resulting from infection and inflammation [9, 43-47].

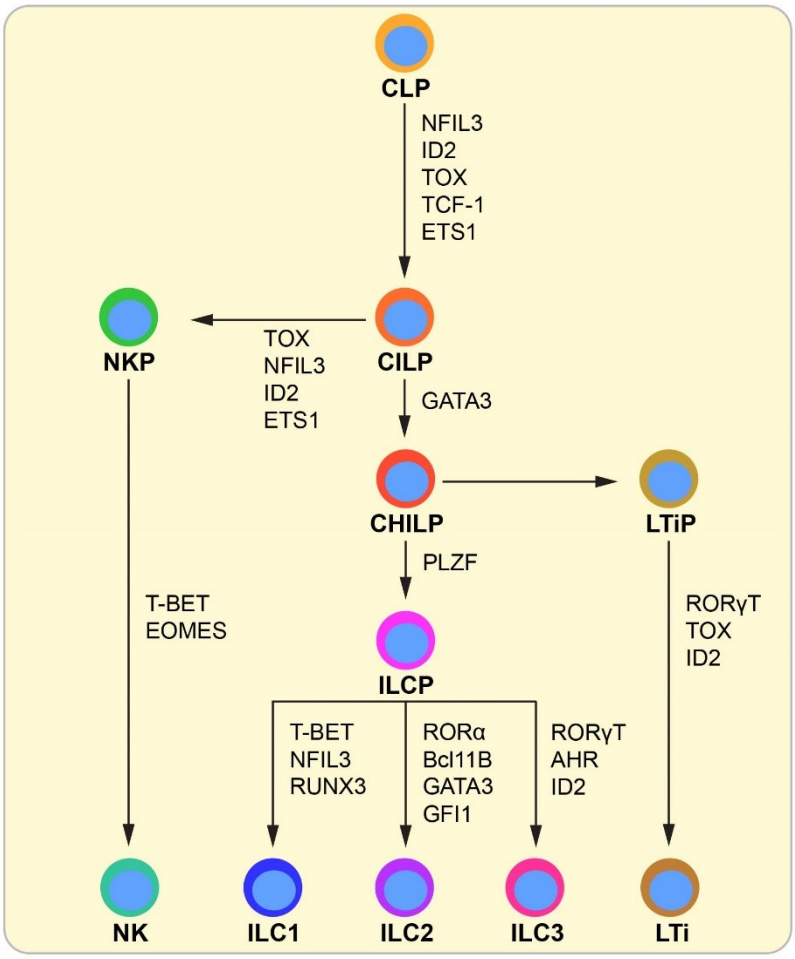

Figure 1. Development of ILCs.

Schematic description of ILC development is shown mainly based on findings from mice. This figure is adapted from [4].

Abbreviations: CILPs (common innate lymphoid progenitors), CLPs (common lymphoid progenitors), CHILPs (common helper innate lymphoid progenitors), LTiPs (lymphoid tissue inducer progenitors), ILCP (innate lymphoid cell precursors), NFIL3 (nuclear factor IL-3 induced), Id2 (inhibitor of DNA binding 2), TOX (thymocyte selection-associated high mobility group box protein), TCF-1 (T cell factor 1), ETS1 (avian erythroblastosis

virus E26 homolog-1), GATA3 (GATA binding protein 3), PLZF (promyelocytic leukemia zinc finger), T-bet (T-box transcription factor), Eomes (Eomesodermin), RUNX3 (runt-related transcription factor 3), RORα (RAR-related orphan receptor α), Bcl11b (B cell lymphoma/leukemia 11B), Gfi1 (growth factor independent 1), RORγt (RAR-related orphan receptor γt) and AhR (Aryl hydrocarbon receptor).

B. Interleukin-33 (IL-33)

IL-33 is a cytokine of IL-1 family which was originally identified as an inducer of type 2 immunity activating Th2 cells and mast cells [48]. Phylogenetic study shows that IL-33 protein in mammals is evolutionarily conserved and is closely related to IL-18 among the IL-1 family members [48]. IL-33 exerts cytokine activity by binding to a heterodimer receptor complex composed of its specific receptor ST2 and co-receptor IL-1 receptor accessary protein (IL-1RAcP) [48, 49]. IL-33 was first described as a nuclear protein known as nuclear factor from high endothelial venules (NF-HEV) because of its nuclear localization [50]. Following study showed that IL-33 is a chromatin-associated nuclear cytokine *in vivo* and that its nuclear domain within the N terminus is necessary and sufficient for nuclear localization and chromatin association [51].

IL-33 functions as an alarmin which is released from cells after cell injury to alert immune system during trauma or infection [52-55]. The role of IL-33 as an alarmin is further supported by evidence that it is constitutively expressed in normal human tissues, full length IL-33 is biologically active and it is released to the extracellular space after injury or necrotic cell death [54, 56, 57]. IL-33 is constitutively expressed in various types of

cells in humans and mice at the steady state [56, 58]. The major source of IL-33 includes endothelial cells, epithelial cells in barrier tissues and fibroblast-like cells such as fibroblastic reticular cells in lymphoid organs [56, 58, 59]. However, species-specific differences in cell types expressing IL-33 have been noted. In mice, IL-33 is not constitutively expressed along vascular tree, although it can be detected in some vascular beds [58, 60, 61]. In the lungs, IL-33 is expressed by the lung airway epithelial cells in humans, while it is expressed by alveolar type II pneumocytes in mice [53, 62, 63]. The expression of IL-33 can be further increased during inflammation, although it is already expressed in the steady state. In humans, IL-33 levels are increased in the airway epithelial cells from patients with chronic obstructive pulmonary disease (COPD) and in skin keratinocytes and blood vessels from patients with atopic dermatitis [53, 62, 64]. In mice, IL-33 expression is increased in alveolar type II pneumocytes after helminth infection, exposure to cigarette smoke or intranasal allergen challenge [53, 63, 65, 66]. Activated fibroblasts, fibroblast-like cells and myofibroblasts are also important sources of IL-33 in diseases related to tissue fibrosis and wound repair [67-69].

Regulation of IL-33 is critical because IL-33 has a profound function in pro-inflammatory responses. Full length IL-33 can be cleaved to shorter mature forms by inflammatory proteases from neutrophils and mast cells [70, 71]. Mature forms are 10- to 30-fold more potent than full length IL-33 in activating mast cells and ILC2s. There are several mechanisms restricting IL-33 activity. During apoptosis, processing of IL-33 by apoptotic caspases such as caspase 3 and caspase 7 could be important to suppress the potent pro-inflammatory effect of IL-33 [54, 57]. IL-33 contains a site for cleavage by apoptotic caspases which produce biologically inactive forms of IL-33 [54, 57]. A soluble form of

ST2 (sST2) has been shown to function as a decoy receptor in neutralizing IL-33 activity in serum [60, 72]. Following release, IL-33 can be inactivated rapidly in the extracellular space by oxidation of cysteine residues and the formation of disulfide bonds in the cytokine domain [73].

IL-33 binding to ST2 leads to IL-1RAcP recruitment and the formation of a signaling complex recruiting signaling adaptor molecules such as myeloid differentiation primary response protein 88 (MYD88), IL-1R-associated kinase 1 (IRAK1), IRAK4 and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) [48, 74]. The cluster of these signaling molecule complex activates mitogen-activated protein kinases (MAPKs) such as JNK, ERK and p38 and nuclear factor-κB (NF-κB), which drive the proliferation, survival, type 2-associated cytokine secretion and amphiregulin (AREG) expression by cells expressing ST2 [48, 74]. The IL-33/ST2 signaling pathway is depicted in ***Figure 2***.

Stimulation of lymphoid and myeloid cells by IL-33 results in their proliferation and survival and their production and secretion of type 2 cytokines such as IL-5 and IL-13 [75]. Th2 cells, regulatory T (Treg) cells and ILC2s are lymphoid cells known to express ST2. Th2 cells were the first cells shown to express ST2 and exert type 2 functions [76]. IL-33 induces the proliferation and AREG expression of ST2-expressing Treg cells [77, 78]. ST2-expressing ILC2s are also stimulated by IL-33 and produce IL-5 and IL-13 [8, 25, 26]. ILC2s activated by IL-33 enhance type 2 immune responses and help tissue repair through AREG production [8, 9, 25, 26]. Among myeloid cells, macrophages and dendritic cells (DCs) express ST2 [79-81]. DCs stimulated by IL-33 can mediate Th2 cell polarization and support Treg cell expansion through IL-33-induced secretion of IL-2 [79, 81, 82]. IL-2 that mast cells produce by IL-33 stimulation also mediates Treg cell expansion [83].

Collectively, IL-33 plays a key role in innate and adaptive immunity contributing to regulation of infection and inflammation.

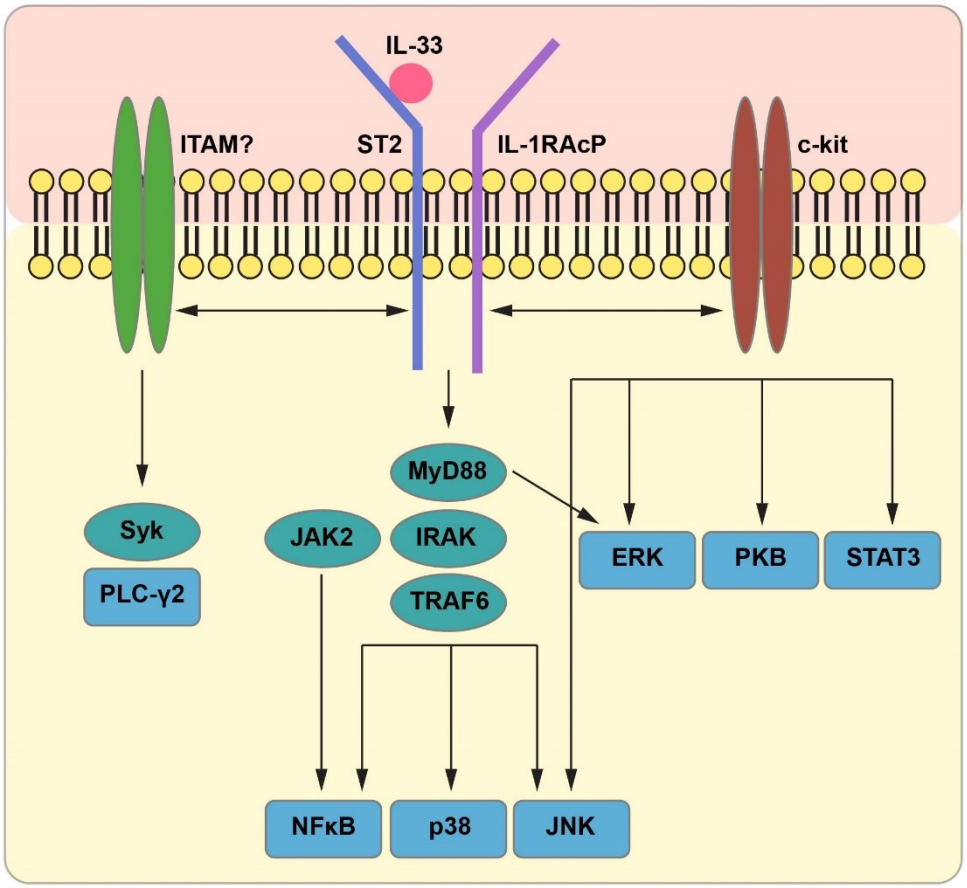

Figure 2. IL-33/ST2 signaling pathway.

IL-33 binds to ST2 and then IL-1RAcP which forms a receptor complex. IL-33 signaling through ST2 is dependent on Myd88 which activates intracellular signaling molecules in the downstream. This figure is adapted from [84].

Abbreviations: IL-1RAcP (IL-1 receptor accessory protein), IRAK (IL-1 receptor-associated kinase), ITAM (immunoreceptor tyrosine-based activation motif), JAK (Janus

kinase), NFκB (nuclear factor κB), PKB (protein kinase B), PLC (phospholipase C), STAT3 (signal transducer and activator of transcription 3), Syk (GRB2-associated-binding protein 2, GAB2), TRAF6 (tumor necrosis factor receptor-associated factor 6).

C. Pericarditis

Pericarditis is inflammation of the pericardium that is the most common form of pericardial disease [85-89]. Affected patients are usually young and middle-aged individuals and recurrences are frequently observed [85, 86]. Pericarditis represents 0.2% of all hospital cardiovascular admissions [87]. Approximately 5% of patients with non-ischemic chest pain are diagnosed with pericarditis in emergency departments in North America and Western Europe [88, 89].

The etiology of pericarditis could be an infectious or non-infectious cause (***Table 1***) [90-92]. Pericarditis may be a part of manifestations of systemic disease or a primary condition independent of systemic disease [93-95]. The exact etiology is difficult and challenging to determine because many mild cases resolve without a diagnosis and determination of etiology depends on its magnitude of investigation. In addition, epidemiological data are limited for pericarditis. However, it is important to diagnose and treat pericarditis because failure to treat pericarditis in a timely manner might prolong disease and increase recurrences [96]. In developing countries, tuberculosis is the most likely underlying disease accounting for about 70% of pericarditis diagnoses which is associated with a high mortality [93, 94]. Tuberculous pericarditis is less than 5% of all cases in developed countries, which is much less common compared with frequency observed in developing

countries [97-100]. In North America and Western Europe, about 80 to 90% of pericarditis cases are diagnosed as idiopathic and most of those cases are assumed to be viral [88, 89]. In a recent study including 933 hospitalized patients diagnosed with acute pericarditis, the etiologies of pericarditis were reported as a following order: idiopathic (55%), autoimmune or post-cardiac injury syndromes (24%), neoplastic (9%), bacterial (3.1%) and tuberculosis (0.5%) [100]. This study suggests that pericarditis in hospitalized patients are more complicated cases and reveal a higher risk of a non-idiopathic etiology. Furthermore, in developed countries, aging certainly contributes to the etiology of pericarditis with the use of cardiovascular intervention in the elderly population which possibly increases the risk of complications happening in the pericardium [96, 100].

Table 1. Etiology of pericarditis

Group	Frequency	Etiological agents
Infectious cause		
Virus	Common	Enteroviruses (coxsackieviruses and echoviruses), Herpesviruses (Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6)), Adenoviruses, Parvovirus B19 (possible overlap with viral etiologic agent of myocarditis)
Bacteria	Common or rare	*Mycobacterium tuberculosis* (common in developing countries), *Coxiella burnetii*, *Borrelia burgdorferi,* Rarely other microorganisms (*Pneumococcus, Meningococcus, Gonococcus, Streptococcus, Staphylococcus, Haemophilus, Chlamydia, Mycoplasma, Legionella, Leptospira, Listeria and Providencia stuartii*)
Fungi	Rare	*Histoplasma* species (more likely in immunocompetent patients) *Aspergillus*, *Blastomyces* and *Candida* species (more likely in immunocompromised host)
Parasite	Rare	*Echinococcus* and *Toxoplasma* species
Non-infectious cause		
Autoimmune and autoinflammatory	Common	Systemic autoimmune diseases (especially systemic lupus erythematosus, Sjogren syndrome, rheumatoid arthritis, scleroderma) Systemic vasculitides (e.g. eosinophilic granulomatosis with polyangiitis or allergic granulomatosis, previously named Churg-Strauss syndrome, Horton disease, Takayasu disease, Behcet syndrome) Autoinflammatory diseases (familial Mediterranean fever, tumor necrosis factor receptor–associated periodic syndrome, Still disease) Other (sarcoidosis, inflammatory bowel diseases)
Neoplastic	Common or rare	Primary tumors (rare; pericardial mesothelioma) Secondary metastatic tumors (common; lung and breast cancer, lymphoma)
Metabolic	Common	Uremia, myxedema, anorexia nervosa
Traumatic and iatrogenic	Common	Early onset: Direct injury (penetrating thoracic injury, esophageal perforation); indirect injury (nonpenetrating thoracic injury, radiation injury) Delayed onset: Pericardial injury syndromes (post–myocardial infarction syndrome, postpericardiotomy syndrome); posttraumatic, including after iatrogenic trauma (e.g, coronary percutaneous intervention, pacemaker lead insertion, and radiofrequency ablation)
Drug related	Rare	Lupus-like syndrome (procainamide, hydralazine, methyldopa, isoniazid, phenytoin) Antineoplastic drugs (often associated with cardiomyopathy and may cause pericardiopathy): doxorubicin, daunorubicin, cytosine arabinoside, 5-fluorouracil, cyclophosphamide Penicillins (as hypersensitivity pericarditis with eosinophilia)
Other	Common or rare	Common: amyloidosis, aortic dissection, pulmonary arterial hypertension and chronic heart failure Rare: congenital partial and complete absence of the pericardium

This table is based on [88, 90-92, 100].

Clinical diagnosis of pericarditis can be made based on diagnostic criteria (***Table 2***). Clinical presentation of patients with pericarditis is mostly chest pain. Additional signs and characteristics in acute pericarditis could include pericardial friction rubs, an electrocardiogram (ECG) with widespread ST-segment elevation and pericardial effusion [85, 86]. Aforementioned characteristic ECG is reported in less than 60% of patients with acute pericarditis and more commonly found in younger male patients especially associated with myocarditis [88, 101]. ECG can be affected by timing in the course of pericarditis; therefore, it should be carefully interpreted. About 60% of patients with acute pericarditis have a pericardial effusion, which is usually mild and can be found using echocardiography, and a large effusion is associated with an increased risk of complications [88, 97]. The absence of a pericardial effusion does not exclude pericarditis. The international guidelines on pericardial diseases were published first in 2004 and updated in 2015 by the European Society of Cardiology (ESC) [91, 92]. In addition to criteria specified above, a consensus expert statement on integrated cardiovascular imaging of pericardial diseases was proposed by the American Society of Echocardiography [102]. Specific clinical criteria for the diagnosis of acute and recurrent pericarditis are summarized in ***Table 2***. Pericardial fluid analysis could be used for diagnostic purpose of certain types of pericarditis but limited data are available and pericardiocentesis is usually performed only with some patients with acute pericarditis. In addition, biochemical and hematologic tests are not helpful for diagnosis of distinguishing among the different pericardial diseases due to a considerable overlap of test results among the different pericardial disorders [103].

Table 2. Diagnostic criteria for pericarditis

Pericarditis	Diagnostic criteria
Acute	At least 2 of the 4 following criteria are required for diagnosis: (1) Pericarditic chest pain (2) Pericardial friction rubs (3) Widespread ST-elevation or PR depression on ECG (4) Pericardial effusion (new or worsening)
Incessant	Pericarditis lasting for longer than 4-6 weeks but shorter than 3 months without remission
Recurrent	Recurrence of pericarditis after a documented first episode of acute pericarditis A symptom-free interval of 4-6 weeks or longer
Chronic	Pericarditis lasting for longer than 3 months
Acute or recurrent	Additional supporting findings - Elevation of inflammation markers (i.e. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell count) - Evidence of pericardial inflammation by an imaging technique (i.e. contrast-enhanced pericardium on computed tomography (CT) or pericardial edema and pericardial late gadolinium enhancement on cardiac magnetic resonance imaging (cMRI))

This table is based on [88, 92].

Treatments for pericarditis are determined depending on its type and etiology. Aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) including ibuprofen and indomethacin are the central therapy for acute and recurrent pericarditis with idiopathic or viral etiology. A clinical trial done in patients with postpericardiotomy syndrome showed that ibuprofen and indomethacin were effective in resolution of symptoms and both were significantly more effective than placebo [104]. Corticosteroids such as prednisone were the initial choice for treating pericarditis with pericardial effusions or recurrences which did not respond to aspirin or NSAIDs. However, in a non-randomized study which included 100 patients with recurrent pericarditis, this therapy was shown to be associated with more adverse effects, recurrences and hospitalizations when higher dose of prednisone was used compared to lower dose [105]. Use of colchicine in addition to NSAIDs was suggested to improve remission rates and recurrence rates in acute and recurrent pericarditis compared with NSAIDs only [85, 86]. In a meta-analysis including seven studies of therapy for pericarditis,

colchicine was associated with a reduced risk of treatment failure and recurrences [106]. The most common adverse effect associated with colchicine was gastrointestinal symptoms, especially diarrhea, which was reported in 8% of patients with colchicine treatment [86]. For patients with refractory recurrent pericarditis which is not responsive to any therapy, the last option is pericardiectomy. Although there are two studies which are limited by their retrospective design, the result of this surgery is controversial in terms of its efficacy [107, 108].

D. Pericarditis murine model

Currently, there is no murine model specific to pericarditis. However, it is critical to generate and use a mouse model of pericarditis to improve our understandings on the mechanism of how pericarditis develop and what immune cells are involved in the progression of inflammation. One study showed that BALB/c mice infected with coxsackievirus B3 (CVB3) with the treatment of recombinant mouse IL-33 intraperitoneally every other day from day 1 to day 9 post-infection increased CVB3-induced myocarditis and pericarditis compared to PBS-treated controls [109]. Histology and flow cytometry analysis indicated that IL-33 treatment increased the number of eosinophils in the heart of CVB3-induced myocarditis compared to PBS treatment. Recombinant ST2 treatment which block IL-33 did not lead to eosinophilia in both myocardium and pericardium of mice infected with CVB3 [109]. In this study, IL-33 treatment also induced pericarditis with increased eosinophils in uninfected mice which was similar in terms of phenotype to that observed in CVB3-induced myocarditis with IL-

33 treatment [109]. From these findings, we adapted a mouse model of IL-33-induced pericarditis and used in our studies. BALB/c mice are administered with 1 µg of recombinant mouse IL-33 intraperitoneally every other day for a total of five injections, and inflammation is examined via histology and flow cytometry (

Figure 3).

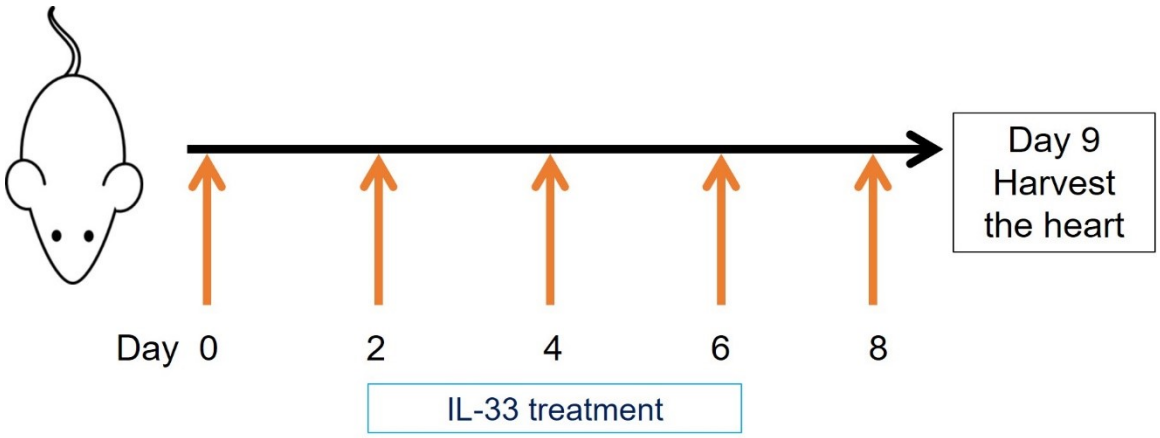

Figure 3. Murine model of pericarditis induced by IL-33.

BALB/c mice are injected with 1 µg of recombinant mouse IL-33 intraperitoneally every other day on days 0, 2, 4, 6 and 8 in 100 µl phosphate buffered saline (PBS). The inflammation is assessed at day 9 by harvesting the heart and using histology and flow cytometry.

Chapter 2. IL-33 in Inflammatory Diseases

A. IL-33 in cardiovascular diseases

A role for IL-33 in cardiovascular diseases was first considered to be protective in pressure overload mouse model [72]. In this study, IL-33 treatment reduced hypertrophy and fibrosis and improved survival after pressure overload performed by transverse aortic constriction (TAC) in mice [72]. IL-33 produced and secreted by endothelial cells in the setting of pressure overload is important for inducing a selective systemic inflammatory response [60]. IL-33 prevented cardiomyocyte apoptosis and improved cardiac function and survival after experimental myocardial infarction [110]. In atherosclerosis, high levels of IL-33 and ST2 were expressed by vascular endothelial and smooth muscle cells [111]. IL-33 treatment reduced atherosclerosis development in apolipoprotein E-deficient ($ApoE^{-/-}$) mice on a high-fat diet by induction of type 2 immune response switching from atherosclerotic type 1 immune response [111]. While the role for IL-33 in the cardiovascular diseases listed above is beneficial or protective, it can be pathogenic in a certain setting of cardiac inflammation. IL-33 treatment promoted eosinophilic pericarditis in CVB3-induced viral myocarditis and sST2 treatment improved systolic functions of the heart [109]. A component of IL-33 signaling, ST2, was proposed as a prognostic marker for acute myocardial infarction [112]. Increased levels of serum sST2 happened after myocardial infarction and sST2 concentrations in serum were correlated with impaired left ventricular function and poor prognosis [112]. In addition, elevated sera sST2 was associated with an increased risk of heart failure in male patients with myocarditis who were not order than age 50 years [113]. IL-33/ST2 axis has not progressed to be used as

therapeutic targets of cardiovascular diseases except for the use of sST2 as a biomarker, however, it should be carefully assessed to use IL-33 as a therapeutic since it could induce activation of immune system leading to adverse effects.

B. IL-33 in asthma and allergic inflammation

Many studies have shown the importance of IL-33 in airway Th2 inflammatory diseases such as asthma and allergic rhinitis. IL-33 expression levels in bronchial tissue correlate with asthma severity in humans [114]. *IL33* genetic variants are implicated in the risk of asthma and the susceptibility to allergic rhinitis [115, 116]. In mice, IL-33 treatment induced airway inflammation through ILC2 activation resulting in type 2 cytokine production, eosinophil infiltration to the lungs and M2 macrophage polarization [35, 48, 66, 117]. IL-33 was shown to play a crucial role in mediating allergic rhinitis in experimental murine models with allergen challenge [118, 119]. Atopic dermatitis is an allergic inflammatory disease in the skin that shares the similar role for IL-33 in the pathogenesis to one shown in lung airway inflammation. Increased levels of IL-33 were reported in the skin of patients with atopic dermatitis [39, 64]. Transgenic mice expressing IL-33 in keratinocytes developed spontaneous dermatitis with the activation of ILC2s [40]. Blocking IL-33 using anti-IL33 antibody was effective to ameliorate inflammation in experimental allergic rhinitis and asthma [120, 121]. Therefore, IL-33 induce type 2 cytokine production and pathology at barrier tissues such as the lungs and skin and targeting IL-33 in asthma and allergic disorders might be a good therapeutic option.

C. IL-33 in rheumatoid arthritis

IL-33 is detected in the synovium of patients with rheumatoid arthritis (RA) [51, 122]. Higher IL-33 levels in synovial fluid and serum were correlated with more severe disease [122]. Single-nucleotide polymorphism (SNP) of the *IL33* gene was associated with lower serum levels of IL-33 and a decreased susceptibility to RA [123]. In collagen-induced arthritis (CIA) mouse model, IL-33 treatment exacerbated joint inflammation in a mast cell-dependent manner [124]. Exacerbation of inflammation was accompanied by increased expression levels of proinflammatory cytokines such as IFNγ, TNFα and IL-17 [124]. Blocking IL-33 signaling through ST2 by using sST2, knockout of ST2 or anti-ST2 antibody, decreased the severity of joint inflammation in mouse models of RA [124-126]. Therefore, targeting IL-33 is a potential therapeutic option for RA and it might be a good approach in targeting chronic RA by inhibiting the release of proinflammatory cytokines. However, efficacy of IL-33 neutralization needs to be evaluated and blocking IL-33 need to compete against current treatments for RA which are successful.

D. IL-33 in gastrointestinal diseases

IL-33/ST2 signaling axis is important for maintaining the integrity of epithelial cell layers in the gastrointestinal tract [127]. Esophageal expression of IL-33 was higher in patients with active eosinophilic esophagitis (EoE) compared to control individuals [128]. Increased expression of IL-33 was associated with the development of pediatric EoE [129]. In addition, IL-33 treatment induced transmural inflammation and mucosal

hyperproliferation in the esophagus in mice [129]. IL-33 is highly expressed in the mucosa of intestine which suggest its important role in inflammatory bowel disease (IBD). Mucosal IL-33 levels were increased in Crohn disease and ulcerative colitis which was correlated with the severity of disease [130, 131]. This suggests the use of IL-33 as a biomarker of IBD. However, similar to its role in the cardiovascular diseases, IL-33 can play both beneficial and pathogenic roles in gastrointestinal inflammatory diseases [127]. In a dextran sodium sulfate (DSS)-induced colitis model, IL-33$^{-/-}$ mice showed decreased inflammation and mortality but delayed recovery at later timepoint [132]. Similarly, ST2$^{-/-}$ mice were resistant to DSS-induced colitis due to the protection granted from absence of ST2 in non-hematopoietic cells [130]. The absence of ST2 also enhanced wound healing in the colon [130]. On the other hand, IL-33 administration can ameliorate trinitrobenzene sulfonic acid (TNBS)-induced colitis and T cell transfer-induced colitis [133, 134]. IL-33 induced the expansion of gut-associated Treg cells which overcome the effect of IL-23, a proinflammatory cytokine in IBD which usually restrains Treg responses [134]. IL-33 can also protect and restore intestinal tissue homeostasis by activating ILC2s to produce AREG [135]. These controversial effect of IL-33 could be due to different experimental murine models of colitis or the timing and dose of IL-33. Furthermore, acute nature of disease models used in mice might contribute to confounding because chronic inflammation is usually observed in patients with IBD. The balance between IL-33-induced inflammation and repair should be further addressed in these models to evaluate the potential therapeutic application of IL-33/ST2 axis.

E. IL-33 in kidney diseases

Studies have shown both pathogenic and beneficial functions of IL-33 in kidney diseases using animal models. The pathogenic role of IL-33 has been reported in kidney ischemia-reperfusion injury (IRI), cisplatin-induced acute kidney injury (AKI) and ovalbumin-induced nephrotoxicity models [136-138]. In IRI model, IL-33 treatment induced more severe renal fibrosis [136]. IL-33 worsened cisplatin-induced AKI with an increase of T cell infiltration, serum creatine, acute tubular necrosis (ATN) and apoptosis [137]. By contrast, a recent study revealed a protective role for IL-2-IL-33 fusion protein in kidney IRI models, which was mediated by the expansion of renal ILC2s [139]. IL-33 treatment for a short term also led to the expansion of renal ILC2s and protected from adriamycin-induced glomerulosclerosis [140]. It is likely that IL-33/ST2 signaling pathways functions differently depending on types of renal diseases.

F. IL-33 in metabolic disorders

Expression of IL-33 and ST2 were found in adipose tissues [61, 141, 142]. Low serum levels of IL-33 were correlated with high body mass index [143]. These findings suggest IL-33 might be associated with obesity and diabetes. IL-33 treatment to adipocyte cultures *in vitro* reduced expression of genes related to adipogenesis and lipid metabolism [142]. $ST2^{-/-}$ mice fed high-fat diet increased body weight and fat mass and showed impaired insulin secretion and glucose regulation compared to WT mice fed the same diet [142]. The protective role of IL-33 in adipose tissue might be exerted by increased production of type 2-associated cytokines and polarization of M2 macrophages and by the maintenance of

Treg cells in adipose tissue [141, 142]. In addition, IL-33 is critical for the maintenance of ILC2s in white adipose tissue and for limiting adiposity in mice by enhancing caloric expenditure through a process called beiging in which white adipose tissue is conversion to brown adipose tissue [8, 144, 145]. Brown adipose tissue plays a role in transferring energy into heat potentially protecting from metabolic disorders such as type 2 diabetes.

G. IL-33 in the central nervous system

High levels of IL-33 expression are observed in the central nervous system (CNS) [48]. IL-33 is expressed by macroglia, astrocytes and oligodendrocytes and ST2 is expressed by microglia [55, 146]. In mice, IL-33 treatment attenuated experimental autoimmune encephalomyelitis (EAE) [147]. Also, IL-33 can promote the differentiation of M2-like microglia and Treg cells limiting glial scaring in experimental ischemic stroke and spinal cord injury (SCI) [55, 148, 149]. In Alzheimer's disease, β-amyloid is accumulated in the brain which triggers chronic inflammation and leads to microglia activation and synaptic and neuronal dysfunction. In humans, IL-33 expression was decreased in the brain of patients with Alzheimer's disease and serum levels of sST2 were elevated in patients with mild cognitive impairment [150, 151]. IL-33 administration reversed synaptic plasticity impairment and memory deficits in APP/PS1 mice, a commonly used mouse model of Alzheimer's disease [150]. Furthermore, IL-33 modulated innate immune function by polarizing microglia towards an anti-inflammatory phenotype and reducing the expression of pro-inflammatory genes [150]. Collectively, IL-33 likely mediates neuroprotective

functions in various brain inflammation and injury models. IL-33 might have a potential to be used as a therapeutic option for CNS inflammation and injury and Alzheimer's disease.

The summarized roles of IL-33 in various inflammatory diseases are listed in ***Table 3***.

Table 3. Roles of IL-33 in inflammatory diseases

Inflammatory diseases	Role of IL-33	Protective/ Pathogenic	Refs
Cardiovascular diseases	IL-33 reduced hypertrophy and fibrosis in pressure overload model	Protective	[72]
	IL-33 prevented cardiomyocyte apoptosis and improved cardiac function in myocardial infarction	Protective	[110]
	IL-33 reduced atherosclerosis development in ApoE$^{-/-}$ mice	Protective	[111]
	IL-33 promoted eosinophilic pericarditis in CVB3-induced myocarditis	Pathogenic	[109]
	Elevated serum levels of sST2 is correlated with poor prognosis of myocardial infarction and with an increased risk of heart failure in male patients ≤50 years old with myocarditis	Protective	[112, 113]
Asthma and allergic inflammation	IL-33 levels were correlated with asthma severity	Pathogenic	[114]
	IL33 genetic variants were related to the risk of asthma and allergic rhinitis	Pathogenic	[115, 116]
	IL-33 treatment induced airway inflammation in mice	Pathogenic	[35, 48, 66, 117]
	IL-33 mediated experimental allergic rhinitis	Pathogenic	[118, 119]
	IL-33 levels were elevated in the skin of patients with atopic dermatitis	Pathogenic	[39, 64]
	Keratinocyte-specific expression of IL-33 in mice led to spontaneous development of dermatitis	Pathogenic	[40]
	Anti-IL-33 treatment reduced inflammation in experimental allergic rhinitis and asthma	Pathogenic	[120, 121]
Rheumatoid Arthritis (RA)	Higher IL-33 levels were correlated with more severe RA	Pathogenic	[122]
	Polymorphism of *IL33* gene was associated with a decreased risk of RA	Pathogenic	[123]
	IL-33 exacerbated collagen-induced arthritis in mice	Pathogenic	[124]
	Blocking IL-33/ST2 signaling reduced joint inflammation in mice	Pathogenic	[124-126]
Gastrointestinal inflammation	Increased levels of IL-33 was associated with eosinophilic esophagitis	Pathogenic	[128, 129]
	IL-33 treatment induced eosinophilic esophagitis in mice	Pathogenic	[129]
	Increased mucosal IL-33 levels were found in patients with IBD	Pathogenic	[130, 131]
	IL-33$^{-/-}$ mice and ST2$^{-/-}$ mice were protected from DSS-induced colitis	Pathogenic	[130, 132]
	IL-33 treatment protected mice from TNBS-induced or T cell transfer-induced colitis	Protective	[133, 134]
Kidney diseases	IL-33 induced renal fibrosis following IRI	Pathogenic	[136]
	IL-33 administration exacerbated cisplatin-induced AKI	Pathogenic	[137]
	IL-33 fused to IL-2 enhanced protection from AKI	Protective	[139]
	IL-33 treatment ameliorated adriamycin-induced glomerulosclerosis	Protective	[140]
Metabolic disorders	Low serum IL-33 levels were correlated with high body mass index in humans	Protective	[143]
	IL-33 reduced body weight and fat mass in mice	Protective	[142]
	IL-33 increased beiging of white adipose tissue and caloric expenditure	Protective	[144, 145]
Central nervous system	IL-33 treatment attenuated EAE in mice	Protective	[147]
	IL-33$^{-/-}$ mice had impaired recovery from CNS injury	Protective	[55]
	IL-33 administration attenuated ischemic stroke with reduced CNS inflammation	Protective	[148]
	IL-33 treatment reduced secondary injury and improved recovery from SCI	Protective	[149]
	Decreased IL-33 levels in the brain were correlated with Alzheimer's disease	Protective	[151]
	Elevated sST2 serum levels were correlated with mild cognitive impairment	Protective	[150]
	IL-33 reversed synaptic plasticity impairment and memory deficits in a mouse model of Alzheimer's disease	Protective	[150]

H. Clinical applications of IL-33/ST2 signaling

There has been progress in the development of IL-33/ST2 axis blocking tools. IL-33 and ST2 have been targets in preclinical studies and pharmaceutical pipelines. IL-33 Trap, a fusion protein comprised of ST2 and IL-1RAcP, ameliorates the pathology of a macular degenerative disease in a murine model [152]. Monoclonal antibodies against cytokines and their receptors have been proposed to be used as clinical therapeutics in diverse types of diseases including cancer and inflammatory disorders [153, 154]. Anti-IL-33 monoclonal antibodies (ANB020, AMG282, REGN3500/SAR440340) and anti-ST2 monoclonal antibodies (GSK3772847 and MSTT1041A) have been under development and phase 1 or 2 clinical trials either have completed or are currently ongoing (***Table 4***). The neutralization of IL-33/ST2 pathway is a practical approach to regulate and treat inflammation caused by IL-33/ST2 axis, however, it should be considered carefully since blocking IL-33 signaling can be either protective or pathogenic depending on tissue and inflammation conditions.

Table 4. Clinical trials of anti-IL-33 and anti-ST2 antibodies

Anti-IL-33	Conditions tested	Phase	ClinicalTrials.gov identifier
ANB020	Eosinophilic asthma	Phase 2	NCT03469934
	Peanut allergy	Phase 2	NCT02920021
	Atopic dermatitis	Phase 2	NCT03533751
	Chronic rhinosinusitis	Phase 2	NCT03614923
AMG282	Chronic rhinosinusitis with nasal polyps	Phase 1	NCT02170337
	Asthma	Phase 1	NCT01928368
REGN3500/SAR440340	Healthy volunteers	Phase 1	NCT02958436
	Asthma, moderate asthma	Phase 1	NCT02999711
	Allergic asthma	Phase 1	NCT03112577
	Atopic dermatitis	Phase 2	NCT03738423
	Atopic dermatitis	Phase 2	NCT03736967
	Asthma	Phase 2	NCT03387852
Anti-ST2	**Conditions tested**	**Phase**	**ClinicalTrials.gov identifier**
GSK3772847	Asthma	Phase 2	NCT03393806
	Asthma	Phase 2	NCT03207243
MSTT1041A	Atopic dermatitis	Phase 2	NCT03747575
	Asthma	Phase 2	NCT02918019
	COPD exacerbation	Phase 2	NCT03615040

Chapter 3. ILC2s in Infection, Inflammation & Homeostasis

ILC2s are known to serve as a critical innate source of type 2 effector cytokines. Although ILC2s have been studied intensely in mucosal tissues, it is important to appreciate that ILC2s are present in diverse locations. The function of ILC2s at these anatomical sites is also diverse and they play an important role in regulating innate and adaptive immunity. Thus, there is a growing interest to understand the role of ILC2s in settings of infection and inflammation. This chapter gives an overview on diverse roles of ILC2s in diseases which shows association with or involvement of ILC2s in disease processes.

A. ILC2s

ILC2s are characterized by their ability to produce type 2-associated cytokines such as IL-5 and IL-13 [8, 25, 26]. They are also shown to be tissue-resident immune cells [30, 155]. ILC2s strongly respond to IL-25, IL-33 and TSLP [25, 38, 39, 156, 157]. IL-33 can induce strong activation of ILC2s *in vitro*, while IL-25 stimulates ILC2s only moderately [8, 11]. IL-2, IL-7 and TSLP alone are not sufficient for the activation of ILC2s *in vitro*, but enhance the effect of IL-33 and boost activation when used in combination [15, 34]. *In vivo*, IL-25 can elicit the expansion of a subset of ILC2s called inflammatory ILC2s (iILC2s) [158]. iILC2s are characterized by high expression of the maturation marker, KLRG1, and IL-25 receptor [158]. iILC2s mediate not only anti-helminth immunity, but

also combat *Candida albicans* infection by expressing RORγt and producing IL-17, although further investigation is needed for precise lineage and their plasticity between ILC2s and ILC3s [158].

ILC2s express the transcription factor GATA3 at levels higher than other ILC subsets, and the development and function of ILC2s are inhibited in the absence of GATA3 [11, 15-17]. In mice, a common marker used to define ILC2s is ST2, a component of the IL-33 receptor, however, some tissue ILC2s such as skin-resident ILC2s do not express ST2 [156]. ST2 expression might be also altered by the state of microenvironment depending on tissue. In human, ILC2s present in peripheral blood lack ST2 [159]. However, human ILC2s express the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) and high levels of CD161 [38].

Cell-to-cell interaction through surface receptors such as ICOS and KLRG1 expressed on ILC2s influences the activation and survival of ILC2s [39, 160, 161]. ICOS and its ligand ICOSL are co-expressed on ILC2s and the interaction between ICOS and ICOSL promotes ILC2 proliferation which might provide evidence of a self-amplifying mechanism [160, 161]. By contrast, in human ILC2s, the interaction of KLRG1 with its ligand E-cadherin has been shown to inhibit type 2 cytokine production by ILC2s [39]. However, since KLRG1 is dispensable for NK cells, *in vivo* functional analysis remains to be investigated. Moreover, prostaglandins and eicosanoids produced by myeloid cells regulate the function of ILC2s. The prostaglandin D_2 (PGD_2) receptor, CRTH2, is expressed on human ILC2s in circulation and regulates the migration and accumulation of ILC2s in lung tissue and their production of IL-13 [162-164]. ILC2s also express the leukotriene D4 receptor, CysLT1R, which stimulates IL-4 production in addition to IL-5 and IL-13, and the receptor

for the TNF-family cytokine, TL1A, which results in stimulatory signals in ILC2s [165-167]. ILC2s also express the receptor for a neuropeptide allowing these cells to receive signals from the enteric nervous system. The neuropeptide VIP, which activates ILC2s, is secreted by enteric neurons and its expression is regulated by circadian rhythm [168].

ILC2s are involved in the innate immune response through type 2 cytokine production and contribute to expulsion of parasites such as *Nippostrongylus brasiliensis* [8, 25]. Besides classic T helper cell cytokines, other effector molecules are also known to be secreted by ILC2s. After resolving infection, ILC2s help to repair tissue damage through their production of AREG [9, 135]. ILC2s also secrete methionine-enkephalin which induces beiging of adipocytes affecting the regulation of adipose function and metabolic homeostasis [144]. ILCs including ILC2s play their roles mainly by secreting soluble molecules.

B. ILC2s in infection

It is well known that ILC2s mediate resistance to helminth infections. Before ILC2s were identified, multiple studies reported that, in the absence of T cells, type 2 immune responses can be initiated under helminth infection or IL-25 administration [169, 170]. The role for ILC2s in parasite infections were mostly investigated using a *N. brasiliensis* infection model [8, 11, 25]. Some other studies showed that ILC2s might also contribute to the clearance of *Strongyloides venezuelensis* and *Trichuris muris* [65, 171]. IL-25 and IL-33 are important for worm expulsion in most settings [25, 172]. IL-25 is secreted by tuft cells in the intestine and stimulates IL-13 release from ILC2s which induce hyperplasia of tuft

cells [172-174]. In order to deal with helminth infection, ILC2s are activated and secrete IL-4, IL-5, IL-13 and AREG. IL-5 is important for eosinophil survival and function, whereas AREG contributes to the repair of epithelial cells. IL-13 leads to smooth muscle contraction, mucus production by goblet cells, recruitment of alternative activated macrophages and eotaxin secretion, which work together to facilitate worm expulsion [8, 25, 171]. In animals infected primarily with *N. brasiliensis*, both ILC2s and Th2 cells cooperate to remove larvae of *N. brasiliensis* in the lungs upon reinfection, which prevents larvae maturation and migration to the stomach and intestines [175]. In addition, IL-9 produced by ILC2s, which act as an amplifier of ILC2 functions using an autocrine mechanism, is important for repair of epithelial cells in the lungs and worm expulsion after *N. brasiliensis* infection [176].

Although ILC2s play a critical role in resistance to parasites that invade through the mucosa in mouse models, little is known about the role of human ILC2s in defense at barrier tissues. It has been shown that the proportions of ILC2s in the blood are reduced in young children infected with *Schistosoma haematobium* and the levels of ILC2s are restored by removal of the parasites after treatment [177]. However, another study has reported that the number of c-Kit+ ILCs in the blood are increased in patients with filaria infection [178]. Since the proportion or number of ILC2s in circulation do not accurately reflect the activation status and expansion of ILC2s in tissues, further investigations are needed to uncover the role of human ILC2s in host defense against parasites.

Lung ILC2s were first identified in influenza infection models [9, 179]. It has been reported that, after influenza infection, ILC2s play a key role in tissue repair by the production of AREG [9]. Alveolar macrophages and NKT cells were reported as sources of IL-33 in

influenza-infected lungs, which is different sources of IL-33 than epithelial cells [179, 180]. Cigarette smoke exposure resulted in an attenuated response of ILC2s and an exacerbated type 1 immune response during influenza infection, which proposes another protective role of ILC2s in the lung [53]. IL-13 derived from lung ILC2s was shown to be responsible for collagen deposition and fibrosis in the lungs of mice treated with *Schistosoma mansoni* eggs [181]. Rhinovirus infection early in life has been linked to asthma development [182]. In mice, rhinovirus infection of neonates induced the expansion of ILC2s secreting IL-13 [183]. It might be due to reduced Treg cells in the lungs, and the formation of airway microbiota induces Treg cells in lungs early in life [184]. Further studies are required to delineate the role of ILC2s in lung infections.

C. ILC2s in inflammation

In the gastrointestinal tract, while ILC1s and ILC3s have been strongly implicated in the pathogenesis of IBD, we are only at the initial stages of defining roles of ILC2s in the process of intestinal inflammation [185]. IL-13 mediates colitis after oxazolone treatment which is partially caused by the IL-25-dependent activation of ILC2s [186]. Crohn's disease patients also have increased numbers of IL-13-producing ILCs in the intestine, suggesting a possible role for ILC2s in the pathogenesis of disease [187]. IL-33, IL-25 and TSLP, which stimulate ILC2s, have been associated with food allergies and eosinophilic esophagitis [188-190]. ILC2s have been shown to be enriched in patients with active eosinophilic esophagitis [191]. In mice, IL-33 treatment increased ILC2s and led to the development of eosinophilic esophagitis [129].

The role of ILC2s in inflammation has been studied extensively in the settings of allergic lung diseases and asthma. Genome-wide association studies have shown that genes related to the susceptibility to allergic lung diseases and asthma such as genes encoding IL-33, the IL-33 receptor, IL-4, IL-5, IL-13 and TSLP are associated with ILC2 [115, 192]. This association is strongly supported by data from mouse models of asthma and rhinosinusitis. Papain induces asthma-like symptoms in *Rag1*$^{-/-}$ mice but not in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice or *Rag*$^{-/-}$ mice that underwent ILC depletion [34]. Challenge with allergen induces asthma-like symptoms in ILC-deficient mice reconstituted with ILC2s [34]. In the lungs, IL-33 seems to be a central cytokine required for ILC2 activation. Purified naïve ILC2s from the lungs of mice require IL-33 for their production of type 2 cytokines [34]. IL-33 is a more potent inducer of ILCs than IL-25 in mice challenged with ragweed or with *Alternaria alternata* [66]. Other studies also suggest that IL-33 is a key activating cytokine of lung ILC2s in type 2 lung inflammation models [35, 193]. By contrast, IL-25 seems to play a more prominent role in intestinal ILC2 activation [25]. This is consistent with reduced ST2 expression on enteric ILC2s [194]. However, intranasal IL-25 administration promotes the emergence of an IL-17RB^{+}KLRG1^{hi} ILC2 population in the lungs [158]. These IL-25-stimulated ILC2s in the lungs may convert to ST2-expressing ILC2s, suggesting that tissue-specific microenvironment might affect the regulation of ILC2s [158]. Other than IL-33 and IL-25, basophil-derived IL-4 has been shown to stimulate ILC2s to promote inflammation in the lungs [195]. The capacity of ILC2s to trigger hyperreactivity in airway is also reported after infection with influenza virus [179].

ILC2s are a potent source of type 2 cytokines in settings of allergic lung inflammation. Naïve ILC2s are primed to produce IL-5 as indicated by increased *Il5* transcription and

shown in IL-5 reporter mice [34, 168]. IL-5 derived from ILC2s is important for eosinophil homeostasis, while IL-13 and eosinophil attracting chemokine, eotaxin, produced locally are critical for eosinophilic lung inflammation induced by allergen challenge [168]. During allergic inflammation in the lungs, ILC2s are a major cellular source of IL-13 which induces goblet cell hyperplasia, mucus production and smooth muscle contraction, all of which unfavorably influencing airflow and lung function. ILC2s promote airway hypersensitivity through IL-25, IL-33 and ICOS signaling [66, 160, 179, 196]. Furthermore, IL-13 derived from ILC2s promote lymph node trafficking of lung dendritic cells in papain-induced lung inflammation model providing a link between type 2 innate immune response and allergen sensitization of Th2 cells [197]. ILC2s are also a source of IL-9 in allergen-induced airway inflammation [198]. In addition to its role in autocrine regulation of ILC2s, IL-9 supports goblet cell hyperplasia and mast cell proliferation in lungs in helminth-induced lung inflammation, which suggests a similar function of IL-9 in lung inflammation induced after antigen challenge [176, 199].

ILC2s expand in number after second challenge with papain in sensitized animals or after repeated challenges with house dust mite (HDM) extract [157, 197]. Moreover, a study shows that ILC2s play an important but secondary role to Th2 cells in recalling response to inhaled ovalbumin antigen [200]. However, another study suggests that ILC2 play a more critical role in an HDM model of chronic asthma [201]. Regardless of the extent of ILC2 contribution to recalling response to allergen, it is important that these studies suggest collaboration between ILC2s and Th2 cells in the context of allergic airway inflammation. In humans, ILC2s have been identified in the lungs of fetal tissue, bronchoalveolar lavage and lungs of healthy subjects [9, 38]. It was reported that patients with asthma exhibit more

ILC2s in circulation [37]. By contrast, a similar number of circulating ILC2s were observed between severe or mild asthma patients and health subjects [164]. Another study showed that corticosteroids, which are commonly used to treat asthma, inhibit ILC2 activation by IL-33 in mice [202]. TSLP is able to confer resistance to the effect of corticosteroids on ILC2s [202]. Further work is needed to understand the translational potential of these findings in preclinical models.

Type 2 inflammation is associated with chronic rhinosinusitis with nasal polyps. The enrichment of activated ILC2s was initially reported in nasal polyps of patients with chronic rhinosinusitis [38]. Nasal polyp ILC2s are a potent source of type 2 cytokines in response to IL-33, IL-25 and TSLP [15, 38]. The association between ILC2s and chronic rhinosinusitis was further established in subsequent studies [203-206]. Nasal polyp ILC2s from patients with chronic rhinosinusitis produced IL-13 when stimulated with IL-33 [205]. Moreover, allergic rhinitis that developed into chronic rhinosinusitis was also associated with an increase in circulating ILC2s after allergen challenge in sensitized patients [207]. Interestingly, corticosteroid treatment in chronic rhinosinusitis with nasal polyps resulted in a decrease of ILC2s in nasal polyps [206]. Also, *Alternaria*-challenged mice showed increased apoptosis of lung ILC2s after treatment with corticosteroids [206]. Thus, it is clear that ILC2s are present in the nasal mucosa and it is likely that they are highly associated with type 2 inflammation occurring in chronic rhinosinusitis with nasal polyps.

Atopic dermatitis is a chronic inflammatory skin disease characterized by increased levels of type 2 cytokines in skin legions. Skin-resident ILC2s were first identified in mice and distinguished from ILC2s in other tissues by their expression of CD103 which is an integrin expressed by other skin-resident immune cells [156, 208]. Skin inflammation, whose

mechanism is dependent on ILC2s, can be induced in mice with calcipotriol, the vitamin D analog, an allergen such as HDM, complexes of IL-2 and anti-IL-2, or by IL-33 overexpression. These sensitization protocols are all associated with a strong ILC2 expansion and activation triggered by TSLP or by IL-25 and IL-33 [39, 40, 156, 208]. Expansion of skin ILC2s is critically dependent on TSLP signaling, although skin-specific IL-33 overexpression can lead to the development of spontaneous dermatitis with increased ILC2s [40, 156]. It remains unclear whether one of these ILC2-stimulating cytokines play a more critical role during atopic dermatitis, however, ILC2s are key mediators of acute type 2 inflammation in the skin.

In humans, there is an enrichment of ILC2s found in the skin lesions of patients with atopic dermatitis [39]. Skin ILC2s are present in healthy subjects and the number was increased in biopsy samples or blood samples from patients with atopic dermatitis [39, 156]. A mechanism of ILC2 suppression was reported in human skin ILC2s. The activation of human skin ILC2s was suppressed by binding of E-cadherin to KLRG1 which is expressed on activated ILC2s [39]. Interestingly, IL-4 derived from basophils can also increase functions of ILC2s and mast cells are present in proximity to skin ILC2s, suggesting a possible interaction and regulation among these immune cells in skin [208, 209]. Collectively, ILC2s appear to play a critical role in driving skin inflammation especially with type 2 immune responses, however, further studies are needed to delineate the exact function and regulation of skin ILC2s.

ILC2s are present not only at mucosal barriers, but also at other tissues. Many diseases accompanying type 2 inflammation may involve ILC2s during their pathogenesis. Liver fibrosis is associated with increased levels of serum IL-33 and it has been shown that ILC2s

are important for IL-13 production induced by IL-33 [210, 211]. IL-13 in turn promotes cholangiocyte hyperplasia in the bile duct and hepatic stellate cell activation which are associated with biliary atresia and hepatic fibrosis, respectively [210, 211]. ILC2s have also been identified in the brain and might play a role in multiple sclerosis. A study showed that ILC2s accumulate in the brain and draining lymph nodes of mice resistant to EAE [212]. The role of ILC2s in meningeal inflammation has been shown in CNS injury [213]. IL-33 is expressed by glia, which promotes recovery following spinal cord injury, and can be released to the cerebrospinal fluid after spinal cord injury suggesting its possible role of activating cells in the meninges [55]. After spinal cord injury, meningeal ILC2s are activated in an IL-33-dependent manner and produce type 2 cytokines suggesting a beneficial role of ILC2s in spinal cord injury [213]. ILC2s are also reported in the aorta where they can produce IL-5 in response to IL-33 stimulation [214]. IL-25 administration reduces atherosclerosis in mice possibly through interactions between ILC2s and B1 B cells leading to natural anti-phosphorylcholine IgM generation [215]. In addition, ILC2s are found to be present in para-aortic adipose tissue and lymph nodes, and they are a major innate cellular source of IL-5 and IL-13 required for mounting protective immunity against atherosclerosis development [216].

D. ILC2s in homeostasis

There is increasing evidence on the role for ILC2s in metabolic homeostasis. Adipose tissues contain many immune cells including ILC2s. Studies have demonstrated that ILC2s contribute to metabolic homeostasis by enhancing type 2 environment in adipose tissue

which is a characteristic of lean individuals. Low-grade type 1 inflammation in white adipose tissue (WAT) is induced by obesity which increases the risk of metabolic diseases. Type 2 cytokines, IL-4 and IL-13, are required for beiging of adipocytes. Beige adipocytes can uncouple the electrochemical gradient in mitochondria from ATP synthesis by expression of the uncoupling protein called UCP-1 [217]. While WAT is enriched for ILC2s, ILC2s are decreased in obesity or in mice on a high-fat diet [144]. Studies demonstrated the importance of IL-33 or IL-25 stimulation of ILC2s in WAT homeostasis [43, 44, 144, 145]. ILC2s in adipose tissue produce IL-5, which is involved in sustaining eosinophils, and IL-13, that is important for polarizing M2 macrophages and regulating adiposity and insulin resistance [43, 144]. ILC2s seem to regulate adiposity and caloric expenditure through several mechanisms. In one proposed mechanism, IL-5 and IL-13 produced by ILC2s leads to IL-4 secretion by eosinophils. The IL-4 directly controls the fate of adipocyte precursors expressing IL-4 receptor and results in promoting pre-adipocyte differentiation to beige adipocytes [145]. In another mechanism, ILC2s produce methionine-enkephalin peptides that induce upregulation of UCP-1 in adipocytes and promote beiging of WAT [144]. Although a role for ILC2s in metabolic homeostasis has been studied, the underlying mechanism by which ILC2s interact with adipocytes and other components in adipose tissue needs more investigation. In addition to the role for ILC2s in adipose tissue, a recent study has reported that ILC2s in the pancreas promote insulin secretion [218]. ILC2s stimulated by IL-33 can elicit dendritic cells and macrophages to produce retinoic acid, which in turn promotes insulin secretion by β cells and regulates glucose levels [218].

ILC2s appear to play an important role in health and diseases. According to findings in many studies, it is obvious that ILC2s are a critical component of immune system in infection, inflammation and metabolic homeostasis. A growing body of evidence shows that ILC2s are regulated by their microenvironment which fine-tunes their function in a tissue-specific manner. Moreover, we have recently found that cardiac ILCs are a resident population and they are type 2-committed in steady state [219]. ILCs found in the heart showed ILC progenitor-like characteristics, however they were unable to differentiate into ILC1s or ILC3s [219]. It is highly possible that ILCs might play a role in maintaining homeostasis and regulating inflammation in the heart. We speculate that cardiac ILCs are a tissue-resident immune cell population which could drive cardiac inflammation in a type 2-skewed environment.

While ILCs are implicated in many inflammatory diseases of diverse organs, it is unclear whether ILCs play a role in cardiac inflammation. In addition, the etiology of pericarditis remains largely idiopathic and the underlying mechanism by which eosinophilic pericarditis is induced is poorly understood. More studies are required to fill the knowledge gaps in the pathogenesis of pericarditis and understandings in roles for ILCs in the heart and cardiac inflammation. The aim of our experimental works was to examine the role of ILCs in inflammatory heart disease using a murine model of IL-33-induced eosinophilic pericarditis. We hypothesized that IL-33 stimulates cardiac ILC2s to produce type 2 cytokines such as IL-5 and IL-13, which drives production of eotaxin and eosinophil trafficking to the heart resulting in the development of pericarditis. In Chapter 4, we will review (1) the background of our experimental study, (2) materials and methods we used

to test the hypothesis, and (3) results from the experiments and their implications. In Chapter 5, we will discuss the overall conclusions and suggest future directions.

Chapter 4. Role of ILC2s in the Heart & Cardiac Inflammation

Summary

Innate lymphoid cells (ILCs) play an important role in inflammation in mucosal organs, however, the role for ILCs in the heart and cardiac inflammation has not been studied. The goal of our work was to investigate the role of group 2 innate lymphoid cells (ILC2s) in inflammatory heart disease. Here we found a new role of ILCs during cardiac inflammation. We identified an accumulation of cardiac ILC2s in IL-33-induced eosinophilic pericarditis. ILC2s, not T and B cells, were required for pericarditis development. ILC2s transferred to the heart of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice restored their susceptibility to eosinophil infiltration. Moreover, ILC2s directed cardiac fibroblasts to produce eotaxin-1 to promote eosinophil trafficking to the heart. We also found that eosinophils resided in the mediastinal cavity of naïve mice, serving as a reservoir of eosinophils for non-vascular trafficking to the heart. Eosinophils transferred to the mediastinal cavity of eosinophil-deficient ΔdblGATA1 mice following IL-33 treatment migrated more effectively to the heart compared to intravenously transferred eosinophils. Our data demonstrate a pathogenic role of ILC2s in IL-33-induced pericarditis, interaction of ILC2s with cardiac fibroblasts leading to eotaxin-1 production and eosinophil trafficking from the mediastinal cavity to the heart.

Background

Immune cells that reside in the heart play an important role in both cardiac homeostasis and progression and modulation of inflammatory heart diseases. The previous view that immune cells found in the heart originated from the bone marrow and trafficked via the blood has been replaced with the current understanding that many cardiac immune cells are resident populations [220]. These resident cells are recruited to the heart during embryonic development and self-renew until adulthood. Great efforts have been devoted to describe the types of myeloid cell populations in mouse and human hearts including macrophages [221, 222]. Mast cells have also been found in mouse and human hearts [223-225]. In addition, the heart contains lymphocytes such as T cells and B cells [226]. We recently found that cardiac innate lymphoid cells (ILCs) are also a resident population in both human and mouse hearts [219].

ILCs are recently identified innate immune cells that serve important roles in lymphoid tissue formation, repair of damaged tissues and homeostasis as well as in immunity against infectious microorganisms [2, 36, 227]. ILCs are categorized into three groups based on their unique expression of transcription factors, surface markers and the production of effector cytokines [1, 228]. Group 1, 2 and 3 innate lymphoid cells (ILC1s, ILC2s and ILC3s) mirror the functions of T helper cells, Th1, Th2 and Th17 cells, respectively. ILC1s are characterized by IFNγ production, ILC2s by IL-5 and IL-13 and ILC3s by IL-17 and IL-22. Natural killer (NK) cells are considered a cytotoxic subset of ILC1s [1]. Distinct from adaptive immune cells such as T cells and B cells, ILCs do not possess antigen

specific receptors [227]. ILCs appear to be tissue resident cells and are maintained mostly by self-renewal in homeostatic settings [30, 155]. It was reported that ILC2s are important for metabolic homeostasis in white adipose tissue and visceral adipose tissue [43, 144]. ILC2s were also shown to be critical for restoring lung tissue homeostasis following acute influenza virus infection [9]. ILC2s are especially important in helminth expulsion and allergic lung inflammation [8, 25, 26, 34, 35, 157].

ILC2s are found in many organs at steady state including lung and mucosal tissues, and can be activated by IL-33, IL-25 and TSLP [8, 25, 26, 36, 156, 229]. After activation, ILC2s produce Th2-associated cytokines, such as IL-5 and IL-13 [8, 25, 26]. These Th2 cytokines have been implicated in eosinophil proliferation, recruitment and homeostasis [26, 168]. The role of ILC2s in infection and inflammation has been investigated mostly in mucosa-associated tissues and skin. ILC2s are beneficial to promote immunity to parasite infection with *N. brasiliensis* [8, 25]. In this helminth infection model, IL-25 and IL-33 were shown to be critical for ILC2 activation and IL-13 produced by ILC2s mediated worm expulsion [25]. In contrast to protective immunity provided by ILC2s, IL-33-activated ILC2s are also involved in the pathogenesis of asthma and atopic dermatitis promoting inflammation in the lungs and skin, respectively [34, 39, 40]. Moreover, enrichment of ILC2s were reported in biopsy samples from patients with eosinophilic esophagitis and atopic dermatitis [39, 191].

IL-33 is a cytokine of the IL-1 family and functions as an alarmin, which is released from cells upon tissue damage or cellular stress [52, 75]. IL-33 is usually expressed and released

from epithelial and endothelial cells, and is also shown to be expressed on stromal cells such as fibroblastic reticular cells in the lymph node [56, 58]. The profiling of IL-33 mRNA expression in mouse tissues revealed that the heart had a relatively low expression of IL-33 compared to mucosal and barrier-related tissues such as lung and skin [48]. The role of IL-33 in the heart has been shown to be beneficial in some mouse models of cardiovascular diseases. IL-33 treatment reduced cardiac hypertrophy in a mouse model of transverse aortic constriction and improved cardiac function and survival in a myocardial infarction mouse model [60, 72, 110]. However, administration of IL-33 induced pericarditis in mice with increased proportion of eosinophils in the heart of naïve and coxsackievirus B3 (CVB3)-infected mice, demonstrating that IL-33 can also play a pathogenic role in the heart [109]. IL-33 has been shown to activate and expand ILC2s [8, 25, 229, 230]. This suggests that the described pathogenic effect of IL-33 in the heart could be mediated by activation of ILC2s.

Here, we show that ILCs in the heart play a pathogenic role and promote cardiac inflammation. Cardiac ILC2s activated by IL-33 caused eosinophilic pericarditis. We identified that ILC2s, not T cells and B cells, were required for IL-33-induced pericarditis. *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice deficient in lymphocytes including ILCs were protected from pericarditis, however, ILC2 transfer to the heart restored the susceptibility of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice to eosinophil infiltration to the heart. We also found that ILC2s induced cardiac fibroblasts to produce eotaxin-1 to direct eosinophil trafficking to the heart. Moreover, we discovered that eosinophils resided in the mediastinal cavity of naïve mice, which serves as a reservoir of eosinophils. We documented that non-vascular recruitment of eosinophils

from the mediastinal cavity was more effective than conventional vascular trafficking of eosinophils to the heart. The eosinophils trafficking from the mediastinal cavity to the heart could explain the development of inflammation preferentially around pericardial area after ILC2 activation with IL-33.

Materials and Methods

Animals

Wild type BALB/cJ (JAX 651), *Rag2*$^{-/-}$ (JAX 8448), *Rag2*$^{-/-}\gamma c^{-/-}$ (JAX 14593), CD45.1/cByJ (JAX 6584), ΔdblGATA1 (JAX 5653) mice were obtained from the Jackson Laboratory (Bar Harbor, ME). ST2$^{-/-}$, IL-13$^{-/-}$ and IL-33$^{cit/+}$ mice were kindly provided by Andrew N.J. McKenzie (Medical Research Council, Cambridge, UK). IL-5Tg mice were kindly provided by James Lee (Mayo Clinic, Scottsdale, AZ). All mice used were 6 to 12-week-old mice on the BALB/c background. Mice were maintained at the Johns Hopkins University School of Medicine specific pathogen-free animal facility. Experiments were performed with age-matched mice in accordance with the guidelines set forth in the Guide for the Care and Use of Laboratory Animals. All methods and protocols were approved by the Animal Care and Use Committee of Johns Hopkins University.

Induction of experimental pericarditis

Mice were injected with 1 μg of recombinant mouse IL-33 (BioLegend) in 100 μl PBS intraperitoneally on days 0, 2, 4, 6 and 8. On day 9 or 10, hearts were harvested for further analysis detailed below.

Assessment of pericarditis severity

Hearts were cut transversely, fixed in SafeFix (Thermo Fisher Scientific), embedded in paraffin, cut into 5 μm-thick sections and stained with H&E (Histoserv, MD). The severity of pericarditis was assessed by scoring infiltration of the area of pericardium around right ventricle (RV) on H&E-stained sections based on histopathology score from 0 to 4 using the following criteria for hematopoietic infiltrates: grade 0, no inflammation; grade 1, <20% of RV is involved and/or mild inflammation; grade 2, 20-50% of RV is involved and/or intermediate inflammation; grade 3, 50-80% of RV is involved and/or severe inflammation; grade 4, >80% of RV is involved and/or severe inflammation with adjacent myocardial infiltrates. Scoring was performed by two blinded pathologists and averaged.

Light microscopy

Images on H&E-stained sections were acquired on the BX43 microscope (Olympus) with the DS-Fi3 camera (Nikon) using NIS-Elements D Software (v. 5.10.01, Nikon).

Echocardiography

Conscious mice were held in a supine position and Doppler echocardiography was performed using Vevo 2100 with a MS550D transducer (FUJIFILM VisualSonics, Ontario, Canada). To measure the Myocardial Performance Index (MPI), tissue Doppler imaging of

the mitral annulus was obtained. Cardiac time intervals, isovolumetric contraction time (IVCT), isovolumetric relaxation time (IVRT) and ejection time (ET), were measured and MPI was calculated based on these parameters [231].

Flow cytometry and cell sorting

Hearts were perfused with PBS through ventricles, cut into small pieces and digested in gentleMACS C Tubes (Miltenyi Biotec) with 3000U Collagenase II and 300U DNase I (Worthington Biochemical Corporation) in HBSS for 30 min at 37°C. To generate single cell suspensions, hearts were mechanically dissociated using GentleMACS dissociator (Miltenyi Biotec) following manufacturer's protocols. Cells in the mediastinal cavity were harvested by lavage with PBS. Blood was collected in PBS with 100U/ml Heparin and overlaid on Histopaque-1119 (Sigma Aldrich) to remove red blood cells. Remaining red blood cells were lysed using ACK buffer (Quality Biological). Cells from heart samples were strained through a 40 μm filter, and cells from other organs were strained through a 70 μm filter. For intracellular cytokine staining, cells were stimulated with 50 ng/ml PMA, 750 ng/ml Ionomycin (Sigma-Aldrich), GolgiStop and GolgiPlug (BD Biosciences) for 4 hours at 37°C before staining. Single cell suspensions were stained with LIVE/DEAD Fixable Aqua (Thermo Fisher Scientific). FcγRII/III was blocked with anti-mouse CD16/CD32 (eBioscience), and markers of interest were stained with flourochrome-conjugated antibodies (BD, BioLegend and eBioscience). Lineage (Lin) used for identifying ILC2s included CD3ε, TCRβ, CD19, B220, CD11b, CD11c, Gr-1, Ter119, FcεRIα and NKp46. To quantify absolute number of cells, viable cells were counted using CountBright Absolute Counting Beads (Thermo Fisher Scientific). Samples were acquired

on BD LSR II or Fortessa (BD Biosciences) and data were analyzed using FlowJo V10 (Tree Star). For cardiac ILC2 sorting, after enzymatic and mechanical dissociation of hearts, single cell suspensions were obtained using Histopaque-1077 (Sigma-Aldrich) according to manufacturer's instructions. Collected mononuclear cells were stained and sorted on BD FACSAria II (BD Biosciences).

Isolation and *in vitro* expansion of cardiac ILC2

Cardiac ILC2s were isolated using FACSAria II (BD Biosciences) from the hearts of IL-33-treated mice and cultured at 37°C in RPMI 1640 (Gibco) with 10% FBS (GE Healthcare Life Sciences), 1% Penicillin-Streptomycin, 2 mM L-Glutamine, 10 mM HEPES, 1mM Sodium Pyruvate (all Quality Biological), 0.1 mM Non-essential amino acids (Sigma Aldrich), 0.05 mM 2-Mercaptoethanol (Gibco). Cytokines, IL-2, IL-7 and IL-33, at a concentration of 25 ng/ml were added in media to expand ILC2s *in vitro*.

Cardiac injection of ILC2

CD45.2^{+} *Rag2*$^{-/-}$*γc*$^{-/-}$ mice were anesthetized with 3.5% isoflurane (Baxter) and tracheal intubation was performed immediately. While on intubation, mice were provided with oxygen with 2% isoflurane by mechanical ventilation system (Model 845, Harvard Apparatus). Pre-operational analgesics (0.05 mg/kg Buprenorphine, Reckitt Benckiser) and paralytics (1 mg/kg Succinylcholine, Henry Schein) were treated before operation. Thoracotomy was done to access the chest cavity and expose the ventricles of the heart. 5x10^{5} CD45.1^{+} cardiac ILC2s were injected into the myocardium on three ventricular

locations using a 29G ½ insulin syringe (BD). Post-operational analgesics (0.05 mg/kg Buprenorphine) were administered during recovery.

Isolation of primary adult mouse cardiac fibroblasts

Hearts were isolated from 6-12-week-old WT BALB/c mice pretreated *i.p.* with PBS with 50 U/ml Heparin. Hearts were cannulated through aorta and perfused for 3 minutes at 37°C with calcium-free perfusion buffer (7.03 g/L NaCl, 1.1 g/L KCl, 0.082 g/L KH_2PO_4, 0.085 g/L Na_2HPO_4, 0.144 g/L $MgSO_4$, 2.38 g/L HEPES, 0.39 g/L $NaHCO_3$, 1 g/L glucose, 3.74 g/L Taurine, 1 g/L 2,3-Butanedione monoxime; all Sigma-Aldrich) and for 8 minutes with the addition of Collagenase II (Worthington Biochemical Corporation) and Protease XIV (Sigma-Aldrich) and 0.03 M CaCl2. The heart was cut into pieces and digested by gentle pipetting for 3 minutes or until large pieces are fully digested. After filtering through a 100 μm strainer and washing with DMEM (Gibco), cardiac fibroblasts in cell suspensions were separated from cardiac myocytes that precipitated rapidly and spontaneously. Cardiac fibroblasts were cultured at 37°C in DMEM with 20% FBS (GE Healthcare Life Siences), Non-essential amino-acids (Sigma Aldrich), Penicillin-Streptomycin, 2 mM L-Glutamine, and 25 mM HEPES (all Quality Biological). Nonadherent cells were washed off after 1 hour. Culture media was changed every day for 5 days until cardiac fibroblasts are confluent. Cardiac fibroblasts were passaged twice before co-culture.

***In vitro* co-culture of cardiac fibroblasts and ILC2**

ILC2s were FACS-sorted from the hearts of IL-33-treated mice and expanded *in vitro* as described above. $1x10^5$ ILC2s were placed on Transwell with 0.4 μm pore polyester

membrane insert (Corning). Cardiac fibroblasts from second passage were co-cultured with ILC2s located on Transwell at 37°C in ILC2 culture media for 24 hours. 25 ng/ml IL-2 and 25 ng/ml IL-7 were included in co-culture and 25 ng/ml IL-33 was added in certain conditions indicated. Cells and supernatants were harvested after co-culture and used for further analysis.

In vivo IL-5 neutralization

To block IL-5 *in vivo*, mice were treated *i.p.* with 300 µg of anti-IL-5 monoclonal antibody (Clone: TRFK5, BioXCell) or isotype antibody (Clone: HRPN, BioXCell) every three days starting from a day before pericarditis induction.

Eosinophil isolation, labeling and transfer to the mediastinal cavity or intravenously

Blood from donor IL-5Tg mice was collected in PBS with 100U/ml Heparin and overlaid on Histopaque-1119 (Sigma Aldrich) to remove red blood cells. Anti-CD90.2 and anti-CD45R(B220) microbeads (Miltenyi Biotec) were used to deplete lymphocytes and enrich eosinophils. Eosinophil purity (78%-95%) was determined by flow cytometry. For labeling, cells were stained with either CellTrace™ Violet (CTV) or CellTrace™ Far Red (CTFR) according to manufacturer's instructions (Thermo Fisher). Recipient mice were anesthetized with 500 µl avertin and 8-10x10^6 cells were injected to the mediastinal cavity or by retro-orbital injection using a 29G ½ insulin syringe (BD).

Quantitative PCR

RNA was extracted in TRIzol (Invitrogen) and cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and executed on MyiQ2 thermal cycler (Bio-Rad) using iQ5 optical system software (Bio-Rad). Acquired data were analyzed by the $2^{-\Delta\Delta Ct}$ method [232]. Threshold cycles were normalized to the expression of *Gapdh* and then to controls. Primers for genes (*Ccl11*: 5'-GAATCACCAACAACAGATGCAC-3' and 5'-ATCCTGGACCCACTTCTTCTT-3', *Ccl24*: 5'-TCTTAGGGCCCTTCTTGGTG-3' and 5'- AATTCCAGAAAACCGAGTGG-3', *Gapdh*: 5'-TTGATGGCAACAATCTCCAC-3' and 5'-CGTCCCGTAGACAAAATGGT-3') were commercially synthesized (Integrated DNA Technologies).

ELISA

Supernatants from cardiac fibroblasts co-culture with ILC2s or sera from mice were stored at -80°C prior to ELISA. Eotaxin-1 was determined by quantitative sandwich ELISA using Mouse CCL11/Eotaxin Quantikine ELISA Kit (R&D Systems) according to manufacturer's protocols. For anti-myosin IgM ELISA, plates were coated with 0.5 µg/well myosin heavy chain α peptide $MyHC\alpha_{614-629}$ (Ac-SLKLMATLFSTYASAD; Genscript) and Alkaline Phosphatase AffiniPure goat anti-mouse IgM, µ chain specific (Jackson ImmunoResearch) at a 1:4000 dilution was used to detect anti-myosin IgM in sera.

Statistics

Two group comparisons were performed using Student's *t*-test for normally distributed data. Multiple group comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. Mann-Whitney U test or Kruskal-Wallis H test for two groups or multiple groups, respectively, was used for nonparametric data. Pearson correlation coefficient, r, was calculated using correlation analysis in Prism 6 (GraphPad Software Inc.). Statistical analysis was conducted in Prism 6. Statistically significant comparisons were represented by asterisks: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Results

Number of ILC2s increases in the heart after IL-33 treatment

ILC2s express IL-33 receptor, which is also known as ST2, and become activated and proliferative upon IL-33 stimulation [1, 36, 228]. To determine whether IL-33 induces an increase of ILC2s in the heart *in vivo*, we used the IL-33-induced pericarditis model in which mice are treated with IL-33 *i.p.* every other day for 9 days [109]. IL-33-treated mice developed severe pericarditis compared to PBS-treated mice (***Figure 4***A). The inflammation mostly affected the pericardium, however, in some cases, adjacent myocardium was also inflamed (***Figure 4***A). We also found that IL-33-treated mice showed increased infiltration of immune cells in other organs including esophagus and lungs (***Figure 5***A and B). We assessed cardiac function of the heart by performing Doppler echocardiography. IL-33-treated mice showed significantly longer isovolumetric relaxation time (IVRT), an indicator of diastolic function, and higher myocardial

performance index (MPI), a useful predictor of global cardiac function, than PBS-treated mice (***Figure 4***B and C). Prolonged IVRT indicates poor myocardial relaxation related to pericardial constriction and high MPI represents both systolic and diastolic dysfunction. This suggests that pericarditis induced by IL-33 resulted in abnormal cardiac function. We analyzed the number of heart-infiltrating $CD45^+$ leukocytes by flow cytometry and found that it was significantly increased in IL-33-treated mice compared to PBS-treated mice, confirming that inflammation occurred in the heart as a result of IL-33 treatment (***Figure 4***D). We identified ILC2s in the heart as $CD45^+Lin^-CD90^+KLRG1^+ST2^+$ (***Figure 5***C). Lin included CD3ε, TCRβ, CD19, B220, CD11b, CD11c, Gr-1, Ter119, FcεRIα and NKp46, and was used to exclude cell populations that may contaminate ILC2s. Both the number and frequency of ILC2s in the heart were dramatically increased upon IL-33 treatment compared to PBS treatment (***Figure 4***E and ***Figure 5***D). We also analyzed other heart infiltrating immune cells and found that the number and frequency of $CD11b^+SiglecF^+$ eosinophils were increased in the heart of IL-33-treated mice and they were the most abundant cells among heart infiltrates (***Figure 4***F and ***Figure 5***E). Number and frequency of other immune cells known to express ST2 including $FcεRIα^+CD117(c\text{-}Kit)^+$ mast cells remained unchanged (***Figure 4***G). In addition, the number of $CD11b^+Ly6G^+$ neutrophils in the heart did not differ between PBS and IL-33 treated mice (***Figure 5***F). Interestingly, we found that the number of $CD19^+$ B cells was greater in the heart of IL-33 treated mice than in the heart of PBS-treated mice (***Figure 5***G). Therefore, we examined if pericarditis induced by IL-33 led to the production of autoantibodies against cardiomyocytes. We found that the level of anti-myosin IgM antibody in serum was significantly higher in IL-33-treated mice than in PBS-treated mice on day 9 (***Figure 5***H). Anti-myosin IgG and IgE

were found at a negligible level in sera of both PBS- and IL-33-treated mice (data not shown). These results indicate that IL-33-induced pericarditis is characterized by an increased number of ILC2s, eosinophils and B cells in the heart.

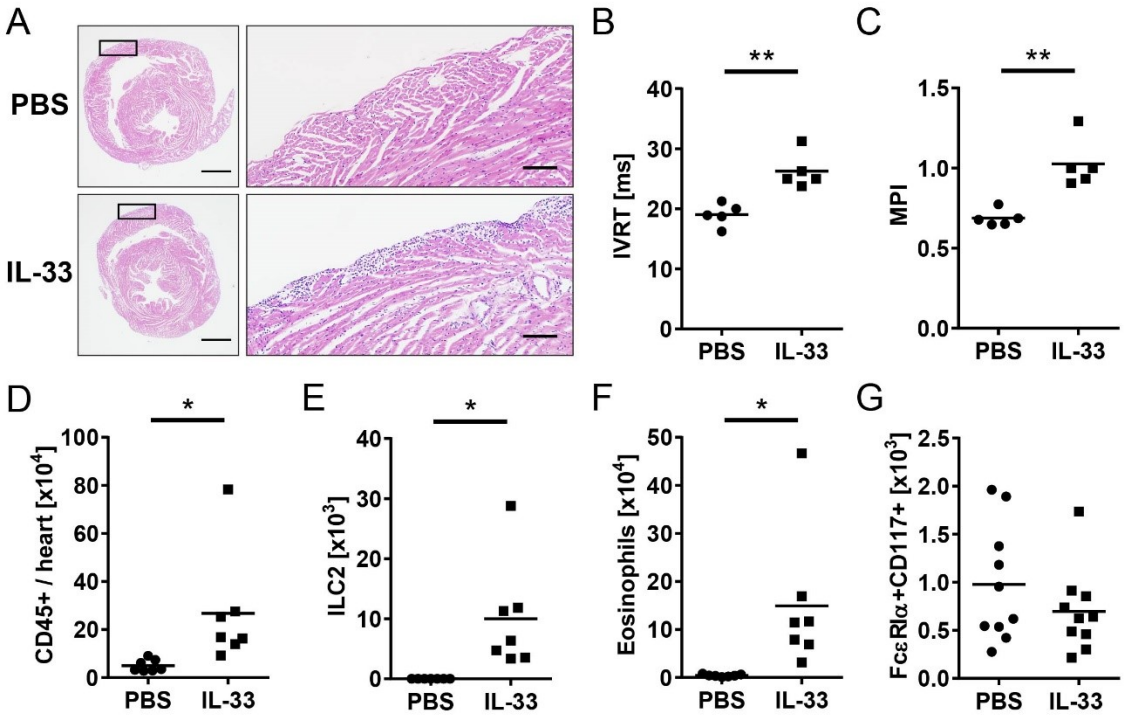

Figure 4. ILC2s increase in the heart following IL-33 treatment.

(A) Representative images of H&E-stained heart sections of the median mice treated with either PBS or IL-33. Areas marked by rectangles are shown as enlarged images in the right panels. Scale bars: 1 mm (left) and 100 μm (right). (B) Isovolumetric relaxation time (IVRT) and (C) myocardial performance index (MPI) of the heart from mice treated with PBS or IL-33 was assessed by Doppler echocardiography on day 9 post-PBS or IL-33 treatment. (D) Total number of heart-infiltrating $CD45^+$ leukocytes was determined by

flow cytometry. Number of (E) ILC2s, (F) eosinophils and (G) mast cells in the hearts. Absolute cell counts per heart were calculated using counting beads for flow cytometry (see Methods). Data are representative of three independent experiments. Data are displayed as the mean. Unpaired t-test (B-G) was used for statistical analysis. *, $P < 0.05$; **, $P < 0.01$.

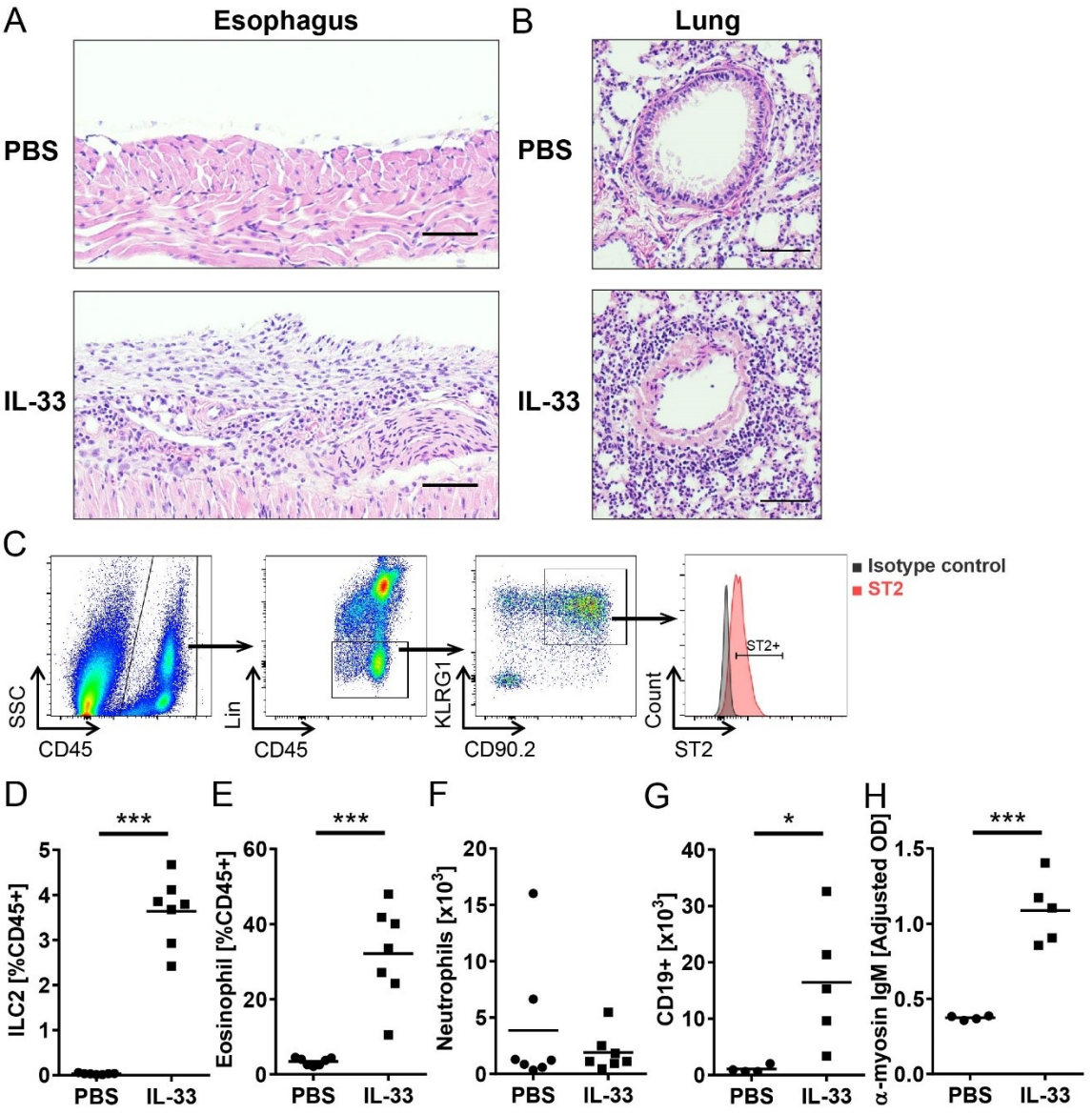

Figure 5. IL-33 treatment induces inflammation in esophagus and lungs and increases the number of B cells in the heart.

(A) Representative images of H&E-stained esophagus section of mice treated with PBS or IL-33. Scale Bars: 50 μm (B) Representative images of H&E-stained lung section of mice treated with PBS or IL-33. Scale Bars: 50 μm (C) Gating strategy of ILC2s in the heart by flow cytometry. Lin includes CD3ε, TCRβ, CD19, B220, CD11b, CD11c, Gr-1, Ter119, FcεRIα and NKp46. Frequency of (D) ILC2s and (E) eosinophils in the hearts. Number of (F) neutrophils and (G) B cells in the hearts. (H) α-myosin IgM in sera of PBS and IL-33-treated animals measured by ELISA. Data are representative of two independent experiments and displayed as the mean. Unpaired t-test (D-H) was used for statistical analysis. *, $P < 0.05$; ***, $P < 0.001$.

IL-33/ST2 signaling pathway is critical for the ILC accumulation in the heart and development of pericarditis

To determine whether the development of pericarditis is dependent on IL-33/ST2 signaling pathway, $ST2^{-/-}$ (*Il1rl1*$^{-/-}$) mice were treated with IL-33. $ST2^{-/-}$ mice were completely protected from the development of IL-33-induced pericarditis, whereas WT mice developed pericarditis (***Figure 6***A and B). The number of total leukocyte infiltrates was significantly reduced in the heart of $ST2^{-/-}$ mice compared to WT mice (***Figure 6***C). To further confirm the dependency of ILC2 accumulation in the heart on IL-33 signaling, we analyzed $Lin^{-}CD90^{+}KLRG1^{+}$ ILC population in the heart of $ST2^{-/-}$ mice after IL-33 treatment. We found that ILCs were significantly reduced in the heart of $ST2^{-/-}$ mice

compared to WT mice (***Figure 6***D and E). The number of eosinophils was also decreased in the heart of $ST2^{-/-}$ mice (***Figure 6***F). This demonstrates that IL-33 signaling through ST2 is important for pericarditis development and IL-33/ST2 signaling axis is required for ILC2 accumulation in the heart. Next, we assessed whether exogenous IL-33 treatment is involved in a positive feedback loop resulting in increased endogenous expression of IL-33. IL-$33^{cit/+}$ reporter mice treated with IL-33 showed higher expression of IL-33 compared to mice treated with PBS, which indicates the presence of a positive feedback loop of IL-33 expression (***Figure 6***G and H). In addition, the number of IL-33 expressing cells was increased after IL-33 treatment (***Figure 6***I). Most of IL-33 expressing cells were $CD45^-$ $CD31^-$ stromal cells, and among them, IL-33 expressing $Sca1^+$ fibroblasts were increased in number in the heart of IL-33-treated mice (***Figure 6***J). $Sca1^+$ fibroblasts were the major IL-33-expressing cells in the heart, suggesting that cardiac fibroblasts have a capacity to produce IL-33 in both naïve state and during cardiac inflammation (***Figure 6***K). We found a correlation of IL-33 and ST2 expression in IL-33-induced pericarditis, indicating the existence of a feed-forward loop where IL-33 induces greater ST2 expression and IL-33 production by fibroblasts (***Figure 7***A). Furthermore, we examined whether endogenous IL-33 is important to induce pericarditis. We found that the number of total $CD45^+$ heart infiltrating leukocytes was reduced in IL-$33^{-/-}$ mice compared to WT mice upon IL-33 treatment (***Figure 7***B). The number of myeloid cells and eosinophils was also decreased in the heart of IL-$33^{-/-}$ mice (***Figure 7***C and D). The lack of endogenous IL-33 affected $Lin^-CD90^+KLRG1^+$ ILC population, which decreased in the heart of IL-$33^{-/-}$ mice (***Figure 7***E). These data suggest that endogenous IL-33 is a required component to induce severe pericarditis induced by exogenous IL-33 administration. Taken together, these data suggest

that IL-33 signaling through ST2 is important for ILC accumulation in the heart and pericarditis development.

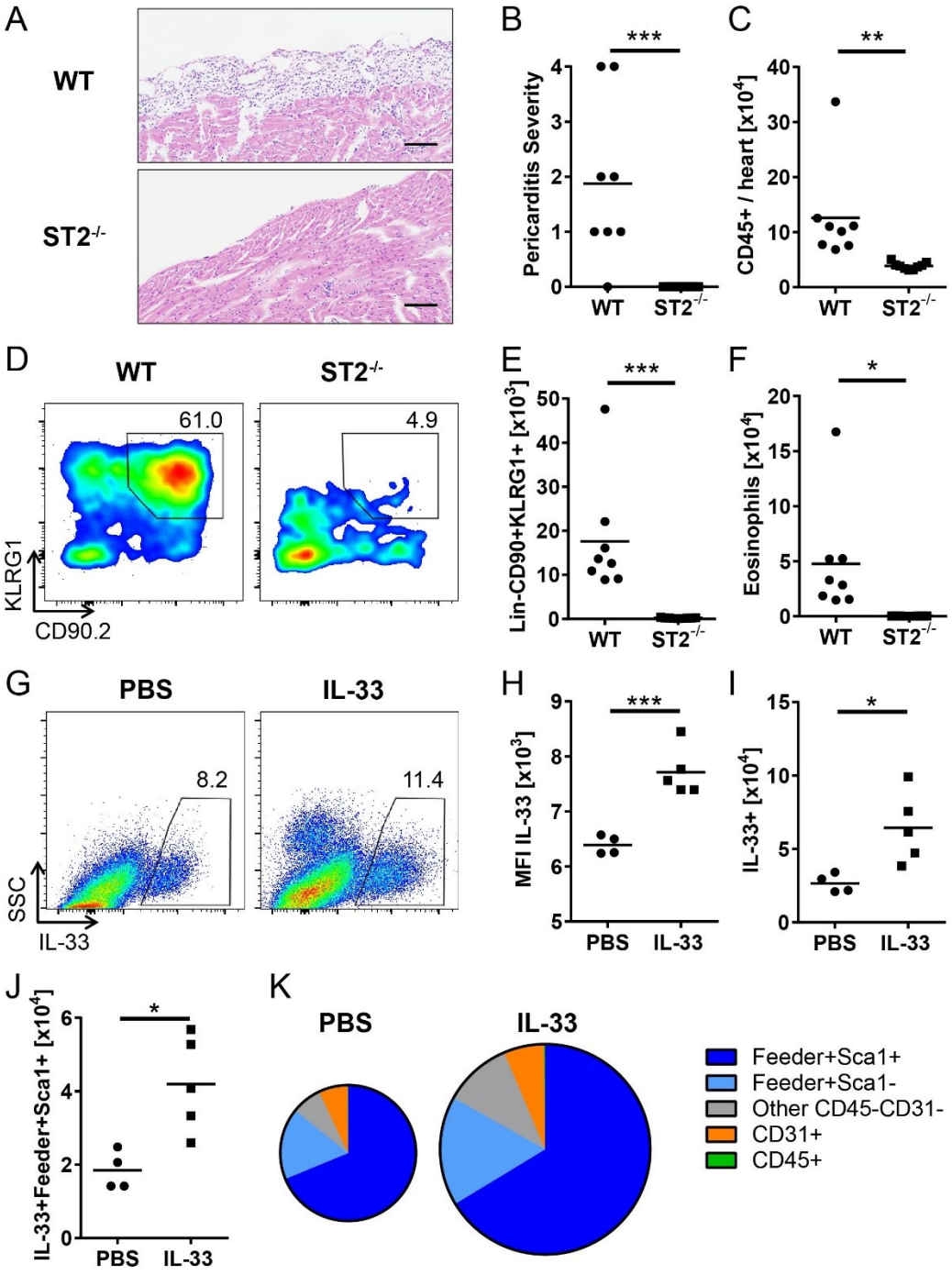

Figure 6. IL-33/ST2 axis is critical for pericarditis development.

(A) Representative images of H&E-stained heart sections of WT and $ST2^{-/-}$ mice. Scale bars: 100 μm. (B) Severity of pericarditis was scored on H&E-stained heart sections. (C) Number of heart-infiltrating $CD45^+$ leukocytes was determined by flow cytometry. (D) Representative flow cytometry plots of $CD45^+Lin^-$ cells. Gates show frequency of $CD90^+KLRG1^+$ ILCs in the heart of WT and $ST2^{-/-}$ mice. Number of (E) $Lin^-CD90^+KLRG1^+$ ILCs and (F) eosinophils in the hearts. (G) Representative flow cytometry plots of cardiac viable cells. Gates show frequency of $IL\text{-}33^+$ cells in the heart of mice treated with PBS or IL-33. (H) Mean fluorescence intensity (MFI) of IL-33 expression in cardiac viable cells. (I) Number of IL-33-expressing cells in the hearts. (J) Total number of $IL\text{-}33^+Sca1^+$ cardiac fibroblasts. (K) Mean frequency of different IL-33-expressing populations. Mean was quantified using values in a PBS-treated group (n=4) and an IL-33-treated group (n=5). Areas of pie charts are proportional to total $IL\text{-}33^+$ cells in the heart. Data are representative of two independent experiments and displayed as the mean. Mann-Whitney U test (B) or unpaired t-test (C, E-F and H-J) was used for statistical analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

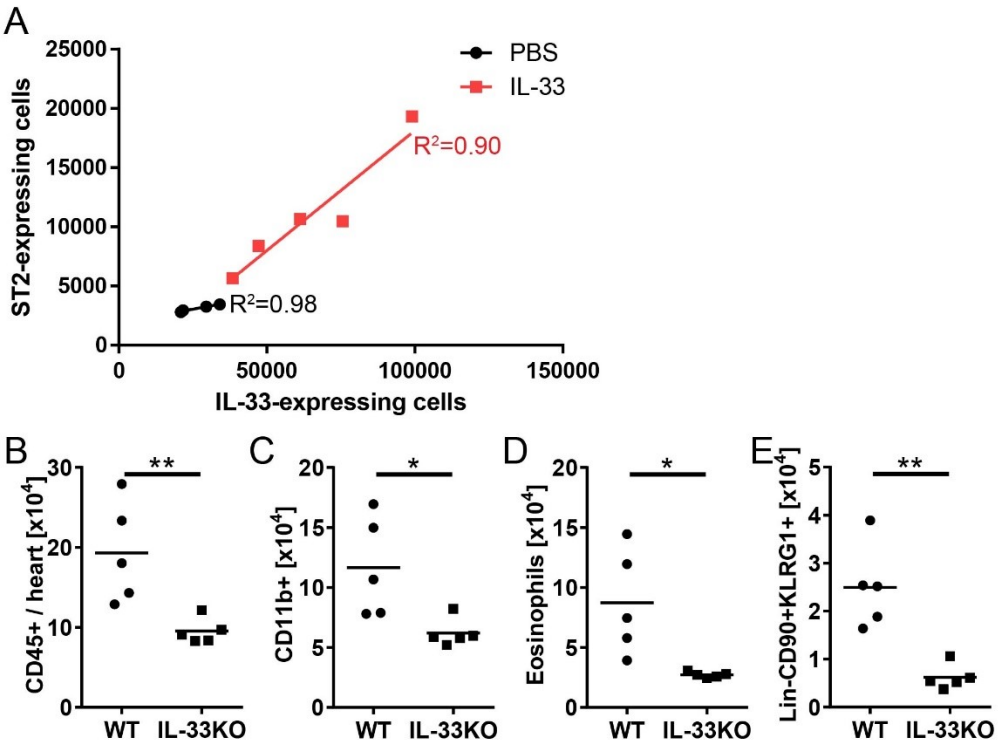

Figure 7. Endogenous IL-33 expression is increased by IL-33 treatment which correlates to ST2 expression and important for pericarditis development.

(A) Correlation between the number of IL-33-expressing cells and the number of ST2-expressing cells in the heart. Pearson correlation coefficient, r, was calculated and r^2 is shown. Number of (B) $CD45^+$ leukocytes, (C) $CD11b^+$ myeloid cells, (D) eosinophils and (E) $Lin^-CD90^+KLRG1^+$ ILCs in the hearts of WT and IL-$33^{-/-}$ mice. Data are representative of two independent experiments.

Unpaired t-test (B-E) was used for statistical analysis. *, $P < 0.05$; **, $P < 0.01$.

ILCs are required for IL-33-induced pericarditis development and sufficient to rescue ILC-deficient mice susceptibility to eosinophil infiltration

To determine whether ILCs are required for the induction of pericarditis, we treated WT, *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice with IL-33. While *Rag2*$^{-/-}$ mice deficient in T cells and B cells developed pericarditis comparable to WT mice, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice, which lack all lymphocytes plus ILCs, were completely protected from the development of pericarditis (***Figure 8***A and B). Thus, the adaptive immune response is not necessary for the induction of pericarditis by IL-33. We assessed leukocytes in the heart and found a significantly reduced number of CD45^{+} cells in the heart of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice compared to both WT and *Rag2*$^{-/-}$ mice (***Figure 8***C). ILC2s were increased in the heart of WT and *Rag2*$^{-/-}$ mice treated with IL-33, whereas ILC2s were not found in the heart of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (***Figure 8***D). IL-33-treated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice showed smaller number of eosinophils, but more neutrophils in the heart than WT and *Rag2*$^{-/-}$ mice (***Figure 8***E and ***Figure 9***A). These data suggest that ILCs are required for the development of pericarditis induced by IL-33 and adaptive immune cells such as T and B cells are not involved in the pathogenesis of IL-33-induced pericarditis. To confirm that IL-33-induced pericarditis is ILC2-dependent, we investigated the ability of transferred ILC2s to reverse the resistance of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice to pericarditis development. We FACS-sorted CD45^{+}Lin^{-}CD90^{+}KLRG1^{+}ST2^{+} ILC2s from the hearts of CD45.1^{+} WT donor mice after IL-33 treatment (***Figure 9***B). Intravenous transfer of ILC2s was not successful and we were not able to detect any transferred ILC2s in the heart of recipient mice (data not shown). Therefore, we expanded FACS-sorted CD45.1^{+} ILC2s *in vitro* and injected them directly to the myocardium of ILC-deficient CD45.2^{+} *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ recipient mice followed by IL-33 treatment (***Figure 9***C). Recipient

mice in a control group were injected with media in the myocardium followed by IL-33 treatment. We were able to detect CD45.1^{+} ILC2s in the hearts of ILC2-transferred CD45.2^{+} *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice on day 9, indicating that ILC2s injected into the myocardium remained (***Figure 8***F). The number of heart-infiltrating CD45.2^{+} leukocytes was increased in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice injected with ILC2s compared to mice injected with media (***Figure 8***G). Eosinophils were significantly increased in the hearts of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice injected with ILC2s compared to mice injected with media, providing evidence of a critical role for cardiac ILC2s in eosinophil recruitment to the heart during the development of pericarditis (***Figure 8***H and I). To summarize, ILCs, but not T cells and B cells, are required for pericarditis development and adoptive transfer of cardiac ILC2s directly to the heart is sufficient to rescue *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice susceptibility to eosinophilic cardiac inflammation induced by IL-33.

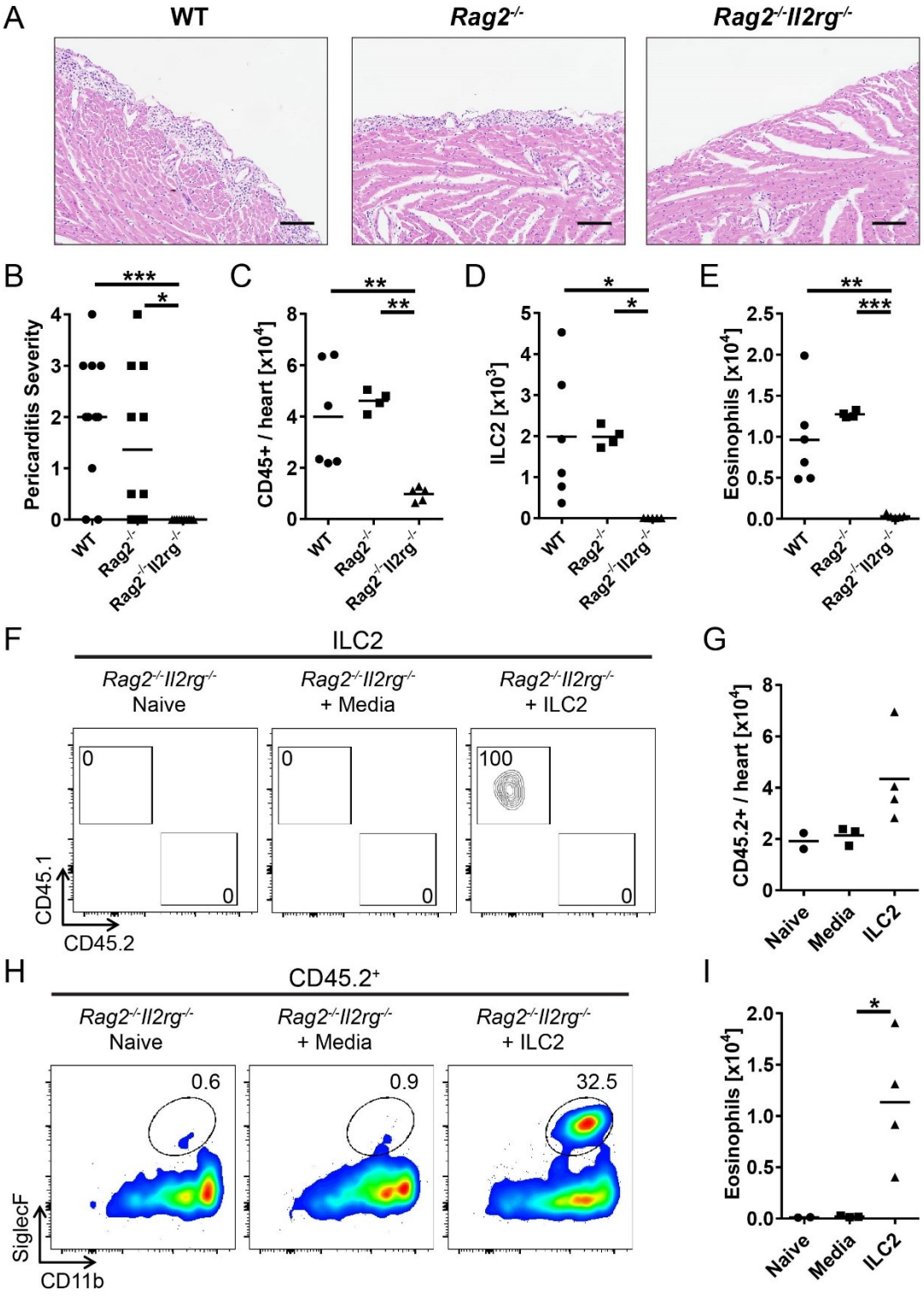
A
WT
Rag2-/-
Rag2-/-Il2rg-/-
B
Pericarditis Severity
C
CD45+ / heart [x10^4]
D
ILC2 [x10^3]
E
Eosinophils [x10^4]
WT
Rag2-/-
Rag2-/-Il2rg-/-
F
ILC2
Rag2-/-Il2rg-/- Naive
Rag2-/-Il2rg-/- + Media
Rag2-/-Il2rg-/- + ILC2
0
100
CD45.1
CD45.2
G
CD45.2+ / heart [x10^4]
Naive
Media
ILC2
H
CD45.2+
0.6
0.9
32.5
SiglecF
CD11b
I
Eosinophils [x10^4]

Figure 8. ILCs are required for the development of pericarditis and ILC2s are sufficient to induce eosinophilic infiltration to the heart.

(A) Representative images of H&E-stained heart sections of WT (left), *Rag2*$^{-/-}$ (middle) and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (right) mice. Scale bars: 100 μm. (B) Severity of pericarditis was scored on H&E-stained heart sections. (C) Total number of heart-infiltrating CD45^{+} leukocytes was determined by flow cytometry. Number of (D) ILC2s and (E) eosinophils in the hearts. (F) Representative flow cytometry plots of ILC2s found in the heart of naïve *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice injected with media or ILC2s followed by IL-33 treatment. (G) Number of heart-infiltrating CD45.2^{+} leukocytes. (H) Representative flow cytometry plots of CD45.2^{+} cells. Gates show frequency of CD11b^{+}SiglecF^{+} eosinophils in the heart of naïve *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice injected with media or ILC2s followed by IL-33 treatment. (I) Number of eosinophils in the hearts. Data are representative of two independent experiments and displayed as the mean. Kruskal-Wallis H test (B) or one-way ANOVA followed by Tukey's post-hoc test (C-E, G and I) was used for statistical analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

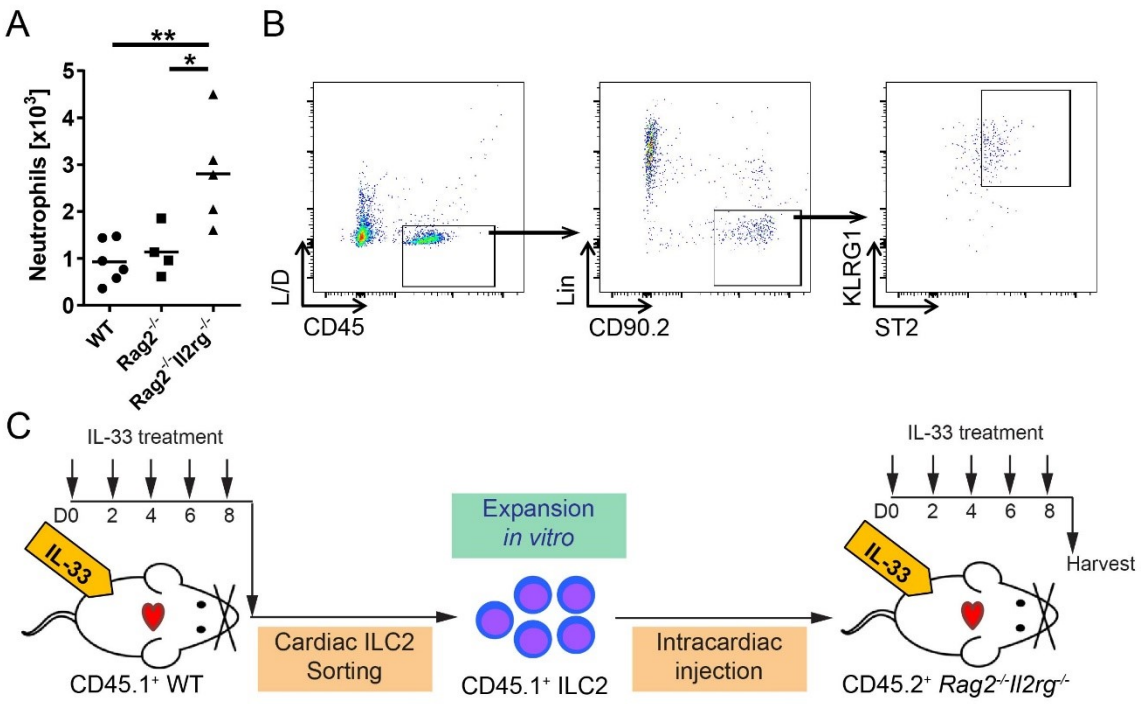

Figure 9. Neutrophils are increased in the heart of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice treated with IL-33 and cardiac ILC2s are isolated from the heart of IL-33 treated mice for transfer to ILC-deficient mice.

(A) Number of neutrophils in the heart of WT, *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. (B) Gating strategy for isolating cardiac ILC2s from IL-33-treated CD45.1^{+} mice by FACS. ILC2s were identified as CD45^{+}Lin^{-}CD90.2^{+}KLRG1^{+}ST2^{+}. (C) Schematic description of CD45.1^{+} WT cardiac ILC2 transfer into the myocardium of CD45.2^{+} *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ by cardiac injection. Data are representative of two independent experiments and displayed as the mean. One-way ANOVA followed by Tukey's post-hoc test (A) was used for statistical analysis. *, $P < 0.05$ **, $P < 0.01$.

ILC2s drive cardiac fibroblasts to upregulate CCL11/eotaxin-1 gene expression

Eotaxin production is known to induce eosinophil trafficking into organs following a chemoattractant gradient [233, 234]. We previously found that the eotaxin-CCR3 pathway is the main mechanism for eosinophil trafficking to the heart [235]. We found that the expression of *Ccl11*, a gene encoding eotaxin-1, was upregulated in the heart of IL-33-treated mice compared to PBS-treated mice, while the expression of *Ccl24* encoding eotaxin-2 was comparable between groups (***Figure 10***A and ***Figure 11***A). Our previous work showed that cardiac fibroblasts produce a diverse set of cytokines and chemokines in response to different Th environments [236]. Specifically, in a Th2 environment during eosinophilic cardiac inflammation, cardiac fibroblasts are the main source of eotaxin-1 [235]. To investigate whether ILC2s and cardiac fibroblasts cooperate to attract eosinophils to the heart in IL-33-induced pericarditis, we devised a co-culture system in which cardiac fibroblasts are co-cultured with ILC2s separated by 0.4 μm transwells (***Figure 10***B). This enabled ILC2s to interact with cardiac fibroblasts through soluble factors such as cytokines, but not through direct contact. We found that cardiac fibroblasts significantly upregulated *Ccl11* expression when co-cultured with ILC2s in the presence of IL-33, compared to cardiac fibroblasts cultured without ILC2s in the absence or presence of IL-33 (***Figure 10***C). *Ccl24* expression by cardiac fibroblasts did not significantly differ (***Figure 11***B). Eotaxin-1 concentration in cell culture supernatant was also significantly increased in the co-culture condition where cardiac fibroblasts are cultured with ILC2s in the presence of IL-33 (***Figure 10***D). In summary, ILC2s are able to stimulate cardiac fibroblasts to produce eotaxin-1 via soluble factors, suggesting a role for ILC2s and cardiac fibroblasts in the recruitment of eosinophils into the heart.

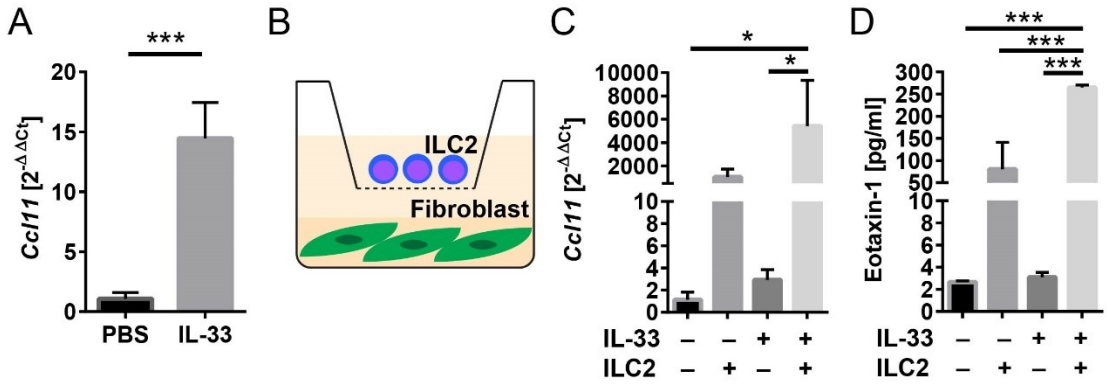

Figure 10. Cardiac fibroblasts increase CCL11/eotaxin-1 expression and production when co-cultured with IL-33-stimulated ILC2s.

(A) Expression of *Ccl11* gene encoding eotaxin-1 in heart homogenates was analyzed by qPCR. (B) Schematic description of cardiac fibroblasts co-culture with ILC2s separated by 0.4 µm transwell. (C) Expression of *Ccl11* in cardiac fibroblasts was analyzed by qPCR. (D) Eotaxin-1 concentrations in cell culture supernatants were measured by ELISA. IL-2 and IL-7 were included in culture media and IL-33 was added where indicated (C and D). Data are representative of two independent experiments and displayed as the mean with SD. Unpaired t-test (A) or one-way ANOVA followed by Tukey's post-hoc test (C and D) was used for statistical analysis. *, $P < 0.05$; ***, $P < 0.001$.

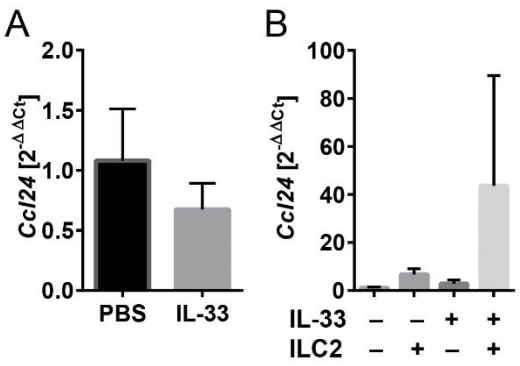

Figure 11. *Ccl24* expression is unchanged in cardiac fibroblasts when co-cultured with IL-33-stimulated ILC2s.

(A) Expression of *Ccl24* gene encoding eotaxin-2 in heart homogenates was analyzed by qPCR. (B) Expression of *Ccl24* in cardiac fibroblasts was analyzed by qPCR. IL-2 and IL-7 were included in culture media and IL-33 was added where indicated. Data are representative of two independent experiments. Data are displayed as the mean with SD. Unpaired t-test (A) or One-way ANOVA followed by Tukey's post-hoc test (B) was used for statistical analysis.

ILC2-derived IL-5 affects the development of IL-33-induced pericarditis

We assessed cytokine production by cardiac ILC2s on day 9 post-IL-33 treatment using flow cytometry. We found that ILC2s in the heart produced both IL-5 and IL-13 after IL-33 treatment (***Figure 12***A). Eighty percent of ILC2s found in the heart produced both IL-5 and IL-13 (***Figure 12***B). To examine the role of IL-5 in the development of pericarditis, we depleted IL-5 in IL-33-treated mice using an anti-IL-5 monoclonal antibody (mAb).

We found that mice injected with IL-5-neutralizing mAb showed a trend of less severe pericarditis by histology after IL-33 administration compared to those injected with isotype control (***Figure 12***C). In addition, we found the total number of leukocytes in the heart was significantly decreased in anti-IL-5-treated mice compared to isotype-treated controls (***Figure 12***D). Eosinophils were also significantly reduced in the heart of anti-IL-5-treated mice compared to isotype treated mice (***Figure 12***E). However, the number of ILC2s was similar between groups (***Figure 12***F). These data show that IL-5 produced by ILC2s drives eosinophil infiltration to the heart and contributes to pericarditis severity. We also examined whether IL-13 plays a role in pericarditis development. IL-13$^{-/-}$ mice showed pericarditis similar to WT mice (***Figure 12***G). We found the number of total leukocytes and eosinophils were analogous between WT and IL-13$^{-/-}$ mice (***Figure 12***H and I). ILC2s did not differ in number between WT and IL-13$^{-/-}$ mice with pericarditis (***Figure 12***J). These data suggest that IL-5 derived from ILC2s affects pericarditis development and cardiac infiltrating immune cells.

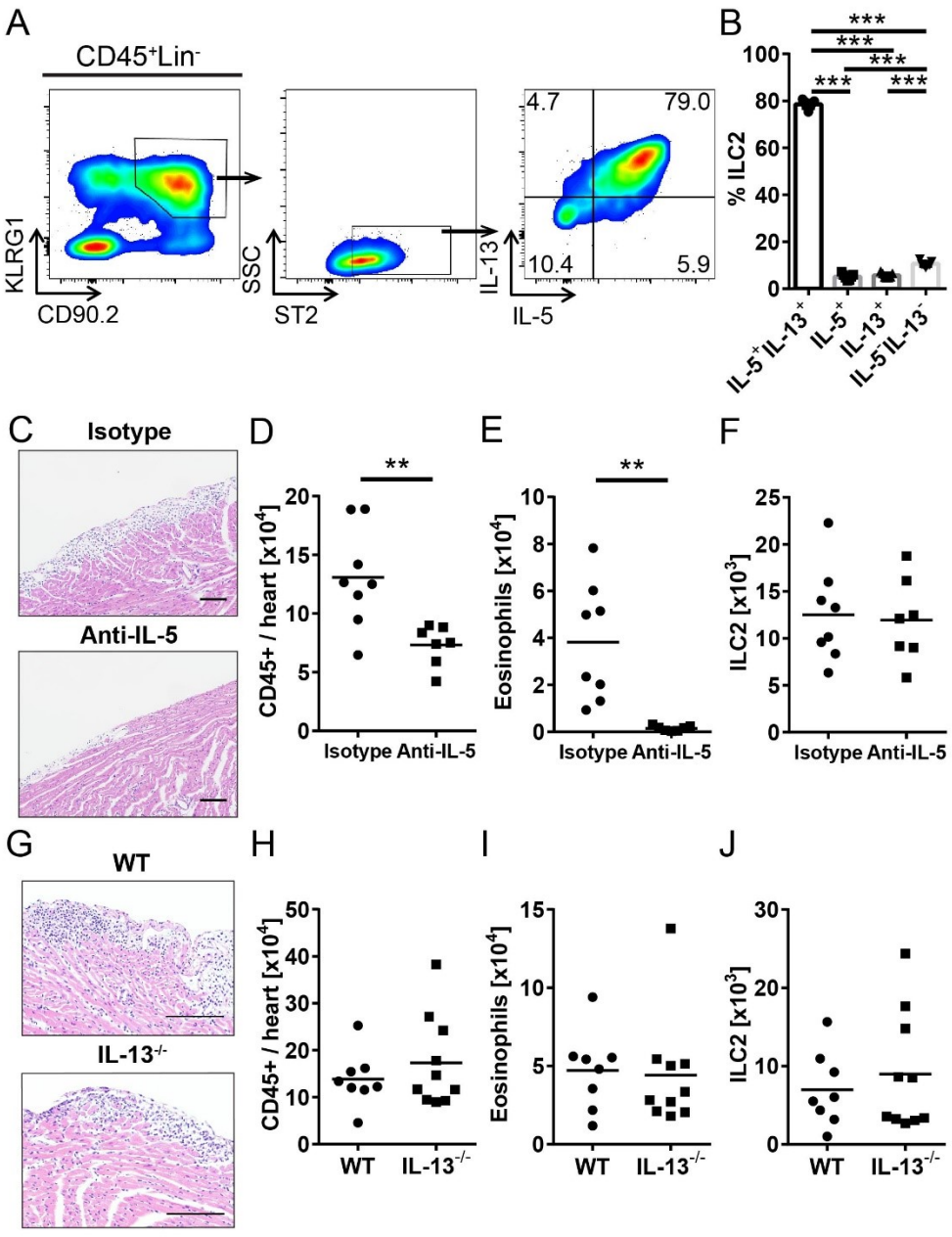

Figure 12. IL-5 produced by ILC2s play a role during the development of pericarditis.

(A) Representative flow cytometry plot of intracellular staining of IL-5 and IL-13 in cardiac ILC2s from IL-33-treated mice. (B) Frequency of ILC2s producing IL-5 and/or IL-13 in the hearts of IL-33-treated mice. (C) Representative images of H&E-stained heart

sections of IL-33-treated mice with isotype or anti-IL-5 administration. Scale bars: 100 μm. (D) Total number of heart-infiltrating $CD45^+$ leukocytes. Number of (E) eosinophils and (F) ILC2s in the hearts of IL-33-treated WT mice with isotype or anti-IL-5 administration. (G) Representative images of H&E-stained heart sections of IL-33-treated WT and IL-$13^{-/-}$ mice. Scale Bars: 100 μm (H) Total number of heart-infiltrating $CD45^+$ leukocytes. Number of (I) eosinophils and (J) ILC2s in the hearts of IL-33-treated WT and IL-$13^{-/-}$ mice. Data are representative of two independent experiments and displayed as the mean. One-way ANOVA followed by Tukey's post-hoc test (B) or unpaired t-test (D-F and H-J) was used for statistical analysis. **, $P < 0.01$; ***, $P < 0.001$.

Eosinophils are present in the mediastinal cavity and can migrate to the heart

Previously, we found that eotaxin-CCR3 pathway is critical for eosinophil migration to the heart during eosinophilic myocarditis [235]. We also showed that ILC2s stimulated by IL-33 promote eotaxin-1 production by cardiac fibroblasts (***Figure 10***C and D). However, given that IL-33-induced cardiac inflammation has a unique disease pattern affecting predominantly the pericardium and myopericardium, we wanted to explore whether eosinophils could migrate from a non-vascular source such as mediastinal cavity, a neighboring serosal cavity. We found that eosinophils resided in the mediastinal cavity of naïve WT mice at a comparable frequency to eosinophils in the heart (***Figure 13***A and B). To examine if eosinophils would be present in the mediastinal cavity in higher numbers in hypereosinophilia, we examined eosinophils in the mediastinal cavity of naïve IL-5 transgenic (IL-5Tg) mice which spontaneously develop tissue and blood eosinophilia [237].

In IL-5Tg mice, we found eosinophils at a high frequency in the mediastinal cavity as well as in the heart and blood (***Figure 13***C and D). Frequency of neutrophils in the mediastinal cavity of both WT and IL-5Tg mice was significantly lower compared to frequency in the blood of these mice, indicating that eosinophils found in the mediastinal cavity were not from blood contamination (***Figure 14***A and B). To determine if eosinophils migrate to the heart preferentially from the mediastinal cavity or through the vasculature, we transferred the same number of eosinophils either to the mediastinal cavity or intravenously to IL-33-treated ΔdblGATA1 mice lacking eosinophils (***Figure 13***E). Both mediastinal cavity and *i.v.* transfers were performed simultaneously to the same animal. Eosinophils injected to the mediastinal cavity and intravenously were labeled with different fluorescent cell tracking dyes, CTV and CTFR, respectively (***Figure 13***E and F). We found eosinophils transferred through both routes in the heart of ΔdblGATA1 mice deficient in eosinophils (***Figure 13***G and H). Eosinophils transferred to the mediastinal cavity were found at a significantly higher frequency in the heart of ΔdblGATA1 mice compared to eosinophils transferred intravenously (***Figure 13***H and I). This indicates that eosinophils can migrate from the mediastinal cavity to the heart more effectively than through vascular routes. Taken together, we demonstrated that eosinophils are present in the mediastinal cavity and that eosinophils can efficiently traffic to the heart from the neighboring serosal cavity. The mediastinal cavity seems to serve as an eosinophil reservoir leading to rapid non-vascular eosinophil trafficking to the heart in IL-33-induced pericarditis model.

In addition, we analyzed expression of integrins and activation marker CD44 on eosinophils found in the mediastinal cavity. Eosinophils are known to express different sets of integrins to mediate their migration to lungs in asthma and also express activation

marker CD44 when stimulated [238, 239]. In naïve mice, mediastinal-cavity eosinophils expressed integrin dimers such as α4β1 (VLA-4, CD49d/CD29) and α4β7 (CD49d/β7) (***Figure 15***A and B). We compared the levels of integrins and CD44 expressed on eosinophils from the heart, mediastinal cavity and blood between naïve and IL-33-treated mice and found differential expression of these markers depending on the location of eosinophils and IL-33 treatment (***Figure 15***C). Interestingly, CD44 expression levels were the highest in mediastinal-cavity eosinophils at steady state, however, blood eosinophils showed the highest CD44 levels after IL-33 treatment. Given that eosinophils from the mediastinal cavity can migrate effectively to the heart, there might be other mechanisms that are currently unclear but important for them to traffic to the heart.

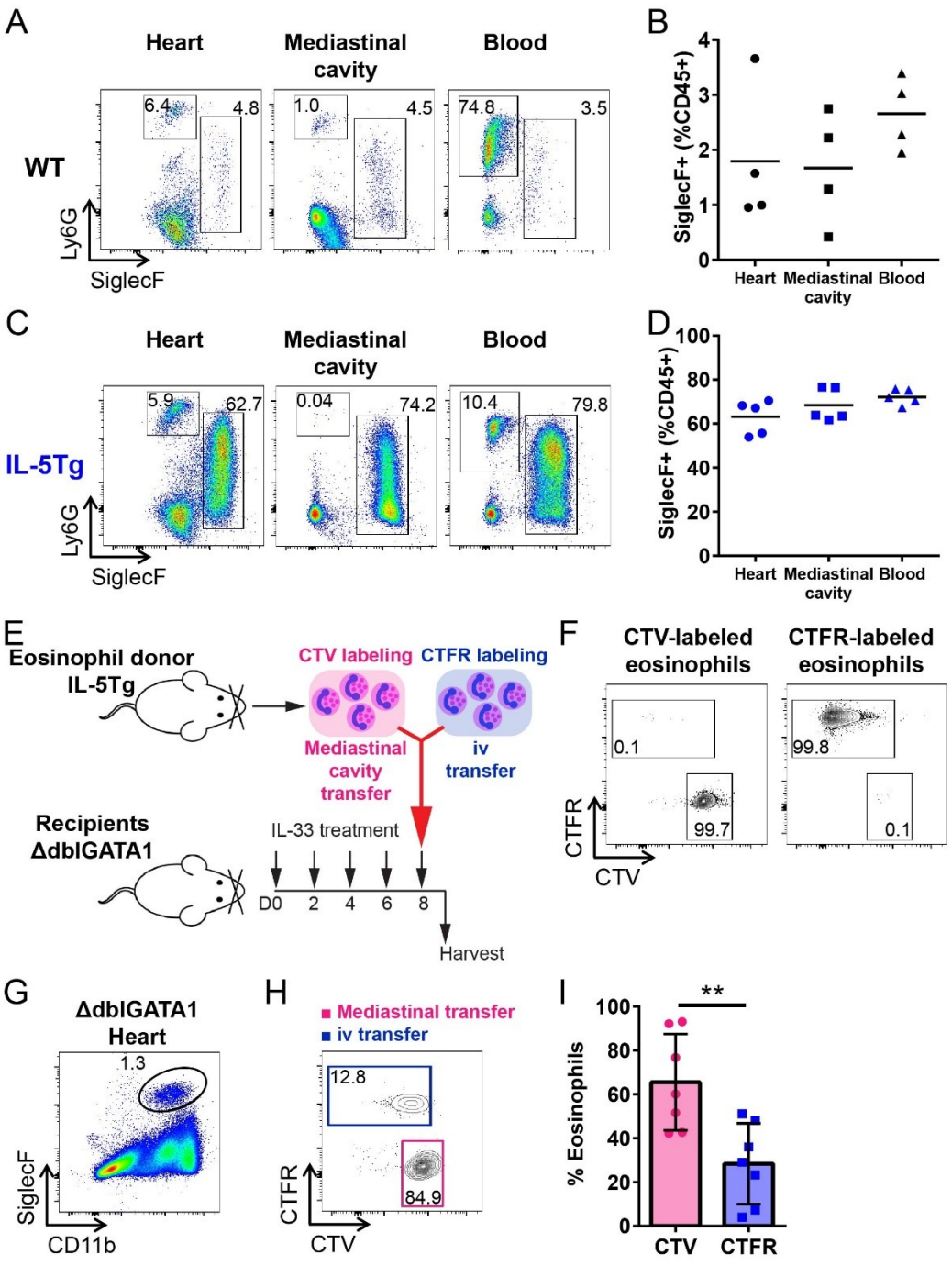

Figure 13. Eosinophils reside in the mediastinal cavity from which these cells can traffic to the heart.

(A) Representative flow cytometry plot of $CD45^+CD11b^+$ cells. Gates show $CD11b^+SiglecF^+$ eosinophils and $CD11b^+Ly6G^+$ neutrophils in the heart, mediastinal cavity and blood of WT naïve mice. (B) Frequency of eosinophils in the heart, mediastinal cavity and blood of WT naïve mice. (C) Representative flow cytometry plot of $CD45^+CD11b^+$ cells. Gates show $CD11b^+SiglecF^+$ eosinophils and $CD11b^+Ly6G^+$ neutrophils in the heart, mediastinal cavity and blood of IL-5Tg naïve mice. (D) Frequency of eosinophils in the heart, mediastinal cavity and blood of IL-5Tg naïve mice. (E) Schematic description of eosinophil transfer to eosinophil-deficient ΔdblGATA1 mice. (F) Flow cytometry plots of CTV- or CTFR-labeled eosinophils. (G) Flow cytometry plot of $CD45^+$ cells in the heart. Gates show $CD11b^+SiglecF^+$ eosinophils found in the heart of ΔdblGATA1 mice treated with IL-33 after eosinophil transfer in the mediastinal cavity and *iv*. (H) CTV- or CTFR-labeled $CD11b^+SiglecF^+$ eosinophils found in the heart of ΔdblGATA1 mice treated with IL-33 after eosinophil transfer. (I) Frequency of CTV- or CTFR-labeled eosinophils found in the heart of ΔdblGATA1 mice treated with IL-33. Concatenated samples (n=7) are shown in G and H. Data are representative of two independent experiments and displayed as the mean. One-way ANOVA followed by Tukey's post-hoc test (B and D) or unpaired t-test (I) was used for statistical analysis. **, $P < 0.01$.

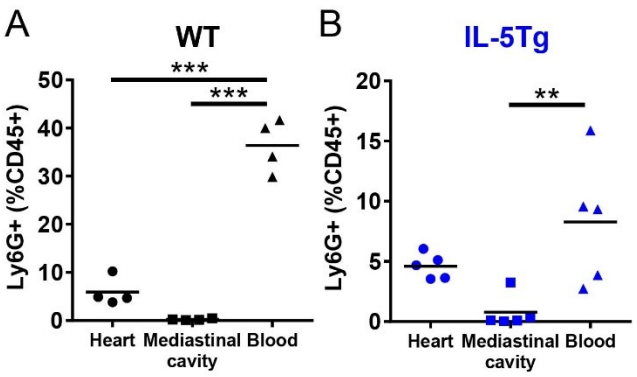

Figure 14. Neutrophils are found less in the mediastinal cavity than in the heart and blood.

(A-B) Frequency of neutrophils in the heart, mediastinal cavity and blood of WT naïve mice (A) and IL-5Tg naïve mice (B). Data are representative of two independent experiments and displayed as the mean. One-way ANOVA followed by Tukey's post-hoc test (A and B) was used for statistical analysis. **, $P < 0.01$; ***, $P < 0.001$.

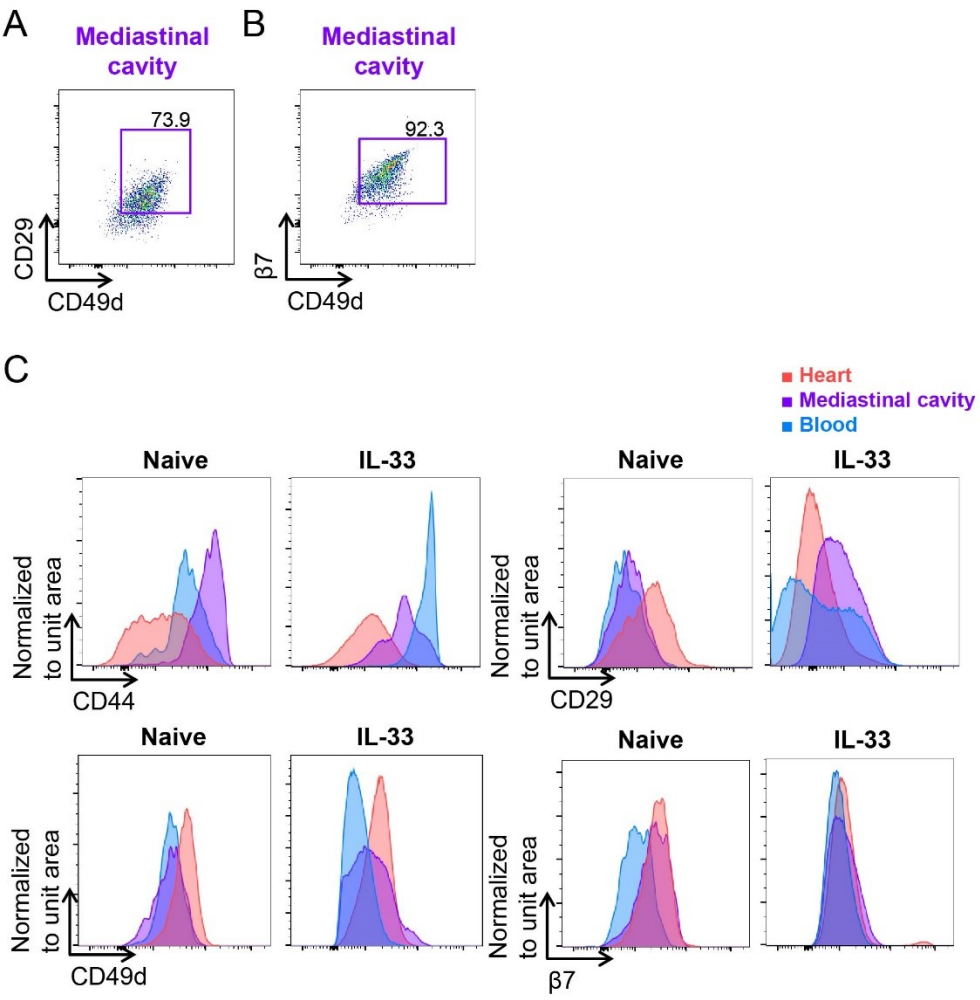

Figure 15. Differential expression levels of integrins and activation marker on eosinophils.

(A-B) Flow cytometry plots of mediastinal-cavity eosinophils in naïve mice expressing (A) VLA-4 (α4β1, CD49d/CD29) and (B) α4β7 (CD49d/β7). (C) Expression levels of CD44, CD29, CD49d and β7 on eosinophils isolated from the heart, mediastinal cavity and blood of naïve and IL-33-treated mice. Concatenated samples (n=5) are shown in each plot. Data are representative of two independent experiments.

Discussion

ILC2s reside in many organs and are potent producers of Th2 cytokines in response to epithelial-derived cytokines, such as IL-33, IL-25 and TSLP. Here we demonstrated that ILC2s accumulate in the heart following IL-33 treatment in mice using a previously described model of IL-33-induced pericarditis [109]. This is the first report of a pathogenic role of ILC2s in the cardiac inflammation, which implicates that cardiac ILC2s activated by IL-33 drive eosinophilic pericarditis in collaboration with cardiac fibroblasts. We identified that ILCs are required for the development of IL-33-induced pericarditis. Given that *Rag2*$^{-/-}$ mice develop comparable cardiac inflammation and pathology to WT mice following IL-33 treatment, we excluded adaptive lymphocytes such as T cells and B cells from being drivers of pericarditis development. The necessity of ILC2s in induction of inflammation has been shown in other organs such as allergen-induced lung inflammation models [34, 196]. Papain causes asthma-like symptoms in *Rag1*$^{-/-}$ mice but not in *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice and ILC-deficient *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice reconstituted with ILC2s develop airway inflammation [34]. While *Rag2*$^{-/-}$ mice lack T cells and B cells, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice are deficient in ILCs in addition to T cells and B cells. Unlike *Rag2*$^{-/-}$ mice, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were protected from IL-33-induced pericarditis, demonstrating ILCs are required for pericarditis development. Our adoptive transfer experiments showed that cardiac ILC2s transferred directly to the heart of pericarditis-resistant *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice elicited cardiac eosinophil infiltration. These findings support the notion that ILCs are tissue resident cells

even during acute inflammation as described by us in the heart and others in different organs [30, 155, 219].

The IL-33-induced pericarditis model allowed us to investigate the pathogenic role of IL-33/ST2 axis in ILC2 activation during cardiac inflammation. IL-33 mediates its effects through binding to its receptor ST2 as we demonstrated in cardiac ILC2 activation [48]. ST2 is known to be expressed not only on ILC2s but also on Th2 cells, B cells, basophils, eosinophils, dendritic cells, mast cells and natural killer T cells [240]. However, using an adoptive transfer experiment, we showed that ILC2s are essential for IL-33-induced cardiac inflammation. It was reported that IL-33 expression increases during cardiac inflammation or after cardiac injury, representing an association between IL-33 and its pathological outcome in cardiac inflammation [60, 241]. ST2 is not only expressed as a membrane-bound form, but is also produced as a soluble form (soluble ST2, sST2) that is released into the circulation. sST2 has been proposed as a prognostic marker for chronic and acute heart failure and aortic stenosis [113, 242-244]. These facts are consistent with our findings that IL-33/ST2 axis plays a key pro-inflammatory role in the heart by stimulating cardiac ILC2s under certain conditions related to increased IL-33 production. It should be noted that IL-33 signaling via ST2 could play a beneficial role in different types of heart diseases such as pressure overload and myocardial infarction by providing improved cardiac function and survival [72, 110].

We showed that IL-33 is expressed in naïve heart suggesting its role in cardiac homeostasis. When mice were treated with exogenous IL-33, its endogenous expression was increased

in the heart, thus offering the evidence for the presence of a positive feedback loop in the heart. This result also suggests that the IL-33-induced pericarditis model does not rely solely on exogenous IL-33 administration, but endogenous IL-33 might contribute to increased IL-33 level in the heart leading to disease development. We identified that $Sca1^{+}$ cardiac fibroblasts are major producers of IL-33 in the heart both in steady state and during pericarditis. This result is in agreement with our previous findings that cardiac fibroblasts are a versatile stromal cell type capable of producing different sets of cytokines and chemokines in response to changes in microenvironment [235, 236]. Although it has been reported that human endothelial cells could be an important source for IL-33, we did not find mouse endothelial cells to be a major producer of IL-33 in the heart [56, 58, 59]. In a myocardial pressure overload mouse model, IL-33 has been shown to be derived from endothelial cells [60]. Cardiomyocytes have been also shown to constitutively produce IL-33 in human heart [245]. Diverse types of cells could produce IL-33 depending on different stimuli and the microenvironment. In our model, we found that cardiac fibroblasts are potent IL-33 producers and increase IL-33 production in response to systemic IL-33 administration, suggesting the existence of a feed-forward loop.

ILC2s produce Th2 cytokines such as IL-5 and IL-13 upon activation with IL-33. We were able to show that IL-33-activated ILC2s induce eotaxin-1 (CCL11) production by cardiac fibroblasts. This is consistent with our previous findings that cardiac fibroblasts are the major CCL11 producers in eosinophilic myocarditis mouse model, and CCL11 expression is induced in cardiac fibroblasts *in vitro* in response to IL-4 and IL-13 [235]. It has been reported that IL-13 potently induces eotaxin expression in lung epithelial cells and

esophagus [246-248]. Furthermore, in humans, eotaxin expression was induced by IL-13 production by various cell types in different organs [249-251]. We found, *in vivo*, that cardiac ILC2s are potent producers of IL-13 upon IL-33 activation. Furthermore, cardiac ILC2s are capable of inducing the production of CCL11 by cardiac fibroblast *in vitro*. Given the ability of IL-13 to induce eotaxin expression, these results support a pathogenic role of cardiac ILC2s in driving eosinophilic pericarditis potentially through IL-13 derived from IL-33-activated ILC2s.

In this study, we found that cardiac ILC2s produce IL-5 and IL-13 which play an important role in developing IL-33-induced pericarditis and that blocking of IL-5 protected mice from eosinophilic pericarditis development. In response to IL-5, eosinophils are released into circulation which might be hindered by blocking IL-5. Several anti-IL-5 agents including mepolizumab, reslizumab and benralizumab, which block IL-5 signaling via IL-5 receptor and reduce blood eosinophils, have been developed, approved and used to treat asthma in a clinical practice [252]. In addition, a Phase 2 clinical trial is currently evaluating the safety and efficacy of benralizumap, an anti-IL-5 receptor biologic, in decreasing eosinophils in patients with hypereosinophilic syndrome (ClinicalTrials.gov Identifier: NCT02130882). As shown in our IL-5 blocking experiment, anti-IL-5 treatment had a profound effect on reducing eosinophils infiltrating to the heart during pericarditis. This finding proposes anti-IL-5/IL-5R agents as a potential treatment option for eosinophilic pericarditis. Future studies should explore clinical biomarkers, such as an increased IL-33 level or an increase in ILCs present in pericardial effusion, for disease management or as therapeutic targets for pericarditis.

IL-33-induced cardiac inflammation has a unique pattern affecting mostly pericardium and myopericardium, thus we explored whether eosinophils could migrate from a non-vascular source such as mediastinal cavity, a neighboring serosal cavity. We identified that eosinophils are present in the mediastinal cavity of naïve mice and that adoptively transferred eosinophils to the mediastinal cavity of IL-33-treated eosinophil-deficient mice are able to traffic to the heart. This suggests that the mediastinal cavity might serve as a reservoir of cardiac infiltrating eosinophils. Immune cells have been previously identified in serous cavities such as peritoneum and pleural cavity [253-256]. Macrophages residing in the peritoneal cavity are rapidly recruited to the injured liver through a non-vascular route and display tissue reparative phenotypes [255]. The pleural space is also shown to possess B1a B cells which can migrate to the lung and produce protective IgM in response to bacterial infection [256]. We found that eosinophils residing in the mediastinal cavity migrate to the heart more effectively than *i.v.* transferred eosinophils. It does not exclude the contribution of circulating eosinophils to pericarditis development, however, it is noteworthy that the mediastinal cavity can serve as a reservoir of eosinophils which might lead to rapid infiltration during inflammation in close proximity to the heart. Interestingly, IL-33 treatment also induces inflammation in lungs and esophagus as we demonstrated here and shown before by others [128, 129]. After treating mice with IL-33, we found pericardial, subserosal and peribronchial eosinophil infiltrates in the heart, esophagus and lungs, respectively. IL-33-induced esophagitis has a subserosal inflammation pattern similar to IL-33-induced pericarditis with the inflammation mostly confined to the subserosa rather than to the mucosa. Based on these observations, we speculate that the

mediastinal cavity could also be a source of eosinophils for other organs located in the mediastinal cavity. Taken together, our findings suggest that ILC2s might direct eosinophils to infiltrate organs not only from the blood, but also from the neighboring serosal cavity where these cells reside locally.

In addition to ILC2s and eosinophils, we found that the number of B cells increase in the heart after IL-33 treatment. Serosal fluid in pericardium contains immune cells including B cells and can become a source of infiltrating leukocytes under certain insults such as exposure following cardiac surgery and myocardial infarction [257, 258]. Whether B cells found in IL-33-induced pericarditis originate from pericardial fluid is unclear. However, the presence of B cells in the heart and anti-myosin IgM antibody in circulation suggests that these factors might play a pathogenic role in myopericarditis. Anti-myosin antibodies were found in sera of patients with myocarditis or myocardial infarction suggesting the presence of these autoantibodies and their potential contribution to long-term myocardial damage and dysfunction in heart diseases [259, 260].

Although many pericarditis case reports have been published, the etiology is still largely unknown. Current treatments for pericarditis mostly aim to resolve symptoms by administrating anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs (NSAIDs, especially indomethacine), colchicine and corticosteroids [85, 261]. Ongoing Phase 3 clinical trial is assessing IL-1 blocker in patients with recurrent pericarditis (ClinicalTrials.gov Identifier: NCT03737110). Another treatment option for severe cases is a pericardiocentesis or pericardiectomy to remove the inflamed pericardium. These

symptomatic therapies, however, do not address a specific cause of pericarditis. One of the problems in developing new targeted therapies is a lack of classification on subtypes of pericarditis. There are several case reports of eosinophilic pericarditis in human [262-266]. In this study, using IL-33-induced eosinophilic pericarditis mouse model, we identified cardiac ILC2s playing a critical role in the pathogenesis of eosinophilic pericarditis. These findings augment our understanding of how ILC2s contribute to cardiac inflammation and provide insights into the targeted therapy for eosinophilic pericarditis.

Chapter 5. Conclusions and Future Directions

We reviewed the roles for IL-33 in diverse inflammatory diseases and potential clinical applications of IL-33/ST2 axis. The effect of IL-33 signaling pathway can be either protective or pathogenic depending on sites of inflammation, inflammation-inducing agents and tissue microenvironments. Thus, care should be taken when therapeutic strategies targeting IL-33/ST2 signaling pathway are made. We also discussed how ILC2s contribute to defending against infection, regulating inflammation and maintaining metabolic homeostasis. ILC2s play a pivotal role in many biological processes not only as effector cells in innate immunity but also as regulators of inflammation and homeostasis in

diverse organs. Further studies are needed to utilize current understandings on the role for ILC2s in different settings for translational applications.

In the experimental study, we established a critical role for ILCs in cardiac inflammation using pericarditis mouse model. We identified the accumulation of ILC2s in the heart with a marked increase of eosinophils infiltrating to the heart during IL-33-induced pericarditis. Diastolic dysfunction was observed in IL-33-treated mice, indicating that inflammation occurring in the pericardium results in not only infiltration of immune cells but also adverse cardiac function. IL-33/ST2 signaling axis is essential to expand cardiac ILCs and drive pericarditis. The lack of IL-33 or ST2 diminished the severity of inflammation with reduced immune cells in the heart. In addition, we revealed the existence of IL-33 feedback loop in which cardiac fibroblast are a major source of endogenous IL-33. Exogenous IL-33 amplifies endogenous IL-33 expression which is needed to drive severe pericarditis. We demonstrated that ILCs are required for pericarditis development whereas adaptive immune cells such as T cells and B cells are not responsible for pericarditis. Cardiac ILC2 transfer to the heart of ILC-deficient mice led to eosinophil infiltration in these mice which are originally resistant to pericarditis upon IL-33 treatment. Moreover, in a co-culture setting, we found the stimulation of cardiac fibroblasts with soluble factors secreted from ILC2s yields upregulation of expression levels of eotaxin-1 and hence increased secretion of eotaxin-1 from cardiac fibroblasts. We showed that cardiac ILC2s are a potent producer of type 2 cytokines, IL-5 and IL-13, *in vivo*. Blocking IL-5 using monoclonal antibody attenuated cardiac inflammation including infiltrating eosinophils. Furthermore, we demonstrated that eosinophils reside in the mediastinal cavity where the heart is located inside. Eosinophils transferred in the mediastinal cavity migrated to the heart more

effectively than intravenously transferred eosinophils. This result suggests that eosinophils in the mediastinal cavity might contribute to cardiac eosinophilic inflammation in addition to circulating eosinophils, although a potential mechanism on eosinophil trafficking from the mediastinal cavity to the heart needs to be elucidated. Our findings suggest that ILC2s play a pathogenic role in driving pericarditis induced by IL-33 and that it might be possible to alleviate eosinophilic pericarditis by blocking IL-33-ST2-ILC2s axis.

Several questions remain to be explored and answered. We were unable to show the location of cardiac ILC2s. Although ILC2s play a pivotal role in driving pericarditis, they are a rare population which is challenging to locate within the heart. We speculate that they might reside near the pericardium given the phenotype of inflammation observed after IL-33 treatment, however, it is also possible that they are present in the myocardium. A recent study showed that ILC2s are present in perivascular regions and localize with fibroblast-like adventitial stromal cells which express IL-33 [267]. Cardiac ILC2s might be also located with cardiac fibroblasts which were shown to express IL-33 in our study. With advanced techniques such as clearing tissue and light sheet fluorescence microscopy, it may be possible to visualize cardiac ILC2s.

It would be of interest to see whether IL-33 and ILC2s are correlated with eosinophilic pericarditis in humans as our results in mice. IL-33 and sST2 have been proposed or are being used as a biomarker in certain cardiovascular diseases [112, 241]. However, supporting evidence is not sufficient to propose the use of IL-33 or sST2 as a prognostic marker for pericarditis. Access to human samples rarely happen since, in many cases, pericarditis is resolved with anti-inflammatory agents and does not require surgery. Patients with a severe pericardial effusion might need to undergo pericardiocentesis.

During the procedure the fluid can be obtained and tested for levels of IL-33. It should be noted that eosinophilic pericarditis is a rare condition, but at the same time, can be one of manifestations of other diseases such as hypereosinophilic syndromes. It would still be intriguing to see whether IL-33/ST2 axis and ILC2s are involved in pericarditis and how these components affect the outcome in humans. IL-1 blocker, a fusion protein comprised of the ligand binding domains of IL-1RI and IL-1RAcP, for the use in recurrent pericarditis is currently being evaluated in a phase 3 clinical trial (ClinicalTrials.gov Identifier: NCT03737110). In addition, as discussed before, there are currently several anti-IL-33 and anti-ST2 being evaluated for the potential use as therapeutics to treat diverse allergic inflammation in clinical trials (***Table 4***). Our findings suggest a possible application of these monoclonal antibodies against IL-33 and ST2 to block IL-33/ST2 signaling in a type 2 cardiac inflammation including eosinophilic pericarditis. Since ILC2s express redundant markers which are shared with other immune cells, it might be difficult to specifically target ILC2s *in vivo*. The neutralization of IL-33/ST2 signaling pathway could be a practical approach to regulate IL-33/ST2-ILC2 axis for the treatment of eosinophilic pericarditis in addition to allergic inflammation.

Future studies will focus on the role of cardiac ILCs at the steady state. The questions to be addressed are: What is the role of cardiac ILCs at steady state? How do ILCs contribute to homeostasis of the heart? What other cells are affected by ILCs at steady state? Are ILCs beneficial or harmful to health of the heart? Recently we have shown that $CD45^+$ leukocytes are a main producer of type 2 cytokines, IL-5 and IL-13 in the heart of naïve WT mice (***Figure 16***A and B). Among these cells, ILCs were a major IL-5 and IL-13 producing population at steady state (***Figure 16***C). We further assessed the role of IL-13

in healthy hearts. IL-13Rα, a component of IL-13 signaling receptor, was expressed by F4/80^{+} cardiac macrophages (***Figure 16***D). This suggests that IL-13-producing ILCs may interact with cardiac macrophages locally. The number of total CD45^{+} leukocytes in the heart was found to be unchanged in naïve IL-13$^{-/-}$ mice compared to WT mice, however, neutrophils were increased in the heart of IL-13$^{-/-}$ mice (***Figure 16***E and F). This suggests a possible role for ILCs in inhibiting neutrophil infiltration to the heart at steady state. Moreover, we found that although the number of cardiac macrophages remained comparable between naïve WT and IL-13$^{-/-}$ mice, the expression of CD206, one of markers for alternative activated macrophages, was slightly upregulated in the heart of IL-13$^{-/-}$ mice (***Figure 16***G, H and I). This result indicates ILCs might perturb the activation status of cardiac resident macrophages. Our data suggest that ILCs might play a role in regulating other resident and infiltrating immune cells in the heart which in turn could affect homeostasis of the heart (***Figure 17***).

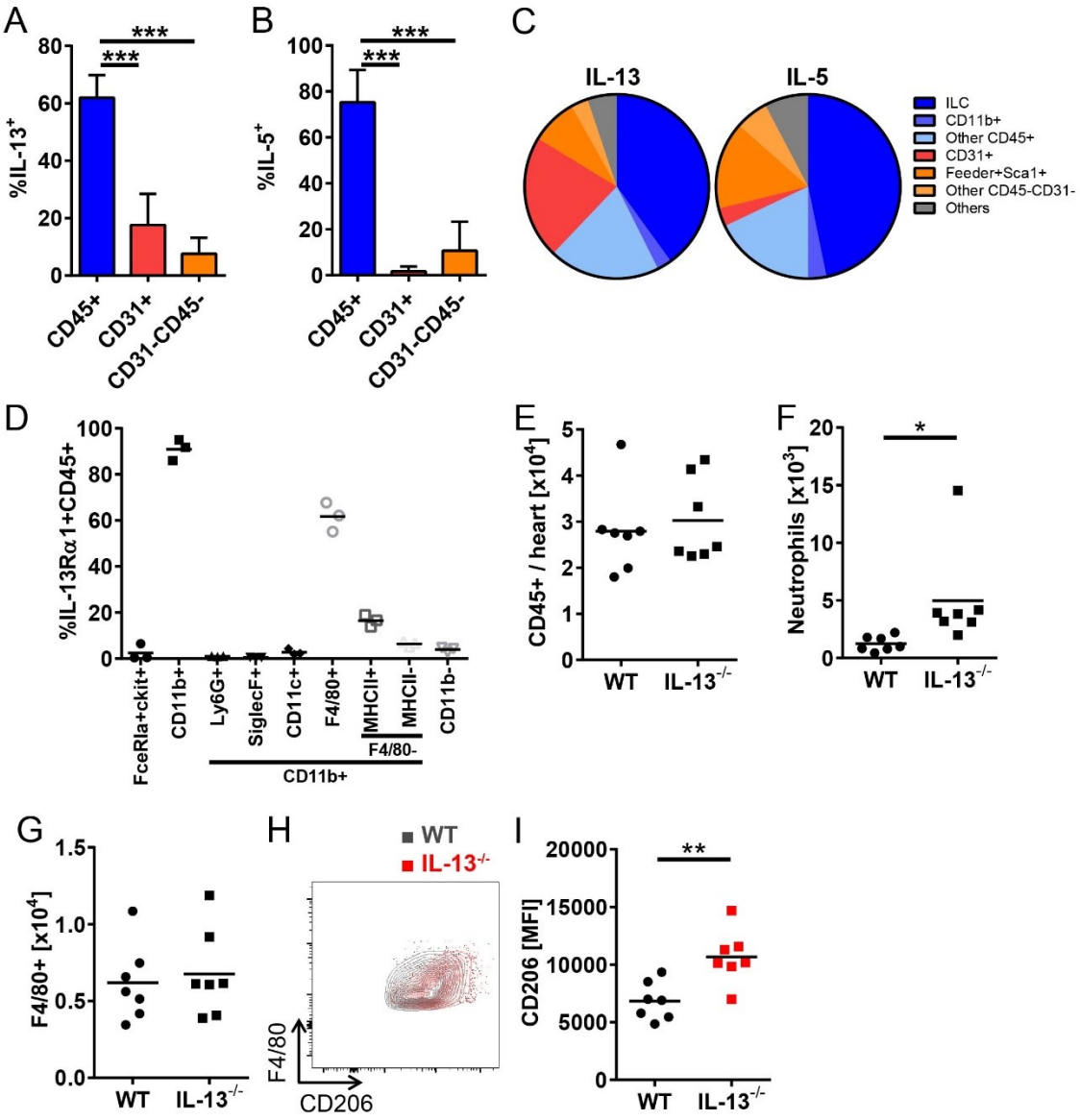

Figure 16. Cardiac ILCs are a key producer of IL-5 and IL-13 at steady state and IL-13 affect neutrophils and macrophages in naïve heart.

(A-B) Frequency of (A) IL-13 and (B) IL-5-expressing populations in the heart of naïve WT mice. Mean was quantified using values in naïve WT mice (n=5). (C) Mean frequency of IL-13 and IL-5 expressing cells in the naïve heart. (D) IL-13Rα-expressing leukocytes

in the heart of naïve WT mice. Severity of pericarditis was scored on H&E-stained heart sections. (E-G) Number of (E) $CD45^{+}$ leukocytes, (F) neutrophils and (G) macrophages in the heart of naïve WT and IL-$13^{-/-}$ mice. (H) Flow cytometry plot of CD206 expression on macrophages in the heart of naïve WT and IL-$13^{-/-}$ mice. (I) Mean fluorescence intensity of CD206 expressed by macrophages. Data are representative of two independent experiments and displayed as the mean. Unpaired t-test (E-G and I) was used for statistical analysis. *, $P < 0.05$; **, $P < 0.01$.

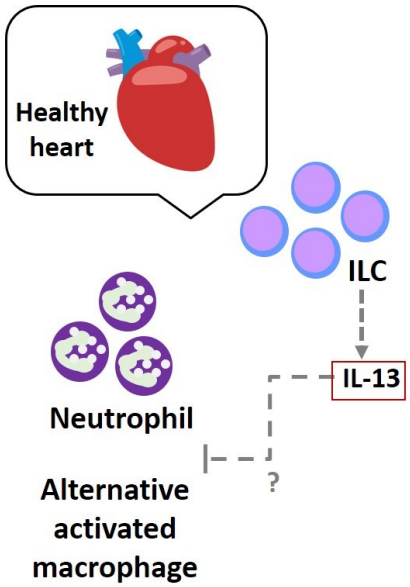

Figure 17. Potential role of cardiac ILCs in the homeostasis of the heart.

Uncovering roles of ILCs at steady state in the heart is of interest as we find ILCs are cardiac resident cells and produce a majority of type 2-associated cytokines in naïve mice.

We currently plan to investigate whether cardiac resident macrophages are altered in naïve IL-13$^{-/-}$ mice in terms of their expression of markers related to activation and regulation. We are able to utilize more diverse markers of macrophages than before to identify the status of macrophages. Both ILCs and macrophages are known to have plasticity to react to microenvironments which enable these cells to respond to different conditions and play an important role both in homeostasis and inflammation. Furthermore, we plan to study the role of ILCs and IL-13 in homeostasis of the heart using aged mice. Cardiac function declines as aging occurs, however, its association with cardiac resident immune cells is poorly understood. Functional studies using echocardiography would provide insight on how ILCs and their production of IL-13 influence cardiac function in a long-term perspective. Although we are currently unable to decide at this point if ILCs are beneficial or harmful to the heart, it would be interesting to see whether ILCs regulate homeostasis of the heart.

In summary, our findings demonstrate that ILCs play a critical role in the heart and cardiac inflammation. ILC2s drive pericarditis development by cooperating with cardiac fibroblasts which in turn could lead to severe inflammation (***Figure 18***). We did not rule out the possibility of other immune cells and stromal cells involved in the pathogenesis of pericarditis. It might be possible that cardiac ILC2s affect other cell types in the heart with unknown mechanisms. Advanced techniques such as single cell RNA sequencing and real time *in vivo* imaging with multiparameter fluorescence microscopy would be of help to further identify cardiac ILCs and investigate roles of cardiac ILCs in the homeostasis and inflammation.

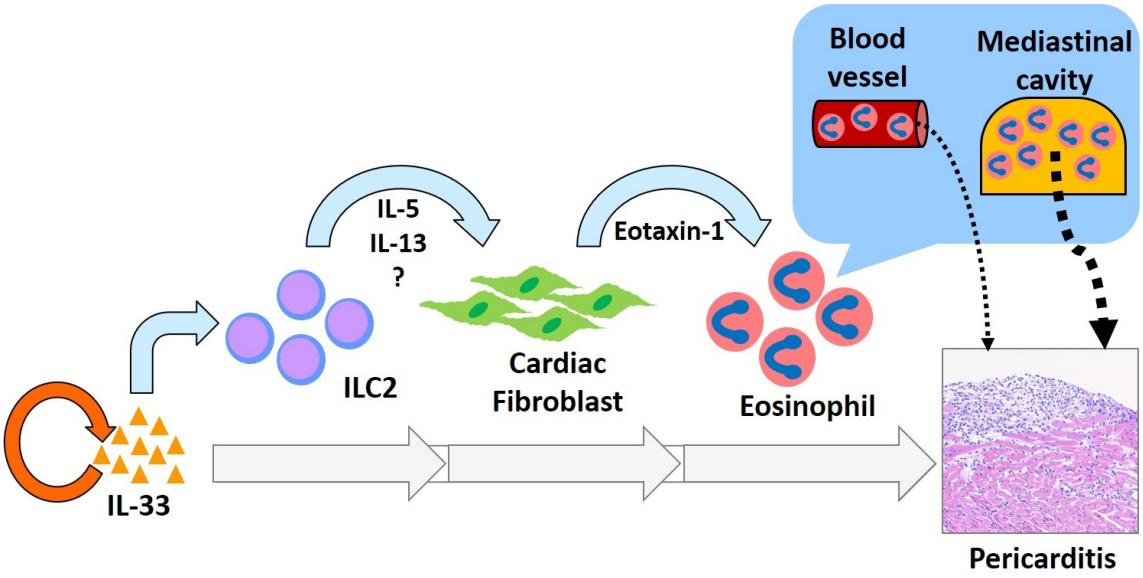

Figure 18. Schematic summary of main findings on the role of ILC2s in pericarditis development.

References

1. Spits, H., et al., *Innate lymphoid cells--a proposal for uniform nomenclature.* Nat Rev Immunol, 2013. **13**(2): p. 145-9.
2. Eberl, G., et al., *Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology.* Science, 2015. **348**(6237): p. aaa6566.
3. Artis, D. and H. Spits, *The biology of innate lymphoid cells.* Nature, 2015. **517**(7534): p. 293-301.
4. Vivier, E., et al., *Innate Lymphoid Cells: 10 Years On.* Cell, 2018. **174**(5): p. 1054-1066.
5. Mebius, R.E., P. Rennert, and I.L. Weissman, *Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells.* Immunity, 1997. **7**(4): p. 493-504.
6. Klose, C.S.N., et al., *Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages.* Cell, 2014. **157**(2): p. 340-356.
7. Constantinides, M.G., et al., *A committed precursor to innate lymphoid cells.* Nature, 2014. **508**(7496): p. 397-401.
8. Moro, K., et al., *Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells.* Nature, 2010. **463**(7280): p. 540-4.
9. Monticelli, L.A., et al., *Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus.* Nat Immunol, 2011. **12**(11): p. 1045-54.
10. Cherrier, M., S. Sawa, and G. Eberl, *Notch, Id2, and RORgammat sequentially orchestrate the fetal development of lymphoid tissue inducer cells.* J Exp Med, 2012. **209**(4): p. 729-40.
11. Hoyler, T., et al., *The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells.* Immunity, 2012. **37**(4): p. 634-48.
12. Yokota, Y., et al., *Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2.* Nature, 1999. **397**(6721): p. 702-6.
13. Diefenbach, A., M. Colonna, and S. Koyasu, *Development, differentiation, and diversity of innate lymphoid cells.* Immunity, 2014. **41**(3): p. 354-365.
14. Daussy, C., et al., *T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow.* J Exp Med, 2014. **211**(3): p. 563-77.

15. Mjosberg, J., et al., *The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells.* Immunity, 2012. **37**(4): p. 649-59.
16. Klein Wolterink, R.G., et al., *Essential, dose-dependent role for the transcription factor Gata3 in the development of IL-5+ and IL-13+ type 2 innate lymphoid cells.* Proc Natl Acad Sci U S A, 2013. **110**(25): p. 10240-5.
17. Furusawa, J., et al., *Critical role of p38 and GATA3 in natural helper cell function.* J Immunol, 2013. **191**(4): p. 1818-26.
18. Wong, S.H., et al., *Transcription factor RORalpha is critical for nuocyte development.* Nat Immunol, 2012. **13**(3): p. 229-36.
19. Yagi, R., et al., *The transcription factor GATA3 is critical for the development of all IL-7Ralpha-expressing innate lymphoid cells.* Immunity, 2014. **40**(3): p. 378-88.
20. Eberl, G., et al., *An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells.* Nat Immunol, 2004. **5**(1): p. 64-73.
21. Satoh-Takayama, N., et al., *Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense.* Immunity, 2008. **29**(6): p. 958-70.
22. Lim, A.I., et al., *Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation.* Cell, 2017. **168**(6): p. 1086-1100 e10.
23. Bernink, J.H., et al., *Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues.* Nat Immunol, 2013. **14**(3): p. 221-9.
24. Fuchs, A., et al., *Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-gamma-producing cells.* Immunity, 2013. **38**(4): p. 769-81.
25. Neill, D.R., et al., *Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity.* Nature, 2010. **464**(7293): p. 1367-70.
26. Price, A.E., et al., *Systemically dispersed innate IL-13-expressing cells in type 2 immunity.* Proc Natl Acad Sci U S A, 2010. **107**(25): p. 11489-94.
27. Cella, M., et al., *A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity.* Nature, 2009. **457**(7230): p. 722-5.
28. Cupedo, T., et al., *Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells.* Nat Immunol, 2009. **10**(1): p. 66-74.
29. Buonocore, S., et al., *Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology.* Nature, 2010. **464**(7293): p. 1371-5.

30. Gasteiger, G., et al., *Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs.* Science, 2015. **350**(6263): p. 981-5.
31. Mackley, E.C., et al., *CCR7-dependent trafficking of RORgamma(+) ILCs creates a unique microenvironment within mucosal draining lymph nodes.* Nat Commun, 2015. **6**: p. 5862.
32. Abt, M.C., et al., *Innate Immune Defenses Mediated by Two ILC Subsets Are Critical for Protection against Acute Clostridium difficile Infection.* Cell Host Microbe, 2015. **18**(1): p. 27-37.
33. Powell, N., et al., *The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells.* Immunity, 2012. **37**(4): p. 674-84.
34. Halim, T.Y., et al., *Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation.* Immunity, 2012. **36**(3): p. 451-63.
35. Bartemes, K.R., et al., *IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs.* J Immunol, 2012. **188**(3): p. 1503-13.
36. Klose, C.S. and D. Artis, *Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis.* Nat Immunol, 2016. **17**(7): p. 765-74.
37. Bartemes, K.R., et al., *Enhanced innate type 2 immune response in peripheral blood from patients with asthma.* J Allergy Clin Immunol, 2014. **134**(3): p. 671-678 e4.
38. Mjosberg, J.M., et al., *Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161.* Nat Immunol, 2011. **12**(11): p. 1055-62.
39. Salimi, M., et al., *A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis.* J Exp Med, 2013. **210**(13): p. 2939-50.
40. Imai, Y., et al., *Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice.* Proc Natl Acad Sci U S A, 2013. **110**(34): p. 13921-6.
41. Villanova, F., et al., *Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis.* J Invest Dermatol, 2014. **134**(4): p. 984-991.
42. Pantelyushin, S., et al., *Rorgammat+ innate lymphocytes and gammadelta T cells initiate psoriasiform plaque formation in mice.* J Clin Invest, 2012. **122**(6): p. 2252-6.

43. Molofsky, A.B., et al., *Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages.* J Exp Med, 2013. **210**(3): p. 535-49.
44. Hams, E., et al., *Cutting edge: IL-25 elicits innate lymphoid type 2 and type II NKT cells that regulate obesity in mice.* J Immunol, 2013. **191**(11): p. 5349-53.
45. Sawa, S., et al., *RORgammat+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota.* Nat Immunol, 2011. **12**(4): p. 320-6.
46. Dudakov, J.A., et al., *Interleukin-22 drives endogenous thymic regeneration in mice.* Science, 2012. **336**(6077): p. 91-5.
47. Taube, C., et al., *IL-22 is produced by innate lymphoid cells and limits inflammation in allergic airway disease.* PLoS One, 2011. **6**(7): p. e21799.
48. Schmitz, J., 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**(5): p. 479-90.
49. Lingel, A., et al., *Structure of IL-33 and its interaction with the ST2 and IL-1RAcP receptors--insight into heterotrimeric IL-1 signaling complexes.* Structure, 2009. **17**(10): p. 1398-410.
50. Baekkevold, E.S., et al., *Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules.* Am J Pathol, 2003. **163**(1): p. 69-79.
51. Carriere, V., et al., *IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo.* Proc Natl Acad Sci U S A, 2007. **104**(1): p. 282-7.
52. Cayrol, C. and J.P. Girard, *IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy.* Curr Opin Immunol, 2014. **31**: p. 31-7.
53. Kearley, J., et al., *Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection.* Immunity, 2015. **42**(3): p. 566-79.
54. Cayrol, C. and J.P. Girard, *The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1.* Proc Natl Acad Sci U S A, 2009. **106**(22): p. 9021-6.
55. Gadani, S.P., et al., *The glia-derived alarmin IL-33 orchestrates the immune response and promotes recovery following CNS injury.* Neuron, 2015. **85**(4): p. 703-9.

56. Moussion, C., N. Ortega, and J.P. Girard, *The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'?* PLoS One, 2008. **3**(10): p. e3331.
57. Luthi, A.U., et al., *Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases.* Immunity, 2009. **31**(1): p. 84-98.
58. Pichery, M., et al., *Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel Il-33-LacZ gene trap reporter strain.* J Immunol, 2012. **188**(7): p. 3488-95.
59. Kuchler, A.M., et al., *Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation.* Am J Pathol, 2008. **173**(4): p. 1229-42.
60. Chen, W.Y., et al., *Myocardial pressure overload induces systemic inflammation through endothelial cell IL-33.* Proc Natl Acad Sci U S A, 2015. **112**(23): p. 7249-54.
61. Molofsky, A.B., et al., *Interleukin-33 and Interferon-gamma Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation.* Immunity, 2015. **43**(1): p. 161-74.
62. Byers, D.E., et al., *Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease.* J Clin Invest, 2013. **123**(9): p. 3967-82.
63. Hardman, C.S., V. Panova, and A.N. McKenzie, *IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation.* Eur J Immunol, 2013. **43**(2): p. 488-98.
64. Savinko, T., et al., *IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors.* J Invest Dermatol, 2012. **132**(5): p. 1392-400.
65. Yasuda, K., et al., *Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice.* Proc Natl Acad Sci U S A, 2012. **109**(9): p. 3451-6.
66. Barlow, J.L., et al., *IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction.* J Allergy Clin Immunol, 2013. **132**(4): p. 933-41.
67. Marvie, P., et al., *Interleukin-33 overexpression is associated with liver fibrosis in mice and humans.* J Cell Mol Med, 2010. **14**(6B): p. 1726-39.
68. Manetti, M., et al., *The IL1-like cytokine IL33 and its receptor ST2 are abnormally expressed in the affected skin and visceral organs of patients with systemic sclerosis.* Ann Rheum Dis, 2010. **69**(3): p. 598-605.

69. Sponheim, J., et al., *Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts.* Am J Pathol, 2010. **177**(6): p. 2804-15.
70. Lefrancais, E., et al., *IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G.* Proc Natl Acad Sci U S A, 2012. **109**(5): p. 1673-8.
71. Lefrancais, 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 U S A, 2014. **111**(43): p. 15502-7.
72. Sanada, S., et al., *IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system.* J Clin Invest, 2007. **117**(6): p. 1538-49.
73. Cohen, E.S., et al., *Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation.* Nat Commun, 2015. **6**: p. 8327.
74. Lott, J.M., T.L. Sumpter, and H.R. Turnquist, *New dog and new tricks: evolving roles for IL-33 in type 2 immunity.* J Leukoc Biol, 2015. **97**(6): p. 1037-48.
75. Molofsky, A.B., A.K. Savage, and R.M. Locksley, *Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation.* Immunity, 2015. **42**(6): p. 1005-19.
76. Xu, D., et al., *Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells.* J Exp Med, 1998. **187**(5): p. 787-94.
77. Matta, B.M., et al., *Peri-alloHCT IL-33 administration expands recipient T-regulatory cells that protect mice against acute GVHD.* Blood, 2016. **128**(3): p. 427-39.
78. Arpaia, N., et al., *A Distinct Function of Regulatory T Cells in Tissue Protection.* Cell, 2015. **162**(5): p. 1078-89.
79. Matta, B.M., et al., *IL-33 is an unconventional Alarmin that stimulates IL-2 secretion by dendritic cells to selectively expand IL-33R/ST2+ regulatory T cells.* J Immunol, 2014. **193**(8): p. 4010-20.
80. Turnquist, H.R., et al., *IL-1beta-driven ST2L expression promotes maturation resistance in rapamycin-conditioned dendritic cells.* J Immunol, 2008. **181**(1): p. 62-72.
81. Rank, M.A., et al., *IL-33-activated dendritic cells induce an atypical TH2-type response.* J Allergy Clin Immunol, 2009. **123**(5): p. 1047-54.
82. Besnard, A.G., et al., *IL-33-activated dendritic cells are critical for allergic airway inflammation.* Eur J Immunol, 2011. **41**(6): p. 1675-86.
83. Morita, H., et al., *An Interleukin-33-Mast Cell-Interleukin-2 Axis Suppresses Papain-Induced Allergic Inflammation by Promoting Regulatory T Cell Numbers.* Immunity, 2015. **43**(1): p. 175-86.

84. Palmer, G. and C. Gabay, *Interleukin-33 biology with potential insights into human diseases.* Nat Rev Rheumatol, 2011. **7**(6): p. 321-9.
85. Imazio, M., et al., *A randomized trial of colchicine for acute pericarditis.* N Engl J Med, 2013. **369**(16): p. 1522-8.
86. Imazio, M., et al., *Colchicine in addition to conventional therapy for acute pericarditis: results of the COlchicine for acute PEricarditis (COPE) trial.* Circulation, 2005. **112**(13): p. 2012-6.
87. Kyto, V., J. Sipila, and P. Rautava, *Clinical profile and influences on outcomes in patients hospitalized for acute pericarditis.* Circulation, 2014. **130**(18): p. 1601-6.
88. Imazio, M., et al., *Controversial issues in the management of pericardial diseases.* Circulation, 2010. **121**(7): p. 916-28.
89. LeWinter, M.M., *Clinical practice. Acute pericarditis.* N Engl J Med, 2014. **371**(25): p. 2410-6.
90. Troughton, R.W., C.R. Asher, and A.L. Klein, *Pericarditis.* Lancet, 2004. **363**(9410): p. 717-27.
91. Maisch, B., et al., *Guidelines on the diagnosis and management of pericardial diseases executive summary; The Task force on the diagnosis and management of pericardial diseases of the European society of cardiology.* Eur Heart J, 2004. **25**(7): p. 587-610.
92. Adler, Y., et al., *2015 ESC Guidelines for the diagnosis and management of pericardial diseases: The Task Force for the Diagnosis and Management of Pericardial Diseases of the European Society of Cardiology (ESC)Endorsed by: The European Association for Cardio-Thoracic Surgery (EACTS).* Eur Heart J, 2015. **36**(42): p. 2921-2964.
93. Mayosi, B.M., et al., *Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis.* N Engl J Med, 2014. **371**(12): p. 1121-30.
94. Mayosi, B.M., *Contemporary trends in the epidemiology and management of cardiomyopathy and pericarditis in sub-Saharan Africa.* Heart, 2007. **93**(10): p. 1176-83.
95. Imazio, M., et al., *Aetiological diagnosis in acute and recurrent pericarditis: when and how.* J Cardiovasc Med (Hagerstown), 2009. **10**(3): p. 217-30.
96. Imazio, M. and B.D. Hoit, *Post-cardiac injury syndromes. An emerging cause of pericardial diseases.* Int J Cardiol, 2013. **168**(2): p. 648-52.
97. Imazio, M., et al., *Indicators of poor prognosis of acute pericarditis.* Circulation, 2007. **115**(21): p. 2739-44.

98. Permanyer-Miralda, G., J. Sagrista-Sauleda, and J. Soler-Soler, *Primary acute pericardial disease: a prospective series of 231 consecutive patients.* Am J Cardiol, 1985. **56**(10): p. 623-30.

99. Zayas, R., et al., *Incidence of specific etiology and role of methods for specific etiologic diagnosis of primary acute pericarditis.* Am J Cardiol, 1995. **75**(5): p. 378-82.

100. Gouriet, F., et al., *Etiology of Pericarditis in a Prospective Cohort of 1162 Cases.* Am J Med, 2015. **128**(7): p. 784 e1-8.

101. Imazio, M., et al., *Good prognosis for pericarditis with and without myocardial involvement: results from a multicenter, prospective cohort study.* Circulation, 2013. **128**(1): p. 42-9.

102. Klein, A.L., et al., *American Society of Echocardiography clinical recommendations for multimodality cardiovascular imaging of patients with pericardial disease: endorsed by the Society for Cardiovascular Magnetic Resonance and Society of Cardiovascular Computed Tomography.* J Am Soc Echocardiogr, 2013. **26**(9): p. 965-1012 e15.

103. Ben-Horin, S., et al., *Diagnostic value of the biochemical composition of pericardial effusions in patients undergoing pericardiocentesis.* Am J Cardiol, 2007. **99**(9): p. 1294-7.

104. Horneffer, P.J., et al., *The effective treatment of postpericardiotomy syndrome after cardiac operations. A randomized placebo-controlled trial.* J Thorac Cardiovasc Surg, 1990. **100**(2): p. 292-6.

105. Imazio, M., et al., *Corticosteroids for recurrent pericarditis: high versus low doses: a nonrandomized observation.* Circulation, 2008. **118**(6): p. 667-71.

106. Lotrionte, M., et al., *International collaborative systematic review of controlled clinical trials on pharmacologic treatments for acute pericarditis and its recurrences.* Am Heart J, 2010. **160**(4): p. 662-70.

107. Khandaker, M.H., et al., *Pericardiectomy vs medical management in patients with relapsing pericarditis.* Mayo Clin Proc, 2012. **87**(11): p. 1062-70.

108. Fowler, N.O. and A.D. Harbin, 3rd, *Recurrent acute pericarditis: follow-up study of 31 patients.* J Am Coll Cardiol, 1986. **7**(2): p. 300-5.

109. Abston, E.D., et al., *IL-33 independently induces eosinophilic pericarditis and cardiac dilation: ST2 improves cardiac function.* Circ Heart Fail, 2012. **5**(3): p. 366-75.

110. Seki, K., et al., *Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling.* Circ Heart Fail, 2009. **2**(6): p. 684-91.

111. Miller, A.M., et al., *IL-33 reduces the development of atherosclerosis.* J Exp Med, 2008. **205**(2): p. 339-46.
112. Shimpo, M., et al., *Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction.* Circulation, 2004. **109**(18): p. 2186-90.
113. Coronado, M.J., et al., *Elevated Sera sST 2 Is Associated With Heart Failure in Men </=50 Years Old With Myocarditis.* J Am Heart Assoc, 2019. **8**(2): p. e008968.
114. Prefontaine, D., et al., *Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells.* J Immunol, 2009. **183**(8): p. 5094-103.
115. Moffatt, M.F., et al., *A large-scale, consortium-based genomewide association study of asthma.* N Engl J Med, 2010. **363**(13): p. 1211-1221.
116. Sakashita, M., et al., *Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis.* Clin Exp Allergy, 2008. **38**(12): p. 1875-81.
117. Kurowska-Stolarska, M., et al., *IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation.* J Immunol, 2009. **183**(10): p. 6469-77.
118. Haenuki, Y., et al., *A critical role of IL-33 in experimental allergic rhinitis.* J Allergy Clin Immunol, 2012. **130**(1): p. 184-94 e11.
119. Nakanishi, W., et al., *IL-33, but not IL-25, is crucial for the development of house dust mite antigen-induced allergic rhinitis.* PLoS One, 2013. **8**(10): p. e78099.
120. Kim, Y.H., et al., *Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis.* Allergy, 2012. **67**(2): p. 183-90.
121. Mizutani, N., T. Nabe, and S. Yoshino, *Interleukin-33 and alveolar macrophages contribute to the mechanisms underlying the exacerbation of IgE-mediated airway inflammation and remodelling in mice.* Immunology, 2013. **139**(2): p. 205-18.
122. Tang, S., et al., *Increased IL-33 in synovial fluid and paired serum is associated with disease activity and autoantibodies in rheumatoid arthritis.* Clin Dev Immunol, 2013. **2013**: p. 985301.
123. Li, C., et al., *Genetic variant in IL33 is associated with susceptibility to rheumatoid arthritis.* Arthritis Res Ther, 2014. **16**(2): p. R105.
124. Xu, D., et al., *IL-33 exacerbates antigen-induced arthritis by activating mast cells.* Proc Natl Acad Sci U S A, 2008. **105**(31): p. 10913-8.

125. Leung, B.P., et al., *A novel therapy of murine collagen-induced arthritis with soluble T1/ST2.* J Immunol, 2004. **173**(1): p. 145-50.
126. Palmer, G., et al., *Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis.* Arthritis Rheum, 2009. **60**(3): p. 738-49.
127. Hodzic, Z., et al., *IL-33 and the intestine: The good, the bad, and the inflammatory.* Cytokine, 2017. **100**: p. 1-10.
128. Travers, J., et al., *IL-33 is induced in undifferentiated, non-dividing esophageal epithelial cells in eosinophilic esophagitis.* Sci Rep, 2017. **7**(1): p. 17563.
129. Judd, L.M., et al., *Elevated IL-33 expression is associated with pediatric eosinophilic esophagitis, and exogenous IL-33 promotes eosinophilic esophagitis development in mice.* Am J Physiol Gastrointest Liver Physiol, 2016. **310**(1): p. G13-25.
130. Sedhom, M.A., et al., *Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice.* Gut, 2013. **62**(12): p. 1714-23.
131. Pastorelli, L., et al., *Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis.* Proc Natl Acad Sci U S A, 2010. **107**(17): p. 8017-22.
132. Oboki, K., et al., *IL-33 is a crucial amplifier of innate rather than acquired immunity.* Proc Natl Acad Sci U S A, 2010. **107**(43): p. 18581-6.
133. Duan, L., et al., *Interleukin-33 ameliorates experimental colitis through promoting Th2/Foxp3(+) regulatory T-cell responses in mice.* Mol Med, 2012. **18**: p. 753-61.
134. Schiering, C., et al., *The alarmin IL-33 promotes regulatory T-cell function in the intestine.* Nature, 2014. **513**(7519): p. 564-568.
135. Monticelli, L.A., et al., *IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions.* Proc Natl Acad Sci U S A, 2015. **112**(34): p. 10762-7.
136. Liang, H., et al., *Interleukin-33 signaling contributes to renal fibrosis following ischemia reperfusion.* Eur J Pharmacol, 2017. **812**: p. 18-27.
137. Akcay, A., et al., *IL-33 exacerbates acute kidney injury.* J Am Soc Nephrol, 2011. **22**(11): p. 2057-67.
138. Park, G.H., et al., *Anti-interleukin-33 Reduces Ovalbumin-Induced Nephrotoxicity and Expression of Kidney Injury Molecule-1.* Int Neurourol J, 2016. **20**(2): p. 114-21.
139. Stremska, M.E., et al., *IL233, A Novel IL-2 and IL-33 Hybrid Cytokine, Ameliorates Renal Injury.* J Am Soc Nephrol, 2017. **28**(9): p. 2681-2693.

140. Riedel, J.H., et al., *IL-33-Mediated Expansion of Type 2 Innate Lymphoid Cells Protects from Progressive Glomerulosclerosis.* J Am Soc Nephrol, 2017. **28**(7): p. 2068-2080.
141. Kolodin, D., et al., *Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice.* Cell Metab, 2015. **21**(4): p. 543-57.
142. Miller, A.M., et al., *Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice.* Circ Res, 2010. **107**(5): p. 650-8.
143. Hasan, A., et al., *IL-33 is negatively associated with the BMI and confers a protective lipid/metabolic profile in non-diabetic but not diabetic subjects.* BMC Immunol, 2014. **15**: p. 19.
144. Brestoff, J.R., et al., *Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity.* Nature, 2015. **519**(7542): p. 242-6.
145. Lee, M.W., et al., *Activated type 2 innate lymphoid cells regulate beige fat biogenesis.* Cell, 2015. **160**(1-2): p. 74-87.
146. Hudson, C.A., et al., *Induction of IL-33 expression and activity in central nervous system glia.* J Leukoc Biol, 2008. **84**(3): p. 631-43.
147. Jiang, H.R., et al., *IL-33 attenuates EAE by suppressing IL-17 and IFN-gamma production and inducing alternatively activated macrophages.* Eur J Immunol, 2012. **42**(7): p. 1804-14.
148. Luo, Y., et al., *Interleukin-33 ameliorates ischemic brain injury in experimental stroke through promoting Th2 response and suppressing Th17 response.* Brain Res, 2015. **1597**: p. 86-94.
149. Pomeshchik, Y., et al., *Interleukin-33 treatment reduces secondary injury and improves functional recovery after contusion spinal cord injury.* Brain Behav Immun, 2015. **44**: p. 68-81.
150. Fu, A.K., et al., *IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline.* Proc Natl Acad Sci U S A, 2016. **113**(19): p. E2705-13.
151. Chapuis, J., et al., *Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease.* Mol Psychiatry, 2009. **14**(11): p. 1004-16.
152. Xi, H., et al., *IL-33 amplifies an innate immune response in the degenerating retina.* J Exp Med, 2016. **213**(2): p. 189-207.
153. Buss, N.A., et al., *Monoclonal antibody therapeutics: history and future.* Curr Opin Pharmacol, 2012. **12**(5): p. 615-22.
154. Takatori, H., et al., *Regulatory Mechanisms of IL-33-ST2-Mediated Allergic Inflammation.* Front Immunol, 2018. **9**: p. 2004.

155. Moro, K., et al., *Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses.* Nat Immunol, 2016. **17**(1): p. 76-86.
156. Kim, B.S., et al., *TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation.* Sci Transl Med, 2013. **5**(170): p. 170ra16.
157. Klein Wolterink, R.G., et al., *Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma.* Eur J Immunol, 2012. **42**(5): p. 1106-16.
158. Huang, Y., et al., *IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells.* Nat Immunol, 2015. **16**(2): p. 161-9.
159. Bal, S.M., et al., *IL-1beta, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs.* Nat Immunol, 2016. **17**(6): p. 636-45.
160. Maazi, H., et al., *ICOS:ICOS-ligand interaction is required for type 2 innate lymphoid cell function, homeostasis, and induction of airway hyperreactivity.* Immunity, 2015. **42**(3): p. 538-51.
161. Paclik, D., et al., *ICOS regulates the pool of group 2 innate lymphoid cells under homeostatic and inflammatory conditions in mice.* Eur J Immunol, 2015. **45**(10): p. 2766-72.
162. Wojno, E.D., et al., *The prostaglandin D(2) receptor CRTH2 regulates accumulation of group 2 innate lymphoid cells in the inflamed lung.* Mucosal Immunol, 2015. **8**(6): p. 1313-23.
163. Xue, L., et al., *Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells.* J Allergy Clin Immunol, 2014. **133**(4): p. 1184-94.
164. Barnig, C., et al., *Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma.* Sci Transl Med, 2013. **5**(174): p. 174ra26.
165. Doherty, T.A., et al., *Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production.* J Allergy Clin Immunol, 2013. **132**(1): p. 205-13.
166. Meylan, F., et al., *The TNF-family cytokine TL1A promotes allergic immunopathology through group 2 innate lymphoid cells.* Mucosal Immunol, 2014. **7**(4): p. 958-68.
167. Yu, X., et al., *TNF superfamily member TL1A elicits type 2 innate lymphoid cells at mucosal barriers.* Mucosal Immunol, 2014. **7**(3): p. 730-40.
168. Nussbaum, J.C., et al., *Type 2 innate lymphoid cells control eosinophil homeostasis.* Nature, 2013. **502**(7470): p. 245-8.

169. Fallon, P.G., et al., *Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion.* J Exp Med, 2006. **203**(4): p. 1105-16.
170. Fort, M.M., et al., *IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo.* Immunity, 2001. **15**(6): p. 985-95.
171. Zaiss, D.M., et al., *Amphiregulin, a TH2 cytokine enhancing resistance to nematodes.* Science, 2006. **314**(5806): p. 1746.
172. von Moltke, J., et al., *Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit.* Nature, 2016. **529**(7585): p. 221-5.
173. Howitt, M.R., et al., *Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut.* Science, 2016. **351**(6279): p. 1329-33.
174. Gerbe, F., et al., *Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites.* Nature, 2016. **529**(7585): p. 226-30.
175. Bouchery, T., et al., *ILC2s and T cells cooperate to ensure maintenance of M2 macrophages for lung immunity against hookworms.* Nat Commun, 2015. **6**: p. 6970.
176. Turner, J.E., et al., *IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation.* J Exp Med, 2013. **210**(13): p. 2951-65.
177. Nausch, N., et al., *Group 2 innate lymphoid cell proportions are diminished in young helminth infected children and restored by curative anti-helminthic treatment.* PLoS Negl Trop Dis, 2015. **9**(3): p. e0003627.
178. Boyd, A., J.M. Ribeiro, and T.B. Nutman, *Human CD117 (cKit)+ innate lymphoid cells have a discrete transcriptional profile at homeostasis and are expanded during filarial infection.* PLoS One, 2014. **9**(9): p. e108649.
179. Chang, Y.J., et al., *Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity.* Nat Immunol, 2011. **12**(7): p. 631-8.
180. Gorski, S.A., Y.S. Hahn, and T.J. Braciale, *Group 2 innate lymphoid cell production of IL-5 is regulated by NKT cells during influenza virus infection.* PLoS Pathog, 2013. **9**(9): p. e1003615.
181. Hams, E., et al., *IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis.* Proc Natl Acad Sci U S A, 2014. **111**(1): p. 367-72.
182. Jackson, D.J., et al., *Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children.* Am J Respir Crit Care Med, 2008. **178**(7): p. 667-72.

183. Hong, J.Y., et al., *Neonatal rhinovirus induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells.* J Allergy Clin Immunol, 2014. **134**(2): p. 429-39.
184. Gollwitzer, E.S., et al., *Lung microbiota promotes tolerance to allergens in neonates via PD-L1.* Nat Med, 2014. **20**(6): p. 642-7.
185. Sonnenberg, G.F., *Regulation of intestinal health and disease by innate lymphoid cells.* Int Immunol, 2014. **26**(9): p. 501-7.
186. Camelo, A., et al., *Blocking IL-25 signalling protects against gut inflammation in a type-2 model of colitis by suppressing nuocyte and NKT derived IL-13.* J Gastroenterol, 2012. **47**(11): p. 1198-211.
187. Bailey, J.R., et al., *IL-13 promotes collagen accumulation in Crohn's disease fibrosis by down-regulation of fibroblast MMP synthesis: a role for innate lymphoid cells?* PLoS One, 2012. **7**(12): p. e52332.
188. Blazquez, A.B., L. Mayer, and M.C. Berin, *Thymic stromal lymphopoietin is required for gastrointestinal allergy but not oral tolerance.* Gastroenterology, 2010. **139**(4): p. 1301-9.
189. Chu, D.K., et al., *IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization.* J Allergy Clin Immunol, 2013. **131**(1): p. 187-200 e1-8.
190. Noti, M., et al., *Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis.* Nat Med, 2013. **19**(8): p. 1005-13.
191. Doherty, T.A., et al., *Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis.* J Allergy Clin Immunol, 2015. **136**(3): p. 792-794.e3.
192. Li, J., Y. Zhang, and L. Zhang, *Discovering susceptibility genes for allergic rhinitis and allergy using a genome-wide association study strategy.* Curr Opin Allergy Clin Immunol, 2015. **15**(1): p. 33-40.
193. Salmond, R.J., et al., *IL-33 induces innate lymphoid cell-mediated airway inflammation by activating mammalian target of rapamycin.* J Allergy Clin Immunol, 2012. **130**(5): p. 1159-1166 e6.
194. Halim, T.Y., et al., *Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation.* Immunity, 2012. **37**(3): p. 463-74.
195. Motomura, Y., et al., *Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation.* Immunity, 2014. **40**(5): p. 758-71.

196. Kim, H.Y., et al., *Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity.* J Allergy Clin Immunol, 2012. **129**(1): p. 216-27.e1-6.
197. Halim, T.Y., et al., *Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation.* Immunity, 2014. **40**(3): p. 425-35.
198. Wilhelm, C., et al., *An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation.* Nat Immunol, 2011. **12**(11): p. 1071-7.
199. Townsend, J.M., et al., *IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development.* Immunity, 2000. **13**(4): p. 573-83.
200. Liu, B., et al., *Collaborative interactions between type 2 innate lymphoid cells and antigen-specific CD4+ Th2 cells exacerbate murine allergic airway diseases with prominent eosinophilia.* J Immunol, 2015. **194**(8): p. 3583-93.
201. Christianson, C.A., et al., *Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33.* J Allergy Clin Immunol, 2015. **136**(1): p. 59-68 e14.
202. Kabata, H., et al., *Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation.* Nat Commun, 2013. **4**: p. 2675.
203. Ho, J., et al., *Group 2 innate lymphoid cells (ILC2s) are increased in chronic rhinosinusitis with nasal polyps or eosinophilia.* Clin Exp Allergy, 2015. **45**(2): p. 394-403.
204. Miljkovic, D., et al., *Association between group 2 innate lymphoid cells enrichment, nasal polyps and allergy in chronic rhinosinusitis.* Allergy, 2014. **69**(9): p. 1154-61.
205. Shaw, J.L., et al., *IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps.* Am J Respir Crit Care Med, 2013. **188**(4): p. 432-9.
206. Walford, H.H., et al., *Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness.* Clin Immunol, 2014. **155**(1): p. 126-35.
207. Doherty, T.A., et al., *Allergen challenge in allergic rhinitis rapidly induces increased peripheral blood type 2 innate lymphoid cells that express CD84.* J Allergy Clin Immunol, 2014. **133**(4): p. 1203-5.
208. Roediger, B., et al., *Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells.* Nat Immunol, 2013. **14**(6): p. 564-73.

209. Kim, B.S., et al., *Basophils promote innate lymphoid cell responses in inflamed skin.* J Immunol, 2014. **193**(7): p. 3717-25.
210. McHedlidze, T., et al., *Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis.* Immunity, 2013. **39**(2): p. 357-71.
211. Li, J., et al., *Biliary repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation.* J Clin Invest, 2014. **124**(7): p. 3241-51.
212. Russi, A.E., et al., *Cutting edge: c-Kit signaling differentially regulates type 2 innate lymphoid cell accumulation and susceptibility to central nervous system demyelination in male and female SJL mice.* J Immunol, 2015. **194**(12): p. 5609-13.
213. Gadani, S.P., et al., *Characterization of meningeal type 2 innate lymphocytes and their response to CNS injury.* J Exp Med, 2017. **214**(2): p. 285-296.
214. Perry, H.M., et al., *Helix-loop-helix factor inhibitor of differentiation 3 regulates interleukin-5 expression and B-1a B cell proliferation.* Arterioscler Thromb Vasc Biol, 2013. **33**(12): p. 2771-9.
215. Mantani, P.T., et al., *IL-25 inhibits atherosclerosis development in apolipoprotein E deficient mice.* PLoS One, 2015. **10**(1): p. e0117255.
216. Newland, S.A., et al., *Type-2 innate lymphoid cells control the development of atherosclerosis in mice.* Nat Commun, 2017. **8**: p. 15781.
217. Qiu, Y., et al., *Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat.* Cell, 2014. **157**(6): p. 1292-308.
218. Dalmas, E., et al., *Interleukin-33-Activated Islet-Resident Innate Lymphoid Cells Promote Insulin Secretion through Myeloid Cell Retinoic Acid Production.* Immunity, 2017. **47**(5): p. 928-942 e7.
219. Bracamonte-Baran, W., et al., *Non-cytotoxic Cardiac Innate Lymphoid Cells Are a Resident and Quiescent Type 2-Commited Population.* Frontiers in Immunology, 2019. **10**(634).
220. Gordon, S. and A. Pluddemann, *Tissue macrophages: heterogeneity and functions.* BMC Biol, 2017. **15**(1): p. 53.
221. Epelman, S., et al., *Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation.* Immunity, 2014. **40**(1): p. 91-104.
222. Bajpai, G., et al., *The human heart contains distinct macrophage subsets with divergent origins and functions.* Nat Med, 2018. **24**(8): p. 1234-1245.

223. Ngkelo, A., et al., *Mast cells regulate myofilament calcium sensitization and heart function after myocardial infarction.* J Exp Med, 2016. **213**(7): p. 1353-74.
224. Dvorak, A.M., *Mast-cell degranulation in human hearts.* N Engl J Med, 1986. **315**(15): p. 969-70.
225. Patella, V., et al., *Human heart mast cells. Isolation, purification, ultrastructure, and immunologic characterization.* J Immunol, 1995. **154**(6): p. 2855-65.
226. Ramos, G.C., et al., *Myocardial aging as a T-cell-mediated phenomenon.* Proc Natl Acad Sci U S A, 2017. **114**(12): p. E2420-e2429.
227. Spits, H. and T. Cupedo, *Innate lymphoid cells: emerging insights in development, lineage relationships, and function.* Annu Rev Immunol, 2012. **30**: p. 647-75.
228. Walker, J.A., J.L. Barlow, and A.N. McKenzie, *Innate lymphoid cells--how did we miss them?* Nat Rev Immunol, 2013. **13**(2): p. 75-87.
229. Kim, B.S. and D. Artis, *Group 2 innate lymphoid cells in health and disease.* Cold Spring Harb Perspect Biol, 2015. **7**(5).
230. von Moltke, J. and R.M. Locksley, *I-L-C-2 it: type 2 immunity and group 2 innate lymphoid cells in homeostasis.* Curr Opin Immunol, 2014. **31**: p. 58-65.
231. Gao, S., et al., *Echocardiography in Mice.* Curr Protoc Mouse Biol, 2011. **1**: p. 71-83.
232. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.
233. Rothenberg, M.E., *Eotaxin. An essential mediator of eosinophil trafficking into mucosal tissues.* Am J Respir Cell Mol Biol, 1999. **21**(3): p. 291-5.
234. Borchers, M.T., et al., *In vitro assessment of chemokine receptor-ligand interactions mediating mouse eosinophil migration.* J Leukoc Biol, 2002. **71**(6): p. 1033-41.
235. Diny, N.L., et al., *Macrophages and cardiac fibroblasts are the main producers of eotaxins and regulate eosinophil trafficking to the heart.* Eur J Immunol, 2016. **46**(12): p. 2749-2760.
236. Chen, G., et al., *Sca-1(+) cardiac fibroblasts promote development of heart failure.* Eur J Immunol, 2018. **48**(9): p. 1522-1538.
237. Lee, N.A., et al., *Expression of IL-5 in thymocytes/T cells leads to the development of a massive eosinophilia, extramedullary eosinophilopoiesis, and unique histopathologies.* J Immunol, 1997. **158**(3): p. 1332-44.
238. Barthel, S.R., et al., *Roles of integrin activation in eosinophil function and the eosinophilic inflammation of asthma.* J Leukoc Biol, 2008. **83**(1): p. 1-12.

239. Johansson, M.W., *Activation states of blood eosinophils in asthma.* Clin Exp Allergy, 2014. **44**(4): p. 482-98.
240. Griesenauer, B. and S. Paczesny, *The ST2/IL-33 Axis in Immune Cells during Inflammatory Diseases.* Front Immunol, 2017. **8**: p. 475.
241. Sanchez-Mas, J., et al., *Modulation of IL-33/ST2 system in postinfarction heart failure: correlation with cardiac remodelling markers.* Eur J Clin Invest, 2014. **44**(7): p. 643-51.
242. McCarthy, C.P. and J.L. Januzzi, Jr., *Soluble ST2 in Heart Failure.* Heart Fail Clin, 2018. **14**(1): p. 41-48.
243. Frioes, F., et al., *Prognostic value of sST2 added to BNP in acute heart failure with preserved or reduced ejection fraction.* Clin Res Cardiol, 2015. **104**(6): p. 491-9.
244. Lindman, B.R., et al., *Prognostic utility of novel biomarkers of cardiovascular stress in patients with aortic stenosis undergoing valve replacement.* Heart, 2015. **101**(17): p. 1382-8.
245. Demyanets, S., et al., *Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature.* J Mol Cell Cardiol, 2013. **60**: p. 16-26.
246. Li, L., et al., *Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells.* J Immunol, 1999. **162**(5): p. 2477-87.
247. Matsukura, S., et al., *Interleukin-13 upregulates eotaxin expression in airway epithelial cells by a STAT6-dependent mechanism.* Am J Respir Cell Mol Biol, 2001. **24**(6): p. 755-61.
248. Zuo, L., et al., *IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway.* J Immunol, 2010. **185**(1): p. 660-9.
249. Moore, P.E., et al., *IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK.* Am J Physiol Lung Cell Mol Physiol, 2002. **282**(4): p. L847-53.
250. Takahashi, K., et al., *Regulation of eotaxin-3/CC chemokine ligand 26 expression by T helper type 2 cytokines in human colonic myofibroblasts.* Clin Exp Immunol, 2013. **173**(2): p. 323-31.
251. Blanchard, C., et al., *IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids.* J Allergy Clin Immunol, 2007. **120**(6): p. 1292-300.

252. Farne, H.A., et al., *Anti-IL5 therapies for asthma.* Cochrane Database Syst Rev, 2017. **9**: p. Cd010834.
253. Ha, S.A., et al., *Regulation of B1 cell migration by signals through Toll-like receptors.* J Exp Med, 2006. **203**(11): p. 2541-50.
254. Shimotakahara, A., et al., *Pleural macrophages are the dominant cell population in the thoracic cavity with an inflammatory cytokine profile similar to peritoneal macrophages.* Pediatr Surg Int, 2007. **23**(5): p. 447-51.
255. Wang, J. and P. Kubes, *A Reservoir of Mature Cavity Macrophages that Can Rapidly Invade Visceral Organs to Affect Tissue Repair.* Cell, 2016. **165**(3): p. 668-78.
256. Weber, G.F., et al., *Pleural innate response activator B cells protect against pneumonia via a GM-CSF-IgM axis.* J Exp Med, 2014. **211**(6): p. 1243-56.
257. Butts, B., et al., *Increased Inflammation in Pericardial Fluid Persists 48 Hours After Cardiac Surgery.* Circulation, 2017. **136**(23): p. 2284-2286.
258. Horckmans, M., et al., *Pericardial Adipose Tissue Regulates Granulopoiesis, Fibrosis, and Cardiac Function After Myocardial Infarction.* Circulation, 2018. **137**(9): p. 948-960.
259. Lauer, B., et al., *Autoantibodies against human ventricular myosin in sera of patients with acute and chronic myocarditis.* J Am Coll Cardiol, 1994. **23**(1): p. 146-53.
260. O'Donohoe, T.J., et al., *Significance of Anti-Myosin Antibody Formation in Patients With Myocardial Infarction: A Prospective Observational Study.* Heart Lung Circ, 2019. **28**(4): p. 583-590.
261. Imazio, M., F. Gaita, and M. LeWinter, *Evaluation and Treatment of Pericarditis: A Systematic Review.* Jama, 2015. **314**(14): p. 1498-506.
262. Li, Q., et al., *Images in cardiovascular medicine. Eosinophilic pericarditis and myocarditis.* Circulation, 2002. **105**(25): p. 3066.
263. Spiegel, R., et al., *Eosinophilic pericarditis: a rare complication of idiopathic hypereosinophilic syndrome in a child.* Pediatr Cardiol, 2004. **25**(6): p. 690-2.
264. Herry, I., et al., *Acute life-threatening toxocaral tamponade.* Chest, 1997. **112**(6): p. 1692-3.
265. AbdullGaffar, B. and A. Almulla, *Eosinophilic pericardial effusion in hypereosinophilic syndrome with restrictive cardiomyopathy.* Cytopathology, 2015. **26**(6): p. 388-9.
266. Van den Bosch, J.M., S.S. Wagenaar, and C.J. Westermann, *Asthma, eosinophilic pleuropneumonia, and pericarditis without vasculitis.* Thorax, 1986. **41**(7): p. 571-2.

267. Dahlgren, M.W., et al., *Adventitial Stromal Cells Define Group 2 Innate Lymphoid Cell Tissue Niches.* Immunity, 2019. **50**(3): p. 707-722 e6.

Curriculum vitae

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Born on January 3, 1988 in Seoul, Korea

EDUCATION

Johns Hopkins University School of Medicine, Baltimore, MD, USA **2019**
Ph.D. in Pathobiology (Expected
Advisor: Dr. Daniela Cihakova in August)
Dissertation: The role of innate lymphoid cells in the heart and cardiac inflammation

Yonsei University, Seoul, Korea **2011**
M.S. in Biotechnology
Advisor: Dr. Baik-Lin Seong
Thesis: Studies on TAR RNA mediated HIV-1 Tat protein folding

Yonsei University, Seoul, Korea **2009**
B.S. in Biotechnology
Graduated with high honors

RESEARCH EXPERIENCE

Graduate Researcher (Research advisor: Dr. Daniela Cihakova) **Aug 2014 - Present**
Pathobiology Graduate Program, Johns Hopkins University School of Medicine, Baltimore, MD

- Designed and conducted experiments for a dissertation project investigating the role of innate lymphoid cells in pericarditis development using experimental pericarditis mouse model.

Graduate Researcher (Rotations) **Sep 2013 - Aug 2014**
Pathobiology Graduate Program, Johns Hopkins University School of Medicine, Baltimore, MD

- Role of autoimmune regulator (AIRE) in the regulation of Th17 response
  Advisor: Dr. Daniela Cihakova
- Effect of gold-conjugated PD-1 antibody on T cell activation and metabolism
  Advisor: Dr. Jonathan Powell
- Regulation of two-component regulatory system SenX3-RegX3 of *M. tuberculosis* under stress conditions
  Advisor: Dr. Petros Karakousis

Graduate Researcher (Research advisor: Dr. Baik-Lin Seong) **Mar 2009 - Feb 2011**

Department of Biotechnology, Yonsei University, Seoul, Korea

- Designed and conducted experiments for a thesis project on RNA-mediated protein folding using HIV protein and its interacting RNA.

Undergraduate Intern (Research advisor: Dr. Baik-Lin Seong) **Jun 2008 - Feb 2009**
Department of Biotechnology, Yonsei University, Seoul, Korea

- Conducted experiments on RNA-mediated protein folding using GFP-fused influenza proteins.

Undergraduate Research Assistant (Research advisor: Dr. Baik-Lin Seong) **Jul 2007 - May 2008**
Department of Biotechnology, Yonsei University, Seoul, Korea

- Cloned influenza virus genes and tested expression for the development of recombinant influenza vaccines.
- Constructed recombinant vectors for GFP-fused influenza proteins and conducted expression tests.

TEACHING AND MENTORING EXPERIENCE

Supervisor **Summer 2016**

- Trained and supervised a college student, Katerina Havlik.

Supervisor **Mar 2010 - Feb 2011**

- Trained and supervised two undergraduate students.

Teaching Assistant **Sep 2009 - Dec 2009**
Department of Biotechnology, Yonsei University, Seoul, Korea

- Course: Introduction to Biopharmaceuticals

HONORS AND AWARDS

Senior Student Presentation Winner, 15th Annual Pathobiology Graduate Program Retreat **2018**
Pathobiology Graduate Program, Johns Hopkins University School of Medicine

AAI Trainee Abstract Award, IMMUNOLOGY 2018 **2018**
American Association of Immunologists

Best Abstract Award, 18th Johns Hopkins Autoimmunity Day **2017**
Deborah and Noel Rose Center for Autoimmune Disease Research

AAI Trainee Abstract Award, IMMUNOLOGY 2017 **2017**
American Association of Immunologists

Excellence Award, Poster Conference on the Day of Biomedical Science **2009**
Biomedical Science and Technology Initiative, Yonsei University, Korea

Excellence Presentation Award, Creative Research Conference — **2008**
Department of Biotechnology, Yonsei University, Korea

High Honors — **2nd semester 2008**
Yonsei University, Korea — **1st semester 2008**

Honors — **2nd semester 2007**
Yonsei University, Korea — **2nd semester 2006**
1st semester 2006
2nd semester 2005
1st semester 2005

FELLOWSHIP

Predoctoral Fellowship — **2016 - 2018**
American Heart Association
Award number: 16PRE31170040

Margaret Lee Fellowship — **2013 - 2014**
Department of Pathology, Johns Hopkins University School of Medicine

SCHOLARSHIPS

Internal Scholarship — **Spring 2010**
Yonsei University, Korea — **Fall 2009**
Spring 2009

National Science and Technology Scholarship — **Fall 2008**
The Ministry of Science and Technology, Korea — **Spring 2007**
Fall 2006

PUBLICATIONS

Choi HS, Hou X, Chen G, Bracamonte-Baran W, Talor MV, Čiháková D, *Innate lymphoid cells drive pericarditis development and eosinophil trafficking from the mediastinal cavity*. In preparation.

Hou X, Chen G, Bracamonte-Baran W, **Choi HS**, Diny NL, Sung J, Hughes D, Won T, Wood MK, Talor MV, Hackam DJ, Klingel K, Davogustto G, Taegtmeyer H, Coppens I, Barin JG, Čiháková D, *The Cardiac Microenvironment Instructs Divergent Monocyte Fates and Functions in Myocarditis.* Cell Reports, 2019. 28(1): p. 172-189.e7.

Bracamonte-Baran W, Chen G, Hou X, Talor MV, **Choi HS**, Davogustto G, Taegtmeyer H, Sung J, Hackam DJ, Nauen D, Čiháková D, *Non-cytotoxic Cardiac Innate Lymphoid Cells Are a Resident and Quiescent Type 2-Commited Population.* Frontiers in Immunology, 2019. 10(634).

Barin JG, Talor MV, Diny NL, Ong S, Schaub JA, Gebremariam E, Bedja D, Chen G, **Choi HS**, Hou X, Wu L, Cardamone AB, Peterson DA, Rose NR, Cihakova D, *Regulation of autoimmune myocarditis by host responses to the microbiome.* Exp Mol Pathol, 2017. 103(2): p. 141-152.

Kim JM, **Choi HS**, Seong BL, *The folding competence of HIV-1 Tat mediated by interaction with TAR RNA.* RNA Biol, 2017. 14(7): p. 926-937.

PUBLISHED ABSTRACTS

Choi HS, Hou X, Bracamonte-Baran W, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the heart and cardiac inflammation. IMMUNOLOGY 2018. May 2018.

Choi HS, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the cardiac inflammation. IMMUNOLOGY 2017. May 2017.

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the cardiac inflammation. EMBO conference on Innate Lymphoid Cells 2016. December 2016.

PRESENTATIONS (Presenter is underlined)

Choi H, Hou X, Chen G, Bracamonte-Baran W, Talor MV, Cihakova D. Cardiac group 2 innate lymphoid cells play an essential role in pericarditis development. 19th Johns Hopkins Autoimmunity Day. Johns Hopkins University, Baltimore, MD, June 2019. (Poster presentation)

Choi H, Hou X, Chen G, Bracamonte-Baran W, Talor MV, Cihakova D. Innate lymphoid cells play an essential role in pericarditis development. Johns Hopkins Cardiovascular Research Retreat. Johns Hopkins University, Baltimore, MD, May 2019. (Poster presentation)

Choi H, Hou X, Chen G, Bracamonte-Baran W, Talor MV, Cihakova D. Innate lymphoid cells play an essential role in pericarditis development. 21th Annual Department of Pathology Young Investigators' Day. Johns Hopkins University, Baltimore, MD, March 2019. (Poster presentation)

Choi H. Role of group 2 innate lymphoid cells in cardiac inflammation. 15th Annual Pathobiology Graduate Program Retreat. Johns Hopkins University, Baltimore, MD, September 2018. (Oral presentation)

Choi H, Hou X, Bracamonte-Baran W, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the heart and cardiac inflammation. IMMUNOLOGY 2018. Austin, TX, May 2018. (Oral and poster presentation)

Choi H, Hou X, Bracamonte-Baran W, Chen G, Diny N, Talor MV, Cihakova D. Role of group 2 innate lymphoid cells in cardiac inflammation. 20th Annual Department of Pathology Young Investigators' Day. Johns Hopkins University, Baltimore, MD, March 2018. (Poster presentation)

Choi H. Role of innate lymphoid cells in cardiac inflammation. 18th Johns Hopkins Autoimmunity Day. Johns Hopkins University, Baltimore, MD, June 2017. (Oral presentation)

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The Role of Innate Lymphoid Cells in Cardiac Inflammation. Johns Hopkins Cardiovascular Research Retreat. Johns Hopkins University, Baltimore, MD, May 2017. (Poster presentation)

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the cardiac inflammation. IMMUNOLOGY 2017. Washington D.C., May 2017. (Oral and poster presentation)

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the cardiac inflammation. 19th Annual Department of Pathology Young Investigators' Day. Johns Hopkins University, Baltimore, MD, March 2017. (Poster presentation)

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The role of innate lymphoid cells in the cardiac inflammation. EMBO conference on Innate Lymphoid Cells 2016. Berlin, Germany, December 2016. (Poster presentation)

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The Role of Group 2 Innate Lymphoid Cells in Eosinophilic Pericarditis. Johns Hopkins Cardiovascular Research Retreat. Johns Hopkins University, Baltimore, MD, June 2016. (Poster presentation)

Choi H, Bracamonte-Baran W, Hou X, Diny N, Talor MV, Cihakova D. The role of group 2 innate lymphoid cells in eosinophilic pericarditis. 18th Annual Department of Pathology Young Investigators' Day. Johns Hopkins University, Baltimore, MD, March 2016. (Poster presentation)

Choi H. The role of group 2 innate lymphoid cells in the pathogenesis of pericarditis. Pathology Grand Rounds. Johns Hopkins University, Baltimore, MD, July 2015. (Oral presentation)

Choi H, Diny N, Hou X, Barin JG, Talor MV, Schaub J, Cihakova D. Type 2 innate lymphoid cells in IL-33-induced eosinophilic pericarditis. 17th Annual Department of Pathology Young Investigators' Day. Johns Hopkins University, Baltimore, MD, March 2015. (Poster presentation)

Choi H, Seong B. RNA Interaction-Mediated Protein Folding. 50th Annual Meeting of the American Society for Cell Biology. Philadelphia, PA, December 2010. (Poster presentation)

Son A*, **Choi H***, Kim H, Choi S, Seong B. Evidence for RNA Interaction Mediated Protein Folding. 5th Cold Spring Harbor Laboratory meeting on Neurodegenerative Diseases: Biology and Therapeutics. Cold Spring Harbor, NY, December 2008. (Poster presentation)
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PROFESSIONAL MEMBERSHIPS

Trainee member **2017 - present**
American Association of Immunologists

Student/Trainee member **2016 - 2018**
American Heart Association