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Authors

Magat, Jenna M

Thomas, Joanna L

Dumouchel, Justin P

et al.

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Endogenous IL-33 and its auto-amplification of IL-33/ST2 pathway play an important role in asthma

Jenna M. Magat[1,3], **Joanna L. Thomas**[2,3,4], **Justin P. Dumouchel**[1], **Fiona Murray**[1,5], **Willis X. Li**[1], **Jinghong Li**[1,§]

[1]Department of Medicine, University of California San Diego, La Jolla, CA

[2]Veterans Affairs San Diego Healthcare System, San Diego, CA

[3]Jenna M. Magat and Joanna L. Thomas contributed equally to this work.

[4]Present address: Department of Biomedical Engineering, Mercer University, Macon, GA 31207

[5]Present address: School of Medical Sciences, University of Aberdeen, Aberdeen, UK

Abstract

IL-33 and its receptor ST2 are contributing factors to airway inflammation and asthma exacerbation. The IL-33/ST2 signaling pathway is involved in both the onset and the acute exacerbations of asthma. Here we address the role of endogenous IL-33 and its auto-amplification of the IL-33/ST2 pathway in antigen-dependent and antigen-independent asthma-like models. Wildtype, IL-33 knockout, ST2 knockout mice were intratracheally administrated with either 500 ng of recombinant IL-33 per day for 4 consecutive days, or were sensitized and challenged with ovalbumin (OVA) over 21 days. In wildtype mice, IL-33 or OVA induced similar airway hyperresponsiveness and eosinophilic airway inflammation. IL-33 induced its own mRNA and ST2L mRNA expression in the lung. IL-33 auto-amplified itself and ST2 protein expression in airway epithelial cells. OVA also induced IL-33 and ST2 protein expression. In IL-33 knockout mice, the IL-33 and OVA induced airway hyperresponsiveness and eosinophilic airway inflammation were both significantly attenuated, while IL-33 induced ST2L mRNA expression was preserved, although no auto-amplification of IL-33/ST2 pathway was observed. In ST2 knockout mice, IL-33 and OVA induced airway hyperresponsiveness and eosinophilic airway inflammation were both completely diminished and no IL-33/ST2 auto-amplification was observed. These results suggest that endogenous IL-33 and its auto-amplification of IL-33/ST2 pathway play an important role in the induction of asthma-like phenotype. Thus an intact IL-33/ST2 pathway is necessary for both antigen-dependent and antigen-independent asthma-like mouse models.

Keywords

Interleukin-33; ST2; asthma; auto-amplification

§ Correspondence to: Jinghong Li, M.D., Ph.D., Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of California, San Diego, La Jolla, CA 92093, jinghongli@ucsd.edu.

Introduction

Interleukin-33 (IL-33) was identified as the functional ligand for the IL-1 receptor family member ST2 (1). ST2 has two forms, one is the transmembrane ST2 (ST2L), which transduces the IL-33 signaling. The other one is a soluble ST2 molecule (sST2) due to alternative splicing (2). sST2 acts as a decoy receptor for IL-33 (3). Polymorphisms of human IL-33 and ST2 genes are associated with asthma and increased numbers of eosinophils (4, 5). IL-33 is a contributing factor to airway inflammation and asthma exacerbation (6, 7). The serum concentrations of sST2 are higher in asthma patients during acute exacerbation, suggesting that the host tries to attenuate IL-33 signaling (8, 9). An IL-33 loss-of-function mutation is associated with reduced blood eosinophil counts and people with this mutation are protected from asthma (10).

IL-33 was originally characterized as a nuclear protein (11). It has a dual function of a potent cytokine and a nuclear transcription factor (12, 13). IL-33 binds to its receptor ST2 to augment the production of Th2 cytokines IL-4, IL-5 and IL-13 *in vitro* and *in vivo* (1, 14). It has been shown that epithelial derived IL-33 is a potent stimulator to induce IL-13 producing type 2 innate lymphoid cells (ILC2) (15, 16). ILC2 contribute to antigen-dependent and antigen-independent airway hyperactivity (15, 16). The role of IL-33 nuclear localization and chromatin association was not well established (17). IL-33 was found to sequester nuclear NF-kappaB and dampen proinflammatory signaling (13). We have previously shown that the intratracheal administration of IL-33 for 4 days induced an asthma-like phenotype mouse model with increased production of Th2 cytokines (18). The IL-33/ST2 signaling pathway may work in an autocrine and paracrine manner to exacerbate airway inflammation. In addition, recent data showed multiple feedback circuits involving IL-33 are required for asthma persistence (19).

In this study, we address the role of auto-amplification of IL-33/ST2 pathway in antigen dependent and antigen independent asthma-like mouse models. In IL-33 knockout mice, we found that exogenous IL-33 could activate the IL-33/ST2 pathway but no IL-33 auto-amplification happened. As a result, no robust airway hyperresponsiveness or eosinophilic inflammation were elicited. In ST2 knockout mice, no IL-33/ST2 auto-amplification was observed and no airway hyperresponsiveness and eosinophilic inflammation were found. Endogenous IL-33 and its auto-amplification of IL-33/ST2 pathway play an important role in the induction of asthma-like phenotype.

Materials and methods

IL-33-induced and OVA-induced asthma-like phenotype in mouse models

All studies were performed according to NIH Guidelines for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee of the San Diego VA Healthcare System. Mouse strains studied included C57BL/6 from Jackson Laboratories (Bar Harbor, ME), IL-33 knockout from Dr. Richard Lee (Brigham and Women's Hospital, Harvard Medical School); ST2 knockout from Dr. Shizuo Akira (Research Institute for Microbial Disease, Osaka University, Japan). Asthma-like phenotype mouse models were previously described (18). In brief, IL-33 Model: 6–8-week-old mice

were intratracheally administrated with 500 ng of recombinant IL-33 per day for 4 consecutive days. OVA Model: 6–8-week-old mice were immunized and sensitized to OVA using a 21-day protocol. Mice were immunized by intraperitoneal injection of 50 μg OVA and 1 mg alum in PBS on day 0 and day 7, followed by 4 intratracheal injections of 20 μg OVA in 50 μl of PBS on days 17, 18, 19 and 20. On day 5 for the IL-33 model or on day 21 for the OVA model, the mice were intubated with a 20 gauge IV catheter and placed on a computer-controlled small animal ventilator (Flexivent, SCRIEQ, Montreal, Canada) delivering 2% isoflurane continuously (20). Baseline airway resistance was measured. The animals were then challenged with an ultrasonic aerosol of 1.5, 3, 6, 12 and 24 mg/ml of methacholine. The peak airway resistance (Rrs) with each dose was obtained.

BAL cell counts, real time qPCR and ELISA

Following the methacholine challenges, the mice were euthanized and bronchoalveolar lavage (BAL) fluid were obtained with 0.5 ml of PBS repeated for 3 times. The total cell counts and differentiation of the cells in BAL were studied. One lung was removed for RNA extraction and protein extraction. The other lung was fixed for histological analysis and immunohistochemistry. Total RNA was extracted from lung tissue by using TRIzol (Invitrogen, Carlsbad, CA). cDNA was synthesized and real time qPCR were performed by SYBR Green (Invitrogen, Carlsbad, CA). Expression is shown relative to the reference gene GAPDH. Primers are: Mouse IL-33 F: ACTCCAAGATTTCGCCG; Mouse IL-33 R: CATGCAGTAGACATGGCAGAA; Mouse ST2L F: GTGATAGTCTTAAAAGTGTTCTGG; Mouse ST2L R: TCAAAAGTGTTTCAGGTCTAAGCA; Mouse sST2 F: ACGCTCGACTTATCCTGTGG; Mouse sST2 R: CAGCTCAATTGTTGGACACG. ELISA for IL33 and sST2 were performed on BAL samples with mouse IL-33 and ST2 ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Immunochemhistology and lung inflammation score

Formalin-fixed paraffin embedded specimens were serially sectioned at 5 um intervals and mounted on glass slides. Immunohistochemistry (IHC) for IL-33 and ST2 were performed using the ABC (Avidin-Biotin Complex) Kits (Vector Laboratories, Burlingame, CA). Rabbit anti-mouse ST2 antibody (Abcam, Cambridge, MA), mouse anti-mouse IL-33 antibody (Abcam, Cambridge, MA), rabbit and mouse IgG isotype controls (Vector Laboratories, Burlingame, CA) were used. Lung inflammation score was performed based on the degree of hypertrophy of the airway epithelium and peribronchial/perivascular cellular inflammation on a scale of 0–5 by lung pathologist blinded to the experimental groups. Peribronchial/perivascular infiltrates as: 0 (not present); 1 (<20% of the airways affected); 2 (20–40%); 3 (40–60%); 4 (60–80%), 5, (>80%).

Western blots

Total lung proteins were prepared with RIPA buffer. Proteins were separated in 10% SDS/PAGE and transferred to nitrocellulose membrane. The membrane was blocked for 1 hour in blocking buffer for fluorescent Western Blot (Rockland, Gilbertsville, PA). Proteins were detected by Western blot by using anti-IL-33 antibody (Abcam, Cambridge, MA). Fluorescent-labeled secondary antibody (Li-Cor Biosciences, Lincoln, NE) was used to

detect the protein bands. An Odyssey imaging system imager was used to capture the images (Li-Cor Biosciences, Lincoln, NE).

Statistical Analysis

ANOVA was performed using GraphPad Prism software (La Jolla, CA). Statistical analyses of data were performed using paired and unpaired Student's t-tests or using one-way ANOVA. For qPCR results, log fold changes were used for t-tests. Statistical significance is defined by $p<0.05$.

Results

IL-33 auto-amplifies the IL-33/ST2 pathway by inducing its own and ST2 expression.

We have previously shown that intratracheal administration of recombinant IL-33 can induce airway hyperresponsiveness and eosinophilic airway inflammation in wildtype mice (18). In the IL-33 models, we found IL-33 induced its own mRNA and ST2L mRNA levels in the lung tissue (Figure 1A). We also examined the BAL IL-33 and sST2 protein levels by ELISA. We found that the endogenous IL-33 levels were increased and the sST2 levels were nearly abolished in BAL (Figure 1B, 1C). In the OVA models, although the IL-33 and ST2 mRNA levels were not significantly changed in the lung tissue (Figure 1A), the BAL IL-33 levels were increased and sST2 levels were decreased with similar trends of lesser magnitude compared to the IL-33 models (Figure 1B, 1C).

Airway epithelial cells are the major source of endogenous IL-33 production in auto-amplification of IL-33/ST2 pathway.

To investigate the changes in the IL33/ST2 pathway at the cellular level, we performed IHC studies (Figure 2A). In the saline controls (Figure 2A, left panel), IL-33 was found in the airway epithelial cells, whereas ST2 was minimally detected in the airway epithelium. In the IL-33 models (Figure 2A, middle panel), IL-33 was found in the airway and alveolar epithelial cells, interstitial cells, including lymphocytes, macrophages, and the nucleus of Type II epithelial cells. ST2 was found in epithelial cells and interstitial cells as well. In the OVA models (Figure 2A, right panel), in addition to the airway and alveolar epithelial cells and interstitial cells, IL-33 was also found in smooth muscle cells and the cytoplasm of alveolar macrophages. ST2 was found in the epithelium and interstitial cells. Lung inflammation scores were performed by a lung pathologist blinded to the experimental groups (Figure 2B). Both IL-33 and OVA groups showed significant increased lung inflammation scores compare with saline control group. Examples of lung tissue IL-33 protein levels are shown by Western blots (Figure 2C). Recombinant IL-33 (exogenous) and endogenous IL-33 were distinguishable in sizes. Auto-amplified endogenous IL-33 protein levels in IL-33 models were confirmed.

Endogenous IL-33 is necessary for antigen dependent and antigen independent asthma-like phenotype in vivo.

In order to understand if endogenous IL-33 is necessary for antigen dependent and antigen independent asthma-like phenotype, we utilized the IL-33 knockout mice. In IL-33 knockout mice, recombinant IL-33 induced airway hyperresponsiveness and eosinophilic airway

inflammation were significantly attenuated compared with wildtype mice (Figure 3A, 3B). Exogenous recombinant IL-33 was not sufficient to restore the asthma-like phenotype in IL-33 knockout mice, despite the intact ST2 and other components of downstream IL-33/ST2 pathway. In IL-33 knockout mice, OVA induced airway hyperresponsiveness and eosinophilic airway inflammation were significantly attenuated as well (Figure 3A, 3B).

Exogenous IL-33 can induce ST2L mRNA expression but is not sufficient to induce the endogenous auto-amplification of IL-33/ST2 pathway.

To further understand why exogenous IL-33 was not sufficient to restore the asthma-like phenotype in IL-33 knockout mice, we examined the ST2L, sST2 mRNA in lung tissue and IL-33, sST2 protein levels in BAL. In IL-33 models, we found the exogenous IL-33 induced ST2L mRNA expression was preserved in lung tissue (Figure 4A), whereas the effects of IL-33 on increasing IL-33 protein levels and decreasing sST2 s in the BAL were diminished (Figure 4B, 4C). In OVA models, OVA induced ST2L mRNA expression was attenuated (Figure 4A). The effects of OVA on increasing IL-33 protein levels and decreasing sST2 protein levels in the BAL were not significant (Figure 4B, 4C).

ST2 is necessary for antigen dependent and antigen independent asthma-like phenotype *in vivo*.

ST2 is currently the only known receptor for IL-33 signaling. In ST2 knockout mice, both IL-33 and OVA induced airway hyperresponsiveness or eosinophilic airway inflammation were completely diminished. There were no significant differences in airway hyperresponsiveness and total cell count and cell differentials compared with saline controls (Figure 5A, 5B). In ST2 knockout mice, IL-33 induced auto-amplification of IL-33 was completely diminished (Figure 5C). OVA induced IL-33 was also completely diminished (Figure 5C).

Discussion

IL-33 is expressed in higher levels in endobronchial biopsies from human asthma patients (7). The elevated IL-33 expression was particularly evident in severe asthmatic subjects (6, 7). IL-33 functions both extracellularly as a cytokine through activation of the ST2L receptor and intracellular as a nuclear transcription factor (12, 13). IL-33 can increase ST2 expression in eosinophils (21). In animal models, multiple feedback circuits involving IL-33 are required for asthma persistence (19). In our IL-33 models, we found parallel increases of IL-33 and ST2L mRNA levels in the lung. In both IL-33 and OVA models, we found increased IL-33 and decreased sST2 in BAL. We found epithelial cells are the major source of endogenous IL-33 and ST2 production. These data suggest the auto-amplification of IL-33/ST2 pathway. OVA induced IL-33 and ST2 expression may have a different mechanism. We found enlarged mediastinal lymph nodes in the OVA models, ST2 was significantly increased in the lymph nodes (data not shown). Our data are consistent with the findings that dust mites and other allergens can induce IL-33 (22, 23) and elevate serum sST2 levels (24). Auto-amplification of IL-33/ST2 pathway is necessary for the induction of asthma like phenotype in both IL-33 models and OVA models.

We used immunohistochemistry to examine the major source of IL-33 in lung tissue. IL-33 was localized to the nuclei of airway and alveolar epithelial cells. IL-33 induced IL-33 and ST2 expression localizing at the same epithelial cells and interstitial cells. It was reported that IL-33 accumulated in the nucleus of producing cells and binds to histones and chromatin (25, 26). The function of IL-33 as a nuclear factor was not well established. A recent study showed the only identified protein modulated by IL-33 as a nuclear factor was IL-33 itself (27). Our data provided the evidence that IL-33 auto-amplified itself and ST2 protein expression in airway epithelial cells. It was reported that multiple feedback and feed-forward circuits between epithelial cells and ILC2 were identified (19). IL-33 may regulate IL-33/ST2 pathway in several manners, such as autocrine and paracrine regulation as a cytokine, and transcriptional regulation or epigenetic regulation at the chromatin level as a nuclear factor.

In IL-33 knockout mice, we showed that exogenous recombinant IL-33 induced a modest airway hyperresponsiveness and mild eosinophilic airway inflammation. Exogenous IL-33 induced ST2L mRNA expression was preserved in lung tissue. However, due to the lack of endogenous IL-33 and its auto-amplification of IL-33/ST2 pathway, BAL IL-33 levels and sST2 levels were not changed, leading to only moderate asthma phenotype. These data indicate that exogenous IL-33 was able to induce ST2L mRNA expression based on the intact ST2 and downstream pathway. The IL-33/ST2 signaling was successfully initiated, but failed to maintain due to lack of endogenous IL-33 and its auto-amplification. We conclude that multiple feedback loops of continuous auto-amplification of IL-33/ST2 pathway are necessary for maintaining the asthma-like phenotype in vivo. It was previously reported that IL-33 knockout mice showed attenuated eosinophil and lymphocyte recruitment to the lung in OVA model (28, 29). We observed similar results. OVA can elicit a moderate response due to the intact adaptive immune response in IL-33 knockout mice. In both IL-33 and OVA models, the responses are reduced due to lack of endogenous IL-33. These data indicate that the endogenous IL-33 and its auto-amplification of IL-33/ST2 pathway are necessary for antigen dependent and antigen independent asthma-like phenotype *in vivo*.

We showed that ST2 expression in the airway epithelium and inflammatory cell infiltrate were increased in both IL-33 and OVA induced asthma models. In ST2 knockout mice, both IL-33 and OVA induced airway hyperresponsiveness or eosinophilic airway inflammation were completely diminished. ST2 knockout mice are partially protected from house dust mite induced asthma, especially in the peripheral lung (23). An intact IL-33/ST2 pathway is necessary for both antigen-dependent and antigen-independent asthma-like mouse models.

In summary, endogenous IL-33 and its auto-amplification of IL-33/ST2 pathway play an important role in both antigen dependent and antigen independent asthma-like mouse models. Airway epithelial cells are the major source of endogenous IL-33 production in auto-amplification of IL-33/ST2 pathway. IL-33 and sST2 are possible biomarkers for asthma exacerbation (24, 30) and also could be therapeutic targets to inhibit the IL-33/ST2 auto-amplification. Novel treatment including IL-33 antibodies, sST2, and vaccination against IL-33 are under investigation in asthma (3, 31–33). Our recent study of small molecule inhibitors of the IL-33/ST2 pathway showed beneficial results (18). Clinical trials with antibodies targeting IL-33 have already been initiated (34).

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References

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, 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):479–90. [PubMed: 16286016]
2. Tominaga S, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, and Komatsu N. Presence and expression of a novel variant form of ST2 gene product in human leukemic cell line UT-7/GM. Biochem Biophys Res Commun. 1999;264(1):14–8. [PubMed: 10527832]
3. Hayakawa H, Hayakawa M, Kume A, and Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. J Biol Chem. 2007;282(36):26369–80. [PubMed: 17623648]
4. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, and Cookson WO. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363(13):1211–21. [PubMed: 20860503]
5. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, Helgadottir H, Steinthorsdottir V, Stefansson H, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet. 2009;41(3):342–7. [PubMed: 19198610]
6. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, Pitman N, Mirchandani A, Rana B, van Rooijen N, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. J Immunol. 2009;183(10):6469–77. [PubMed: 19841166]
7. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, Lemiere C, Martin JG, and Hamid Q. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol. 2009;183(8):5094–103. [PubMed: 19801525]
8. Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S, Tominaga SI, and Sugiyama Y. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. Am J Respir Crit Care Med. 2001;164(2):277–81. [PubMed: 11463601]
9. Watanabe M, Nakamoto K, Inui T, Sada M, Honda K, Tamura M, Ogawa Y, Yokoyama T, Saraya T, Kurai D, et al. Serum sST2 levels predict severe exacerbation of asthma. Respir Res. 2018;19(1):169. [PubMed: 30176857]
10. Smith D, Helgason H, Sulem P, Bjornsdottir US, Lim AC, Sveinbjornsson G, Hasegawa H, Brown M, Ketchem RR, Gavala M, et al. A rare IL33 loss-of-function mutation reduces blood eosinophil counts and protects from asthma. PLoS Genet. 2017;13(3):e1006659. [PubMed: 28273074]
11. Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, Brandtzaeg P, Erard M, Haraldsen G, and Girard JP. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. Am J Pathol. 2003;163(1):69–79. [PubMed: 12819012]
12. Haraldsen G, Balogh J, Pollheimer J, Sponheim J, and Kuchler AM. Interleukin-33 - cytokine of dual function or novel alarmin? Trends Immunol. 2009;30(5):227–33. [PubMed: 19359217]
13. Ali S, Mohs A, Thomas M, Klare J, Ross R, Schmitz ML, and Martin MU. The dual function cytokine IL-33 interacts with the transcription factor NF-kappaB to dampen NF-kappaB-stimulated gene transcription. J Immunol. 2011;187(4):1609–16. [PubMed: 21734074]
14. Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC, Komai-Koma M, Pitman N, Li Y, Niedbala W, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. J Immunol. 2008;181(7):4780–90. [PubMed: 18802081]
15. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, Wu X, Kane CM, Neill DR, 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):933–41. [PubMed: 23810766]

16. Kim HY, Chang YJ, Subramanian S, Lee HH, Albacker LA, Matangkasombut P, Savage PB, McKenzie AN, Smith DE, Rottman JB, et al. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. J Allergy Clin Immunol. 2012;129(1):216–27 e1–6. [PubMed: 22119406]
17. Bessa J, Meyer CA, de Vera Mudry MC, Schlicht S, Smith SH, Iglesias A, and Cote-Sierra J. Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. J Autoimmun. 2014;55(33–41.
18. Li J, Saruta K, Dumouchel JP, Magat JM, Thomas JL, Ajami D, Rebek M, Rebek J Jr., and Bigby TD. Small Molecule Mimetics of alpha-Helical Domain of IRAK2 Attenuate the Proinflammatory Effects of IL-33 in Asthma-like Mouse Models. J Immunol. 2018;200(12):4036–43. [PubMed: 29728508]
19. Christianson CA, Goplen NP, Zafar I, Irvin C, Good JT Jr., Rollins DR, Gorentla B, Liu W, Gorska MM, Chu H, 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):59–68 e14. [PubMed: 25617223]
20. Thomas JL, Dumouchel J, Li J, Magat J, Balitzer D, and Bigby TD. Endotracheal intubation in mice via direct laryngoscopy using an otoscope. J Vis Exp. 201486).
21. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, and Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. J Immunol. 2010;185(6):3472–80. [PubMed: 20693421]
22. Castanhinha S, Sherburn R, Walker S, Gupta A, Bossley CJ, Buckley J, Ullmann N, Grychtol R, Campbell G, Maglione M, et al. Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33. J Allergy Clin Immunol. 2015;136(2):312–22 e7. [PubMed: 25746970]
23. Zoltowska AM, Lei Y, Fuchs B, Rask C, Adner M, and Nilsson GP. The interleukin-33 receptor ST2 is important for the development of peripheral airway hyperresponsiveness and inflammation in a house dust mite mouse model of asthma. Clin Exp Allergy. 2016;46(3):479–90. [PubMed: 26609909]
24. Mitchell PD, Salter BM, Oliveria JP, El-Gammal A, Tworek D, Smith SG, Sehmi R, Gauvreau GM, and PM OAB. IL-33 and Its Receptor ST2 after Inhaled Allergen Challenge in Allergic Asthmatics. Int Arch Allergy Immunol. 2018;176(2):133–42. [PubMed: 29694974]
25. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, Bouche G, and Girard JP. 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):282–7. [PubMed: 17185418]
26. Roussel L, Erard M, Cayrol C, and Girard JP. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. EMBO Rep. 2008;9(10):1006–12. [PubMed: 18688256]
27. Gautier V, Cayrol C, Farache D, Roga S, Monsarrat B, Burlet-Schiltz O, Gonzalez de Peredo A, and Girard JP. Extracellular IL-33 cytokine, but not endogenous nuclear IL-33, regulates protein expression in endothelial cells. Sci Rep. 2016;6(34255.
28. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Matsumoto 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):18581–6. [PubMed: 20937871]
29. Louten J, Rankin AL, Li Y, Murphy EE, Beaumont M, Moon C, Bourne P, McClanahan TK, Pflanz S, and de Waal Malefyt R. Endogenous IL-33 enhances Th2 cytokine production and T-cell responses during allergic airway inflammation. Int Immunol. 2011;23(5):307–15. [PubMed: 21422152]
30. Werder RB, Zhang V, Lynch JP, Snape N, Upham JW, Spann K, and Phipps S. Chronic IL-33 expression predisposes to virus-induced asthma exacerbations by increasing type 2 inflammation and dampening antiviral immunity. J Allergy Clin Immunol. 2018;141(5):1607–19 e9. [PubMed: 28947081]
31. Lei Y, Boinapally V, Zoltowska A, Adner M, Hellman L, and Nilsson G. Vaccination against IL-33 Inhibits Airway Hyperresponsiveness and Inflammation in a House Dust Mite Model of Asthma. PLoS One. 2015;10(7):e0133774. [PubMed: 26214807]

32. Lee HY, Rhee CK, Kang JY, Byun JH, Choi JY, Kim SJ, Kim YK, Kwon SS, and Lee SY. Blockade of IL-33/ST2 ameliorates airway inflammation in a murine model of allergic asthma. Exp Lung Res. 2014;40(2):66–76. [PubMed: 24446582]

33. Zoltowska Nilsson AM, Lei Y, Adner M, and Nilsson GP. Mast cell-dependent IL-33/ST2 signaling is protective against the development of airway hyperresponsiveness in a house dust mite mouse model of asthma. Am J Physiol Lung Cell Mol Physiol. 2018;314(3):L484–L92. [PubMed: 29146574]

34. Mitchell PD, El-Gammal AI, and O'Byrne PM. Emerging monoclonal antibodies as targeted innovative therapeutic approaches to asthma. Clin Pharmacol Ther. 2016;99(1):38–48. [PubMed: 26502193]

Key points

- IL-33 auto-amplifies the IL-33/ST2 pathway by inducing its own and ST2 expression.
- Airway epithelial cells are the major source of endogenous IL-33 production.
- An intact endogenous IL-33/ST2 pathway is necessary for asthma-like mouse models.

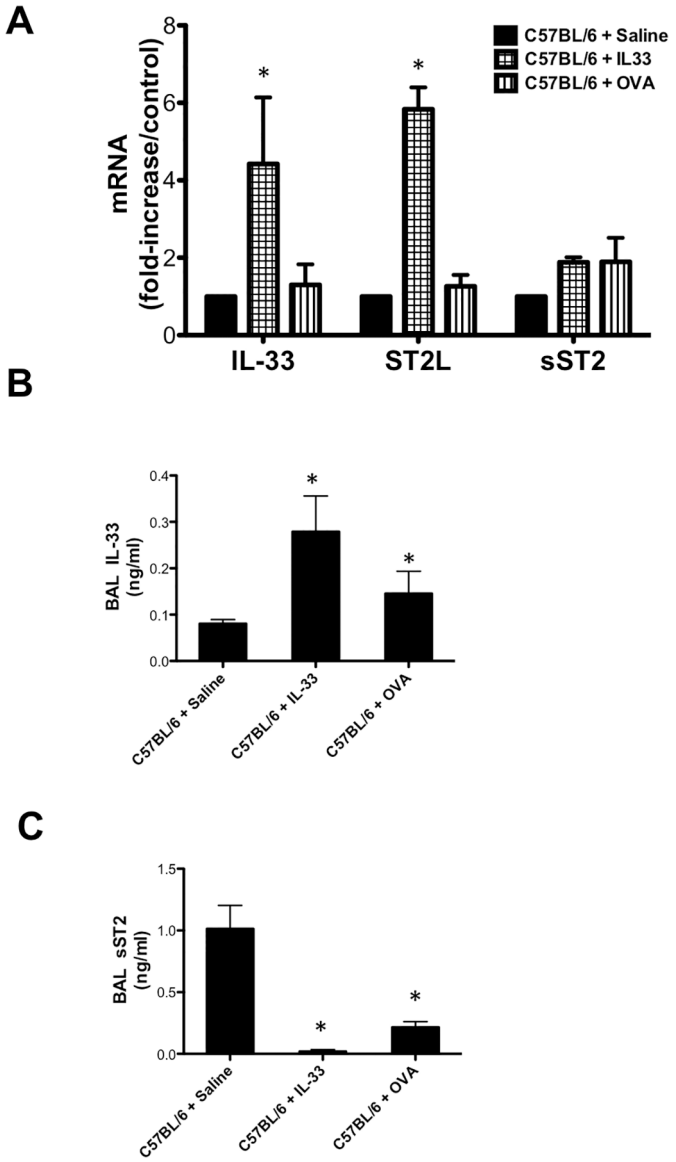

Figure 1. IL-33 auto-amplifies the IL-33/ST2 pathway by inducing its own and ST2 expression.

A. Recombinant IL-33 induces endogenous IL-33 and ST2L mRNA in the lung tissue. Wildtype mice were treated with saline, IL-33 or OVA. Real time qPCR was performed by using IL-33, ST2L, sST2 specific primers. In the IL-33 model, 4-fold increase of IL-33 was observed compared with saline controls (n=6). 5.8-fold increase of ST2L was observed compared with saline controls (n=6). Student's T-test was used to calculate the significance of differences in log fold-changes of the qPCR results, which are normally distributed. * $P < 0.01$ compared with saline controls.

B. IL-33 induces IL-33 in BAL, compared with saline controls (n=6). OVA has moderate increase of IL-33, compared with saline controls (n=6). * $P < 0.01$ compared with saline controls.

C. IL-33 and OVA decreases sST2 in the BAL, compared with saline controls (n=6). * $P < 0.01$ compared with saline controls.

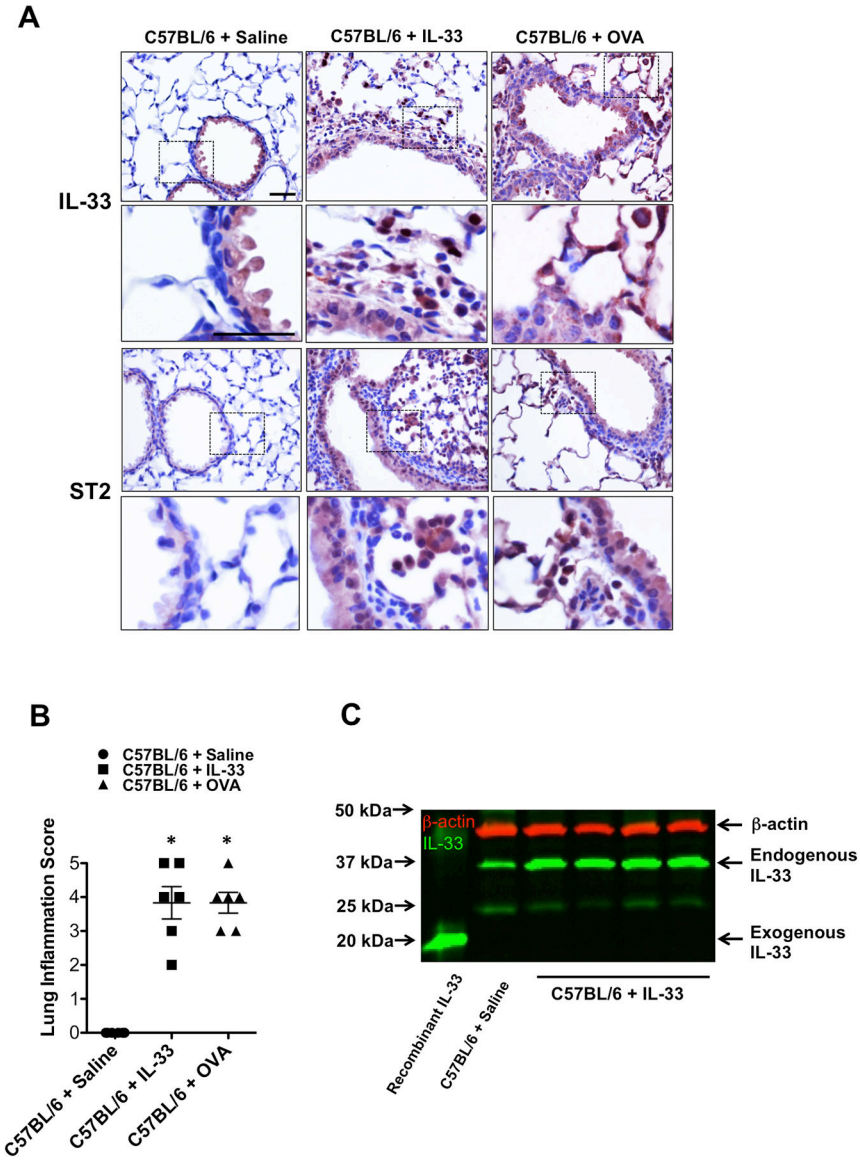

Figure 2. Airway epithelial cells are the major source of endogenous IL-33 production in auto-amplification of IL-33/ST2 pathway.

A. Representative images of IHC lung specimens were shown at Fig. 2A (n=6). Scale bars represent 100 μm at lower or 50 μm at higher magnifications. Higher magnification images are shown for regions indicated by dashed boxes. Left panel, wildtype mice with saline control. IL-33 was mostly found in the airway epithelial cells (top two rows), very less was found in the alveolar epithelial cells. ST2 was minimally detected in the airway epithelium (bottom two rows). Middle panel, wildtype mice with IL-33 models, IL-33 was found in the airway and alveolar epithelial cells, interstitial cells, including lymphocytes, macrophages, and the nucleus of Type II epithelial cells. ST2 was found in epithelial cells and interstitial cells as well. Right panel: wildtype mice with OVA models, in addition to the airway and alveolar epithelial cells and interstitial cells, IL-33 was also found in smooth muscle cells and the cytoplasm of alveolar macrophages. ST2 was found in the epithelium and interstitial cells.

B. Lung inflammation score was performed based on the degree of hypertrophy of the airway epithelium and peribronchial/perivascular cellular inflammation. Both IL-33 and OVA models showed significant increased lung inflammation scores compare with saline control group. * $P < 0.01$ compared with saline controls.

C. Examples of lung tissue IL-33 protein levels are shown by Western blots. Lung tissue proteins from the indicated mice were subject to SDS-PAGE, the resulting membrane was blotted with anti-IL33 (green) and anti-β-actin (red), and were analyzed with an Odyssey imaging system imager. Multiple Western blots were done and representative mages are shown. From left, Lane 1: Recombinant IL-33 protein (exogenous, MW 18.1 kDa). Lane 2: wildtype mice with saline control. Lane 3–6: wild-type mice with IL-33 models. The endogenous IL-33 (lane 2–6) has a MW 35 kDa. In IL-33 group, the endogenous IL-33 is upregulated compared to the saline control group. In all the mice groups, no detectable recombinant IL-33 was found.

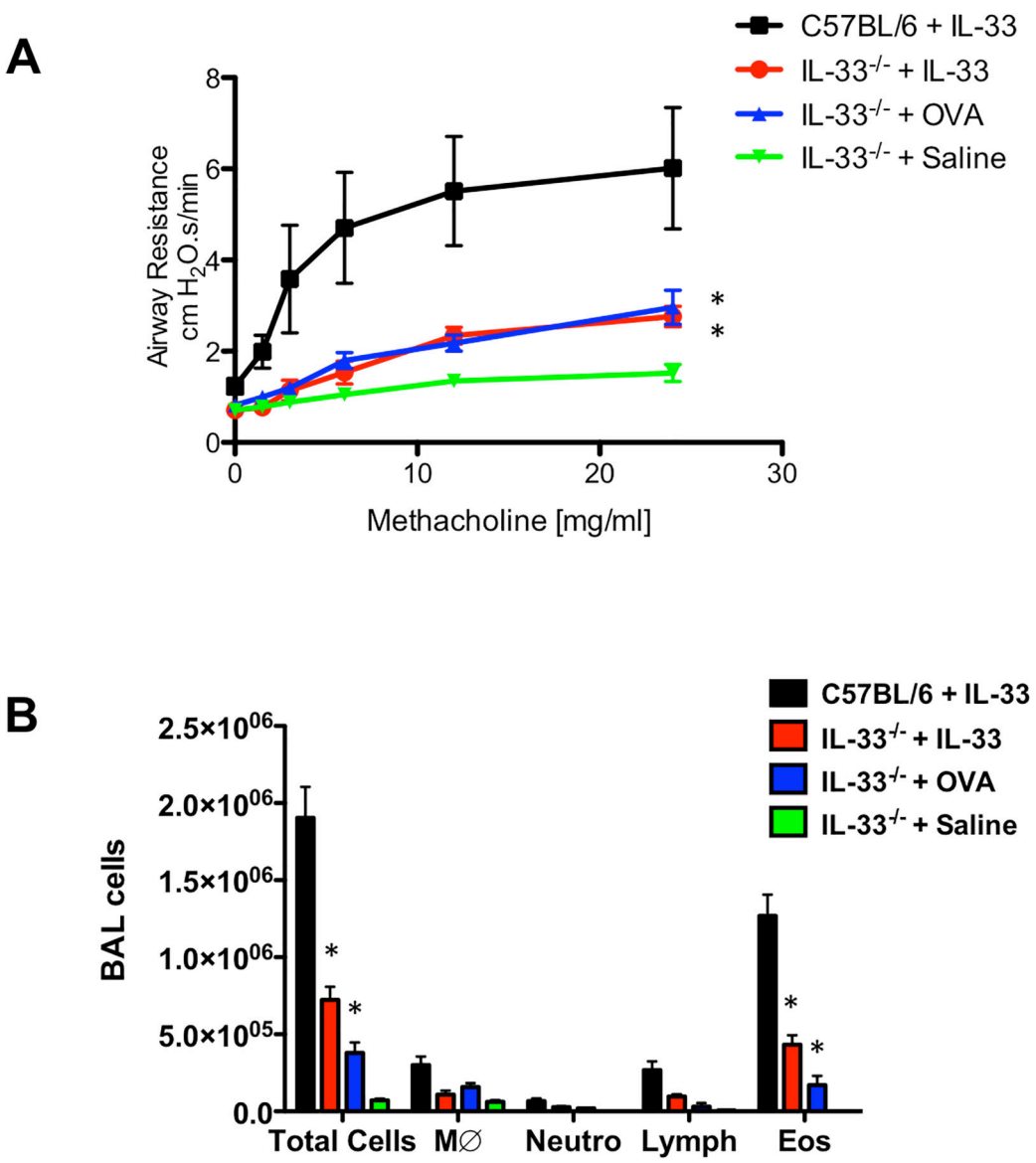

Figure 3. Endogenous IL-33 is necessary for antigen dependent and antigen independent asthma-like phenotype in vivo.

A. IL-33 knockout mice were treated with saline, IL-33 or OVA. IL-33 induced airway hyperresponsiveness was significantly attenuated (n=6). * $P < 0.01$ compared with wildtype with IL-33 model. OVA induced airway hyperresponsiveness was significantly attenuated (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model.

B. In IL-33 knockout mice, IL-33 induced eosinophilic infiltration was significantly attenuated (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model. OVA induced eosinophilic infiltration was significantly attenuated (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model.

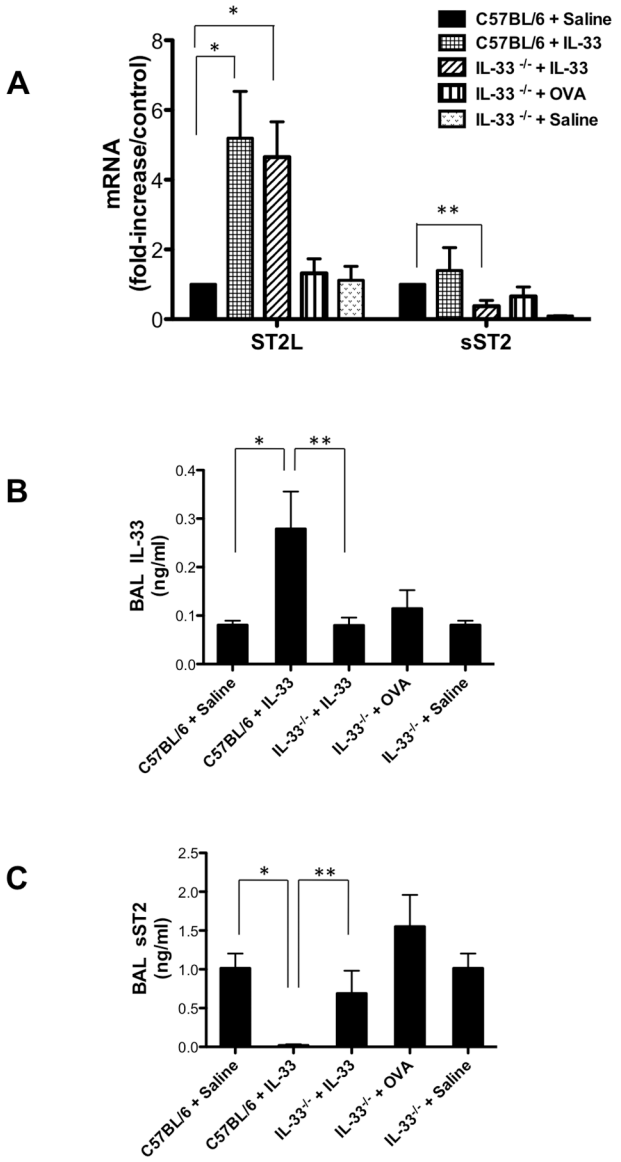

Figure 4. Exogenous IL-33 can induce ST2L mRNA expression but is not sufficient to induce the endogenous auto-amplification of IL-33/ST2 pathway.

A. In IL-33 knockout mice, IL-33 induced ST2L mRNA expression was preserved in the lung (n=6), comparable with wildtype mice saline control. *P < 0.01 compared with wildtype mice saline controls. OVA induced ST2L mRNA expression was attenuated (n=6), not significant compared with wildtype mice saline controls. IL-33 decreased sST2 mRNA expression in the lung (n=6), comparable with wildtype mice saline control. **P < 0.05 compared with wildtype mice saline controls.

B. In IL-33 knockout mice, the effects of IL-33 on increasing IL-33 protein levels in the BAL were diminished (n=6), compared with wildtype mice IL-33 model. ** P < 0.01 compared with wildtype mice IL-33 model. The effects of OVA on increasing IL-33 protein levels in the BAL were not significant compared with saline controls (n=6).

C. The effects of IL-33 on decreasing sST2 protein levels in the BAL were diminished (n=6), compared with wildtype mice IL-33 model. ** P < 0.01 compared with wildtype

mice IL-33 model. The effects of OVA on decreasing sST2 protein levels in the BAL were not significant, compared with saline controls (n=6).

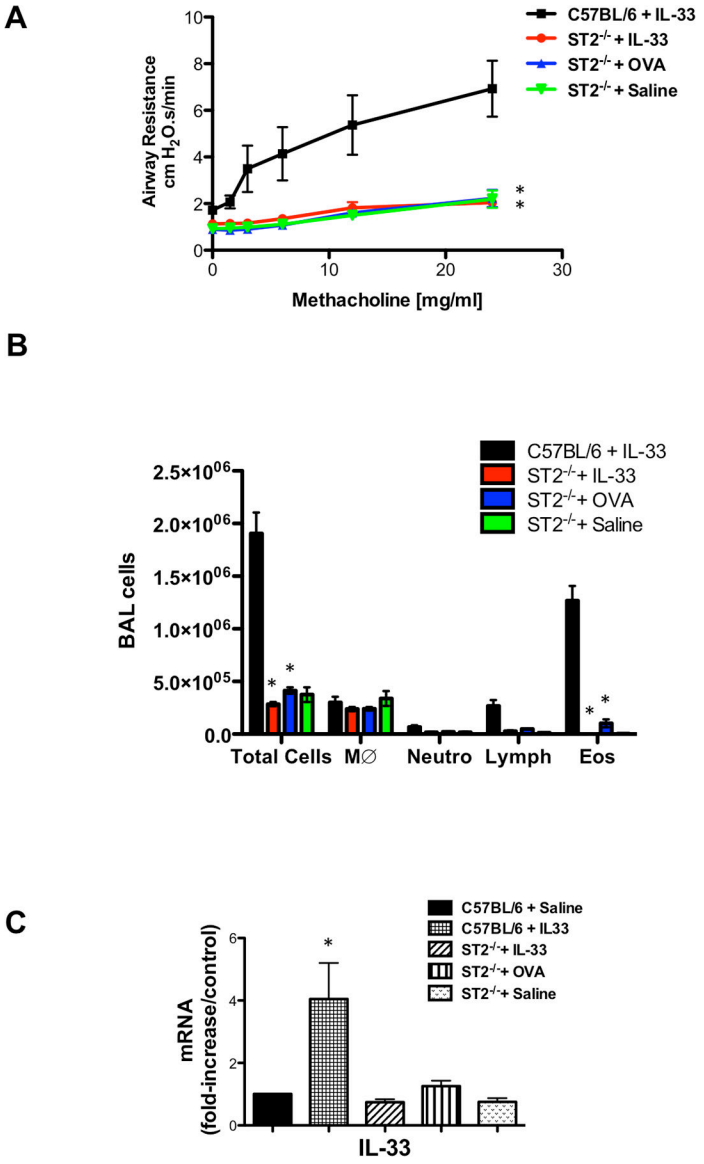

Figure 5. ST2 is necessary for antigen dependent and antigen independent asthma-like phenotype in vivo.

A. ST2 knockout mice were treated with saline, IL-33 or OVA. IL-33 induced airway hyperresponsiveness was completely diminished (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model. OVA induced airway hyperresponsiveness was completely diminished (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model.

B. In ST2 knockout mice, IL-33 induced eosinophilic infiltration was diminished (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model. OVA induced eosinophilic infiltration was diminished (n=6). * $P < 0.01$ compared with wildtype mice IL-33 model.

C. In ST2 knockout mice, recombinant IL-33 induces endogenous IL-33 mRNA in the lung tissue was completely diminished. OVA induced IL-33 mRNA in the lung tissue was also completely diminished. * $P < 0.01$ compared with wildtype mice saline controls.