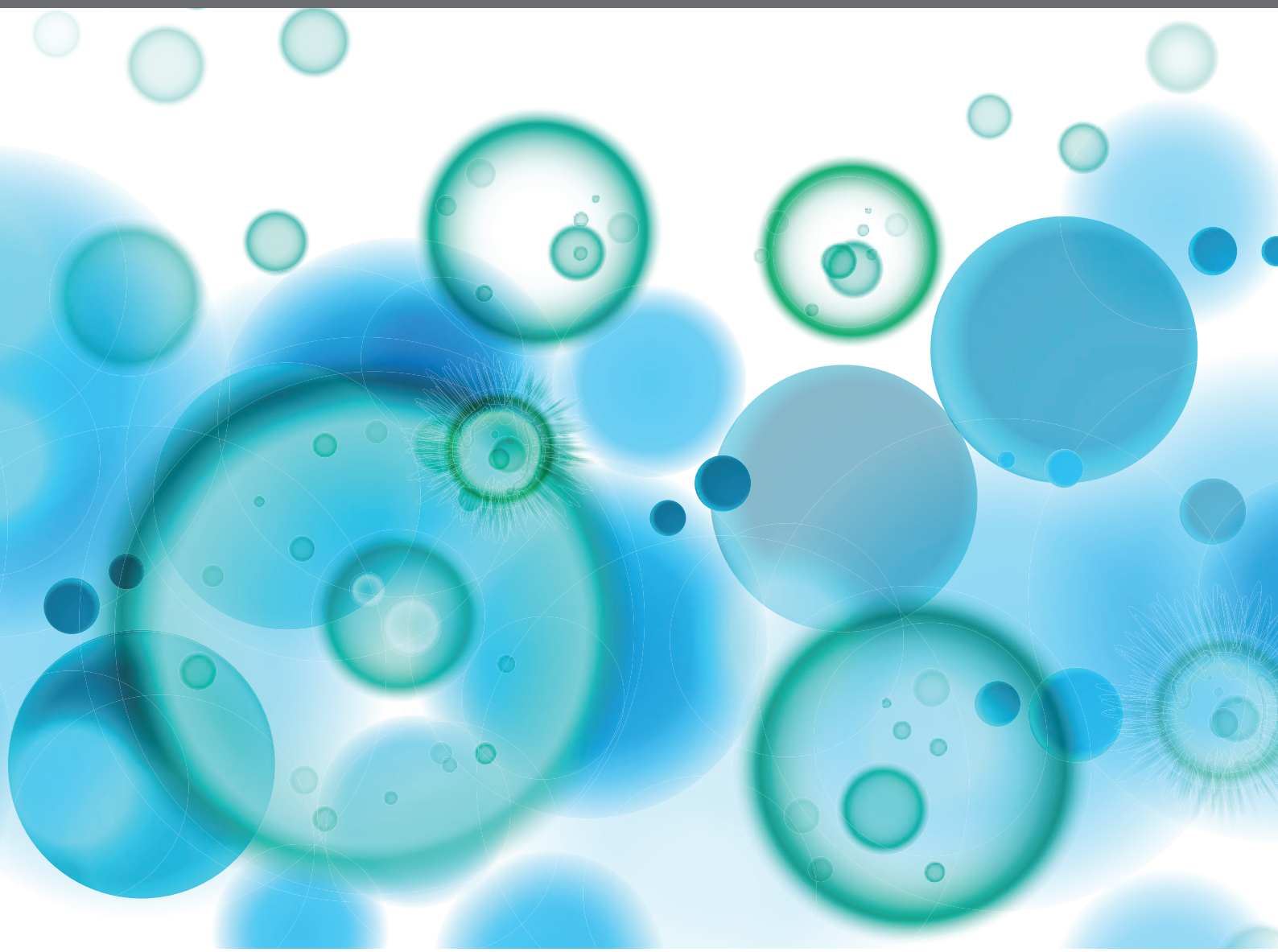
INTERLEUKIN-33 BIOLOGY IN TISSUE DEVELOPMENT, HOMEOSTASIS AND DISEASE

EDITED BY: Hui-Rong Jiang, Jose Carlos Alves-Filho, Fang-Ping Huang and Rong Mu

PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-88966-234-0
DOI 10.3389/978-2-88966-234-0

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

INTERLEUKIN-33 BIOLOGY IN TISSUE DEVELOPMENT, HOMEOSTASIS AND DISEASE

Topic Editors:
Hui-Rong Jiang, University of Strathclyde, United Kingdom
Jose Carlos Alves-Filho, University of São Paulo, Brazil
Fang-Ping Huang, Shenzhen University, China
Rong Mu, Peking University People's Hospital, China

Citation: Jiang, H.-R., Alves-Filho, J. C., Huang, F.-P., Mu, R., eds. (2020). Interleukin-33 Biology in Tissue Development, Homeostasis and Disease. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-234-0

Table of Contents

05 ***Interleukin-33 Receptor (ST2) Deficiency Improves the Outcome of* Staphylococcus aureus*-Induced Septic Arthritis***
Larissa Staurengo-Ferrari, Silvia C. Trevelin, Victor Fattori, Daniele C. Nascimento, Kalil A. de Lima, Jacinta S. Pelayo, Florêncio Figueiredo, Rubia Casagrande, Sandra Y. Fukada, Mauro M. Teixeira, Thiago M. Cunha, Foo Y. Liew, Rene D. Oliveira, Paulo Louzada-Junior, Fernando Q. Cunha, José C. Alves-Filho and Waldiceu A. Verri

19 ***Integrated Expression Profiles Analysis Reveals Correlations Between the IL-33/ST2 Axis and CD8$^+$ T Cells, Regulatory T Cells, and Myeloid-Derived Suppressor Cells in Soft Tissue Sarcoma***
Huoying Chen, Yao Chen, Hongbo Liu, Yi Que, Xing Zhang and Fang Zheng

30 ***Genetic Regulation of the Thymic Stromal Lymphopoietin (TSLP)/TSLP Receptor (TSLPR) Gene Expression and Influence of Epistatic Interactions Between IL-33 and the TSLP/TSLPR Axis on Risk of Coronary Artery Disease***
Shao-Fang Nie, Ling-Feng Zha, Qian Fan, Yu-Hua Liao, Hong-Song Zhang, Qian-Wen Chen, Fan Wang, Ting-Ting Tang, Ni Xia, Cheng-Qi Xu, Jiao-Yue Zhang, Yu-Zhi Lu, Zhi-Peng Zeng, Jiao Jiao, Yuan-Yuan Li, Tian Xie, Wen-Juan Zhang, Dan Wang, Chu-Chu Wang, Jing-Jing Fa, Hong-Bo Xiong, Jian Ye, Qing Yang, Peng-Yun Wang, Sheng-Hua Tian, Qiu-Lun Lv, Qing-Xian Li, Jin Qian, Bin Li, Gang Wu, Yan-Xia Wu, Yan Yang, Xiang-Ping Yang, Yu Hu, Qing K. Wang, Xiang Cheng and Xin Tu

41 ***Interleukin 33 Selectively Augments Rhinovirus-Induced Type 2 Immune Responses in Asthmatic but not Healthy People***
Lisa M. Jurak, Yang Xi, Megan Landgraf, Melanie L. Carroll, Liisa Murray and John W. Upham

51 ***Regulatory Mechanisms of IL-33-ST2-Mediated Allergic Inflammation***
Hiroaki Takatori, Sohei Makita, Takashi Ito, Ayako Matsuki and Hiroshi Nakajima

58 ***IL-33/ST2 Axis in Organ Fibrosis***
Ourania S. Kotsiou, Konstantinos I. Gourgoulianis and Sotirios G. Zarogiannis

73 ***The Pro-tumorigenic IL-33 Involved in Antitumor Immunity: A Yin and Yang Cytokine***
Jean-Jacques Fournié and Mary Poupot

82 ***The Pleiotropic Immunomodulatory Functions of IL-33 and Its Implications in Tumor Immunity***
Claudia Afferni, Carla Buccione, Sara Andreone, Maria Rosaria Galdiero, Gilda Varricchi, Gianni Marone, Fabrizio Mattei and Giovanna Schiavoni

99 ***Interleukin-33 in Systemic Sclerosis: Expression and Pathogenesis***
Liya Li, Honglin Zhu and Xiaoxia Zuo

106 ***IL-33 Prevents MLD-STZ Induction of Diabetes and Attenuate Insulitis in Prediabetic NOD Mice***
Sladjana Pavlovic, Ivica Petrovic, Nemanja Jovicic, Biljana Ljujic, Marina Miletic Kovacevic, Nebojsa Arsenijevic and Miodrag L. Lukic

120 ***Expression and Function of IL-33/ST2 Axis in the Central Nervous System Under Normal and Diseased Conditions***
Karen Fairlie-Clarke, Mark Barbour, Chelsey Wilson, Shehla U. Hridi, Debbie Allan and Hui-Rong Jiang

136 ***Inhibiting Glycolysis and ATP Production Attenuates IL-33-Mediated Mast Cell Function and Peritonitis***
Heather L. Caslin, Marcela T. Taruselli, Tamara Haque, Neha Pondicherry, Elizabeth A. Baldwin, Brian O. Barnstein and John J. Ryan

149 ***Interleukin-33 in Malignancies: Friends or Foes?***
Jia-Xin Shen, Jing Liu and Guo-Jun Zhang

156 ***Deficiency in IL-33/ST2 Axis Reshapes Mitochondrial Metabolism in Lipopolysaccharide-Stimulated Macrophages***
Huadan Xu, Liankun Sun, Yichun He, Xiaofeng Yuan, Junqi Niu, Jing Su and Dong Li

169 ***IL33: Roles in Allergic Inflammation and Therapeutic Perspectives***
Ben C. L. Chan, Christopher W. K. Lam, Lai-Shan Tam and Chun K. Wong

180 ***Pleiotropic Effects of IL-33 on CD4$^+$ T Cell Differentiation and Effector Functions***
Fernando Alvarez, Jörg H. Fritz and Ciriaco A. Piccirillo

194 ***Blockade of IL-33R/ST2 Signaling Attenuates*** **Toxoplasma gondii** ***Ileitis Depending on IL-22 Expression***
Bernhard Ryffel, Feng Huang, Pauline Robinet, Corine Panek, Isabelle Couillin, François Erard, Julie Piotet, Marc Le Bert, Claire Mackowiak, Marbel Torres Arias, Isabelle Dimier-Poisson and Song Guo Zheng

203 ***Divergent Effects of Acute and Prolonged Interleukin 33 Exposure on Mast Cell IgE-Mediated Functions***
Elin Rönnberg, Avan Ghaib, Carlos Ceriol, Mattias Enoksson, Michel Arock, Jesper Säfholm on behalf of ChAMP Collaborators, Maria Ekoff and Gunnar Nilsson

215 ***Interleukin 33/ST2 Axis Components are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer***
Glauben Landskron, Marjorie De la Fuente López, Karen Dubois-Camacho, David Díaz-Jiménez, Octavio Orellana-Serradell, Diego Romero, Santiago A. Sepúlveda, Christian Salazar, Daniela Parada-Venegas, Rodrigo Quera, Daniela Simian, María-Julieta González, Francisco López-Köstner, Udo Kronberg, Mario Abedrapo, Iván Gallegos, Héctor R. Contreras, Cristina Peña, Guillermo Díaz-Araya, Juan Carlos Roa and Marcela A. Hermoso

232 ***Corrigendum: Interleukin 33/ST2 Axis Components are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer***
Glauben Landskron, Marjorie De la Fuente López, Karen Dubois-Camacho, David Díaz-Jiménez, Octavio Orellana-Serradell, Diego Romero, Santiago A. Sepúlveda, Christian Salazar, Daniela Parada-Venegas, Rodrigo Quera, Daniela Simian, María-Julieta González, Francisco López-Köstner, Udo Kronberg, Mario Abedrapo, Iván Gallegos, Héctor R. Contreras, Cristina Peña, Guillermo Díaz-Araya, Juan Carlos Roa and Marcela A. Hermoso

frontiers in Immunology

ORIGINAL RESEARCH
published: 16 May 2018
doi: 10.3389/fimmu.2018.00962

Interleukin-33 Receptor (ST2) Deficiency Improves the Outcome of *Staphylococcus aureus*-Induced Septic Arthritis

*Larissa Staurengo-Ferrari[1], Silvia C. Trevelin[2,3], Victor Fattori[1], Daniele C. Nascimento[3], Kalil A. de Lima[3], Jacinta S. Pelayo[4], Florêncio Figueiredo[5], Rubia Casagrande[6], Sandra Y. Fukada[7], Mauro M. Teixeira[8], Thiago M. Cunha[3], Foo Y. Liew[9], Rene D. Oliveira[10], Paulo Louzada-Junior[10], Fernando Q. Cunha[3], José C. Alves-Filho[3] and Waldiceu A. Verri[1]**

[1] Departamento de Patologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, Brazil, [2] Cardiovascular Division, British Heart Foundation Centre, King's College London, London, United Kingdom, [3] Department of Pharmacology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil, [4] Departamento de Microbiologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, Brazil, [5] Laboratory of Pathology, Faculty of Medicine, University of Brasilia, Brasilia, Brazil, [6] Department of Pharmaceutical Sciences, Healthy Sciences Centre, Londrina State University, Londrina, Brazil, [7] Department of Physics and Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, [8] Laboratório de Imunofarmacologia, Departamento de Bioquímica e Imunologia, Instituto de Ciencias Biologicas (ICB), Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [9] Division of Immunology, Infection and Inflammation, University of Glasgow, Glasgow, United Kingdom, [10] Division of Clinical Immunology, School of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, Brazil

The ST2 receptor is a member of the Toll/IL-1R superfamily and interleukin-33 (IL-33) is its agonist. Recently, it has been demonstrated that IL-33/ST2 axis plays key roles in inflammation and immune mediated diseases. Here, we investigated the effect of ST2 deficiency in *Staphylococcus aureus*-induced septic arthritis physiopathology. Synovial fluid samples from septic arthritis and osteoarthritis individuals were assessed regarding IL-33 and soluble (s) ST2 levels. The IL-33 levels in samples from synovial fluid were significantly increased, whereas no sST2 levels were detected in patients with septic arthritis when compared with osteoarthritis individuals. The intra-articular injection of 1×10^7 colony-forming unity/10 μl of *S. aureus* American Type Culture Collection 6538 in wild-type (WT) mice induced IL-33 and sST2 production with a profile resembling the observation in the synovial fluid of septic arthritis patients. Data using WT, and ST2 deficient ($^{-/-}$) and interferon-γ (IFN-γ)$^{-/-}$ mice showed that ST2 deficiency shifts the immune balance toward a type 1 immune response that contributes to eliminating the infection due to enhanced microbicide effect *via* NO production by neutrophils and macrophages. In fact, the treatment of ST2$^{-/-}$ bone marrow-derived macrophage cells with anti-IFN-γ abrogates the beneficial phenotype in the absence of ST2, which confirms that ST2 deficiency leads to IFN-γ expression and boosts the bacterial killing activity of macrophages against *S. aureus*. In agreement, WT cells achieved similar immune response to ST2 deficiency by IFN-γ treatment. The present results unveil a previously unrecognized beneficial effect of ST2 deficiency in *S. aureus*-induced septic arthritis.

Keywords: interleukin-33, ST2, septic arthritis, *Staphylococcus aureus*, interferon-γ, nitric oxide, Th1, M1 macrophage

OPEN ACCESS

Edited by:
Diana Boraschi, Istituto di Biochimica delle Proteine (CNR), Italy

Reviewed by:
Detlef Neumann, Hannover Medical School, Germany
Fons Van De Loo, Radboud University Medical Center, Netherlands

***Correspondence:**
Waldiceu A. Verri
waverri@uel.br,
waldiceujr@yahoo.com.br

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *02 February 2018*
Accepted: *18 April 2018*
Published: *16 May 2018*

Citation:
Staurengo-Ferrari L, Trevelin SC, Fattori V, Nascimento DC, de Lima KA, Pelayo JS, Figueiredo F, Casagrande R, Fukada SY, Teixeira MM, Cunha TM, Liew FY, Oliveira RD, Louzada-Junior P, Cunha FQ, Alves-Filho JC and Verri WA (2018) Interleukin-33 Receptor (ST2) Deficiency Improves the Outcome of Staphylococcus aureus-Induced Septic Arthritis. Front. Immunol. 9:962. doi: 10.3389/fimmu.2018.00962

INTRODUCTION

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family that can act either as a chromatin-associated nuclear factor or as a classic cytokine (1, 2). Once released, IL-33 binds to the heterodimeric receptor complex consisting of ST2 and IL-1 receptor accessory protein recruiting typical intracellular proteins of the toll-like receptor (TLR)/IL-1 superfamily (3, 4). The transmembrane form of ST2, encoded by the *ST2* gene is expressed by cells including activated Th2 cells (5), mast cells (6), and ILC2 (7). ST2 is alternatively spliced to produce a soluble form (sST2), which acts as an IL-33 scavenger (8). Antibodies targeting ST2, ST2-Fc fusion proteins or ST2 deficient mice contributed to demonstrate that the lack of IL-33/ST2 signaling favors the expansion of Th1 cells and inhibits Th2 cell-mediated immune responses (5, 8–11).

Conversely, IL-33/ST2 signaling has now emerging pleiotropic properties, including type 1 and 3 immunity and regulatory patterns (4, 12). Indeed, IL-33/ST2 has a proinflammatory role in Th1 and Th17 immune responses (13, 14). Both, IL-33 and ST2 are expressed in the human and mouse model of rheumatoid arthritis (RA) synovial tissue, are elevated in the sera and synovial fluids of RA patients and manifest correlation with disease progression (14–18). Endothelial cells and fibroblasts constitutively express high levels of IL-33 mRNA and protein, indicating that they are a key source of IL-33 in the inflamed synovium (11, 16). Thus, IL-33 and ST2 are expressed by joint cells in inflammatory conditions.

During infections, IL-33/ST2 signaling plays dual roles depending on the organ involved and the Th1/Th2 shifting necessary to better control the infectious foci (4). IL-33 is protective during acute phase of sepsis (19), keratitis caused by *Pseudomonas aeruginosa* (20) or *Staphylococcus aureus* wound infection (21) and in parasitic diseases with *Trichuris muris* (22), *Schistosoma mansoni* (23), or *Toxoplasma gondii* (24), whereas it is deleterious during cutaneous and visceral leishmaniasis (10, 25, 26). Thus, strategies targeting IL-33/ST2 pathway should account the cytokine milieu and disease context.

Septic arthritis is considered as one of the most aggressive joint diseases due to its rapidly progressive disease profile, pain, severe joint lesion, and dysfunction even with therapy onset (27, 28). Patients with underlying joint diseases, such as RA are 4- to 15-fold more susceptible to septic arthritis than general population (29). Joint lesions facilitate bacterial colonization together with a reduced immune response due to chronic treatment with disease modifying drugs, corticosteroids and biologic therapies that cause patient immune suppression (30–32). *S. aureus* is the most common cause of SA (31). There is evidence that both drug resistant *S. aureus* such as methicillin-resistant *S. aureus* and non-drug resistant *S. aureus* cause septic arthritis (28, 29, 33, 34). The fast development of joint destruction in septic arthritis supports the urgent need for development of new treatment strategies against *S. aureus* arthritis.

As IL-33 and its receptor ST2 have been recognized as an important axis in joint inflammation and infectious diseases, we therefore, investigated whether ST2 deficiency would influence the outcome and contributing mechanisms of this receptor in *S. aureus*-induced septic arthritis.

MATERIALS AND METHODS

Animals

Male BALB/c [wild-type (WT)], ST2$^{-/-}$ (BALB/c background), C57BL/6 (WT), and interferon-γ (IFN-γ)$^{-/-}$ (C57BL/6 background) mice were used in this study. ST2$^{-/-}$ mice were originally obtained from Dr. Andrew McKenzie (LMB, Cambridge) (15). IFN-γ$^{-/-}$ mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA).

A total of 1,360 mice were used in this study. All mice were housed in standard clear plastic cages with free access to water and food, and temperature of 23°C ± 2 at constant humidity. A 12/12 h light/dark cycle was used with lights on at 6 a.m. and off at 6 p.m. The behavioral tests were performed between 9 a.m. and 5 p.m. in a temperature-controlled room (23°C ± 2). Animal care and handling procedures were in accordance with the International Association for Study of Pain guidelines, and all protocols were approved by the Ethics Committee of the Londrina State University (OF.CIRC.CEUA, process number 20165/2009).

Clinical Samples

Synovial fluid samples from 4 to 5 individuals with septic arthritis and 10 osteoarthritis individuals were collected in order to assess IL-33, sST2, and IFN-γ levels using ELISA kits (R&D Systems, Minneapolis, MN, USA). All individuals were recruited at the Division of Rheumatology, Hospital das Clínicas, Ribeirão Preto Medical School (HC-FMRP), São Paulo, Brazil, and were informed about the aims of the study and provided written consent before participating. The Human Ethics Committee of the FMRP approved this study (Process number 4971/2012).

Mouse Model of *S. aureus*-Induced Arthritis

Staphylococcus aureus was obtained from American Type Culture Collection (ATCC, USA) number 6538. Septic arthritis was induced by local injection of 10^7 colony-forming unity (CFU) of *S. aureus* in 10 μl in sterile PBS into the right knee joints. Intra-articular (i.a.) injection of 10 μl of sterile saline was used as negative control group. To assess the intensity of arthritis, a clinical score was carried out using macroscopic inspection of the knee joints yielding a score of 0–4 for each limb (0—normal, 1—periarticular erythema, 2—articular erythema and edema, 3—function loss with difficult locomotion and articular extension, 4—purulent process with abscess formation) (28).

Assessment of Articular Hyperalgesia

Articular mechanical hyperalgesia was assessed over 27 days post-i.a. infection with *S. aureus* using an electronic pressure meter (IITC 152 Inc., Life Science Instruments California, CA, USA) (35). The electronic pressure-meter apparatus automatically recorded the intensity of the force applied when the paw was withdrawn. The results were expressed as the flexion-elicited withdrawal threshold in grams.

Determination of Knee Joint Edema

Knee joint edema was assessed over 27 days post-i.a. infection with *S. aureus* using a digital caliper (Digmatic Caliper, Mitutoyo

Corp., Kanagawa, Japan). The results were expressed as the difference (delta, Δ) between the diameter measured before (basal) and after induction of articular infection in millimeter.

Quantification of Cytokines

Knee joints were dissected out and frozen with liquid nitrogen. Then, samples were homogenized in a buffer containing a cocktail of protease inhibitors [NaCl 0.4 M, Tween 20 0.05%, bovine albumin 0.5%, phenyl methyl sulphonyl fluoride 0.1 mM, benzethonium chloride 0.1 mM, EDTA 10 mM, aprotinin 20 KI·ml^{-1} (0.01 mg·ml^{-1}) diluted in phosphate buffer saline pH 7.4], centrifuged and the supernatants were used to determine the levels of sST2, IL-33, TNF-α, IL-1β, IFN-γ, IL-4, IL-5, IL-17, and IL-10. The results were expressed as picogram per 100 mg of tissue (36). Supernatants from bone marrow-derived macrophages (BMDMs) culture were also collected to determine the levels of IL-33 and IFN-γ and the results were expressed as picogram/milliliter. All measurements were performed using ELISA kits from R&D Systems (Minneapolis, MN, USA) or eBioscience (San Siego, CA, USA). The minimum sensitivity of the kits was ≥0.7 pg/ml.

Determination of Joint Leukocyte Infiltration and Bacterial Counts

Knee joints cavities were exposed and washed with the aid of a pipette three times with a total volume of 10 µl of sterile saline plus 1 mM EDTA. Each washing procedure used approximately 3.3 µl of saline. The total number of leukocytes was determined in a Neubauer chamber diluted in Turk's solution and differential cell counts were performed in Rosenfeld stained slices using a light microscope. The results were expressed as the number of total of leukocytes, neutrophils, or mononuclear cells × 10^4 (mean ± SEM)/per cavity. The same samples were plated on blood agar to determine the bacterial load in the joints and the results were expressed as CFU per cavity. Additionally, spleen from the same animals were explanted and plated on blood agar to determine the bacterial load. The results were expressed as CFU per spleen.

Histological Analysis

Whole knee joints were removed and fixed in 4% formaldehyde for 2 days before decalcification in 5% formic acid and processing for paraffin embedding. Tissue sections (5 µm) were stained with hematoxylin and eosin. All the slides were coded and assessed in a blinded manner by two observers regarding the degree of synovitis (leukocyte infiltration score) and cartilage destruction.

Proteoglycan Quantification Assay

Chondroitin sulfate from patella samples was quantitated using 1,9-dimethyl-methylene blue assay. The glycosaminoglycan content of samples was calculated from the standard curve of chondroitin sulfate (14).

RT-qPCR

Total RNA was extracted from 1 × 10^6 *S. aureus*-infected BMDMs [multiplicity of infection (MOI): 3] and from whole knee joint samples using TRIzol reagent (Invitrogen). For RT-qPCR, the total RNA was extracted with SV Total RNA Isolation System Kit (Promega, USA) according to the manufacturer's instructions. RT-PCR and qPCR were performed using GoTaq® 2-Step RT-qPCR System (Promega) on a StepOnePlus™Real-Time PCR System (Applied Biosystems®) using primers for *Rankl*, *Rank*, *Opg*, *IFN-γ*, *iNOS*, *IL-33*, and *ST2*. Raw data were normalized to *Gadph* expression and were analyzed by the $2^{-(\Delta\Delta Ct)}$ method.

Western Blot

A total of 2 × 10^6 BMDMs were seeded per well and pretreated with IFN-γ (100 U/ml, Invitrogen), anti-IFN-γ (10 µg/ml, R&D Systems), or medium for 1 h followed by infection with *S. aureus* (MOI = 3) for 18 h. The supernatants were collected to further assess the nitrite production. The cell lysates were collected using RIPA buffer containing CST—Protease/Phosphatase Inhibitor Cocktail (Cell Signaling, USA). Whole knee joint samples using RIPA buffer containing Protease/Phosphatase Inhibitor Cocktail (Cell Signaling, USA) were also collected. Total protein from cells and knee joint samples were quantified and the lysates were mixed with 4× Laemmli sample buffer (Sigma-Aldrich). Antibody against iNOS (1:10,000, Sigma Chemical Co., St. Louis, MO, USA) was used for protein detection after electrophoresis in 10% SDS-PAGE gel, transference into nitrocellulose membrane (Merck Millipore, USA), and blocking. HRP-conjugated secondary antibody was used (KPL, USA). Immunodetection was performed using an enhanced chemiluminescence technique (ChemiDoc XRS System, Biorad Laboratories). The membrane was stripped and reprobed with β-actin (1:5,000, Sigma Chemical Co., St. Louis, MO, USA) as a loading control. Densitometry data were measured after normalization to the control (house-keeping gene, β-actin) using Scientific Imaging Systems (Image Lab 3.0 software; Biorad Laboratories, Hercules, CA, USA). Full scan of the original uncropped western blot is shown in Figure S4 in Supplementary Material.

FITC-Labeling of Staphylococci

Staphylococcus aureus ATCC 6538 was grown to mid-log phase in fresh Muller–Hinton broth. Bacteria were washed twice with sterile PBS and labeled in 0.1 mg/ml FITC (Sigma Chemical Co., St. Louis, MO, USA) for 1 h at 37°C with shaking. Prior to use, bacteria were washed twice with PBS and re-suspended in Hank's solution (Sigma Chemical Co., St. Louis, MO, USA).

Flow Cytometry Analysis

Phagocytosis assay and detection of intracellular cytokines was performed using an FACS Verse (BD Biosciences, San Diego, CA, USA) flow cytometer. Phagocytosis of naïve BMDMs and naïve neutrophils was measured using FITC-labeled *S. aureus* as previously described in Ref. (37). In other set of experiments to assess the intracellular cytokines, draining popliteal lymph nodes (LNs) were collected from naïve and infected mice at indicated times post-infection and processed as a pool. Cell suspensions obtained (1 × 10^6 cells) were stained with fluorochrome-conjugated antibodies for CD4 (H129.19), IFN-γ (B27), IL-4 (11B11), IL-17 (TC11-18H10), or IL-10 (JES5-16E3) from BD Biosciences (San Diego, CA, USA). Data were analyzed with FlowJo software (TreeStar, Ashland, OR, USA).

Killing Assay

To obtain macrophages (BMDM), cells were differentiated during 6 days in RPMI medium plus 20% supernatant from L929 cells (38). To obtain neutrophils, cells were isolated by Percoll gradient (39). BMDM or neutrophils (1×10^6/well) were then pretreated with IFN-γ (100 U/ml), anti-IFN-γ (10 μg/ml), or medium for 1 h followed by infection with *S. aureus* (MOI = 3). Other wells with only bacteria and RPMI medium were prepared as a positive control. The plates were centrifuged for 3 min at 5,000 rpm and returned to the cell incubator for 3 h. The supernatants were collected to further assess the nitrite production. Cells were then lysed by addition of Triton X-100 0.2%. The lysates were serially diluted 1:100,000 in 1 × PBS and plated on blood agar plates and incubated overnight at 37°C. The results were expressed as % of viable bacteria by comparing with the positive control.

Nitrite Determination

Nitrite (NO_2^-) accumulation, as an indication of NO production was measured using Griess reagent. In this assay, 0.1 ml of sample was mixed with 0.1 ml of Griess reagent in a multiwell plate, and the absorbance was read at 550 nm 10 min later. Nitrite concentrations were determined by reference to a standard curve of sodium nitrite (1–200 μmol/l) (40).

Pharmacological Treatment of Mice

In some experiments, infected WT and ST2$^{-/-}$ were treated intraperitoneally with 30 mg/kg of aminoguanidine (AMG; Sigma, Chemical Co., St. Louis, MO, USA), a selective iNOS inhibitor, for 28 consecutive days (once a day). Mice were euthanized at the 28th day post-infection, and inflammatory parameters and bacterial load in joint and spleen tissues were analyzed.

Culture of Macrophages Like-Cells

Peritoneal cells from naïve and infected mice were cultured in RPMI medium for 4 h to allow macrophages to adhere. The floating cells were washed away and the adherent macrophages were challenged with lipoteichoic acid (LTA, 10 μg/ml), a TLR2 agonist plus IFN-γ (100 U/ml) in RPMI for 24 h at 37°C. The supernatants were harvested to assess the NO_2^- accumulation.

Statistical Analysis

Statistical significance was analyzed using PRISM 6.01 (GraphPad Software, USA). The data are expressed as the mean ± SEM. Statistical differences were considered when $P < 0.05$.

RESULTS

IL-33 and sST2 Levels in Synovial Fluids of Septic Arthritis and Osteoarthritis Patients, and a Similar Profile in Mouse Septic Arthritis

First, the IL-33 and sST2 levels in the synovial fluid of patients with septic arthritis and osteoarthritis were determined. Clinical and demographic characteristics of these patients are presented as Table S1 in Supplementary Material. IL-33 levels were higher in the synovial fluid of SA patients than in the synovial fluid OA patients (**Figure 1A**). The levels of sST2 were below limit detection in septic arthritis patients (**Figure 1B**). These clinical results indicate that there are higher levels of IL-33 in septic arthritis primary foci than in osteoarthritis. In mice, the i.a. injection of *S. aureus* induced the increase of IL-33 levels (**Figure 1C**) and decreased of sST2 levels (**Figure 1D**) in the knee joints that lasted for 28 days compared with day 0 (representative group that received only saline). Despite the detection of IL-33 in the synovial fluid and knee joints of septic arthritis patients and mice, respectively, whether IL-33 and its receptor ST2 have a function in disease is unknown.

ST2 Receptor Deficiency Ameliorates *S. aureus*-Induced Septic Arthritis

Considering that septic arthritis triggered the production of IL-33 and reduction of sST2 levels in the synovial fluid of patients

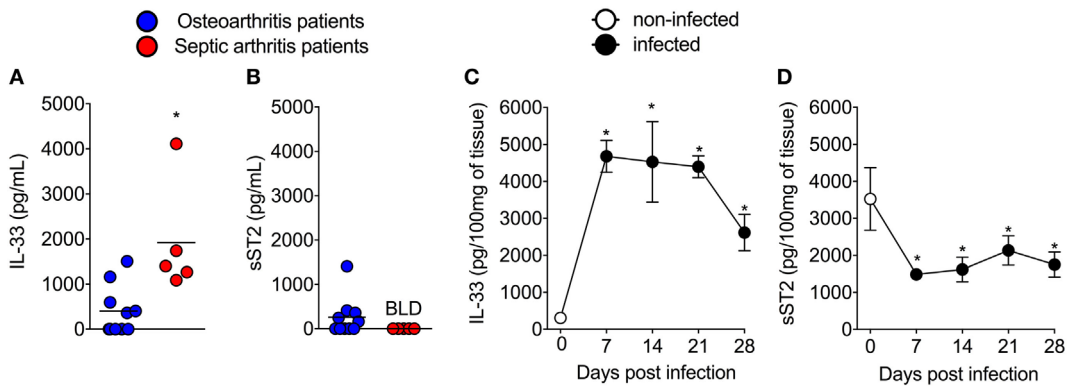


FIGURE 1 | Interleukin-33 (IL-33) and sST2 levels in synovial fluid samples of septic arthritis and osteoarthritis patients, and similar profile in mouse septic arthritis. **(A,B)** Synovial fluid samples from patients with septic arthritis and osteoarthritis were collected and processed to determine the levels of IL-33 and sST2 by ELISA. **(C,D)** *Staphylococcus aureus* or saline (day 0) was injected in in the femur-tibial joint of wild-type and knee joints samples were collected and processed to determine the levels of IL-33 and sST2 at indicated points (7–28 days) post *S. aureus* injection by ELISA. For clinical samples analysis: $n = 5$ for septic arthritis and $n = 10$ for osteoarthritis patients. *$P < 0.05$ vs osteoarthritic patients group **(A,B)**. Kruskal–Wallis test followed by Dunn's test. For mice samples analysis: $n = 6$ per group, representative of two independent experiments. *$P < 0.05$ vs day 0 of infection **(C,D)**. Two-tailed unpaired Student's *t*-test.

(**Figure 1A**), we used a model of *S. aureus*-induced septic arthritis in WT (balb/c) and ST2$^{-/-}$ mice to investigate the disease outcome in the ST2 deficiency scenario. i.a. injection of *S. aureus* increased mechanical hyperalgesia in WT mice as observed by reduction in the mechanical threshold when compared with naïve mice (**Figure 2A**). Interestingly, the *S. aureus*-induced hyperalgesia was similar between WT and ST2$^{-/-}$ mice up to 9 days of infection. However, from 11 days onward, *S. aureus*-induced hyperalgesia started to reduce in ST2$^{-/-}$ compared with WT mice. This result may have, at least, two explanations; ST2$^{-/-}$ mice respond better than WT against *S. aureus* infection or by lacking ST2, these mice would present a reduction of hyperalgesia since IL-33 is a hyperalgesic cytokine (13). Moreover, IL-33 also mediates the paw edema induced by carrageenan (41). Indeed, WT mice presented increased edema when compared with ST2$^{-/-}$ mice (**Figure 2B**). The reduction of hyperalgesia and edema in ST2$^{-/-}$ mice was followed by lower clinical score as well (**Figure 2C**). WT mice also showed increased levels of TNF-α and IL-1β after infection (**Figures 2D,E**, respectively), inflammatory cell recruitment to the knee joint (**Figures 2F–H**), and tissue inflammatory cell infiltration demonstrated histologically (**Figures 2I,K**) when compared with ST2$^{-/-}$ mice. Ultimately, these features resulted in increased cartilage destruction with only parts of cartilage remaining (**Figures 2J,K**), which was confirmed by proteoglycan content loss (**Figure 2L**). These morphological changes were reduced in ST2$^{-/-}$ mice (**Figures 2I–K**). Further, WT mice presented increased mRNA expression of receptor activator of nuclear factor-kappa B ligand (Figure S1A in Supplementary Material) and receptor activator of nuclear factor-kappa B (Figure S1B in Supplementary Material), and reduced mRNA expression of OPG (osteoprotegerin, Figure S1C in Supplementary Material), an expression pattern indicating bone resorption (42). These results indicate that IL-33/ST2 signaling deficiency ameliorated the clinical features of septic arthritis. Therefore, we next examined whether the reduction of disease intensity in ST2$^{-/-}$ mice would be related to reducing

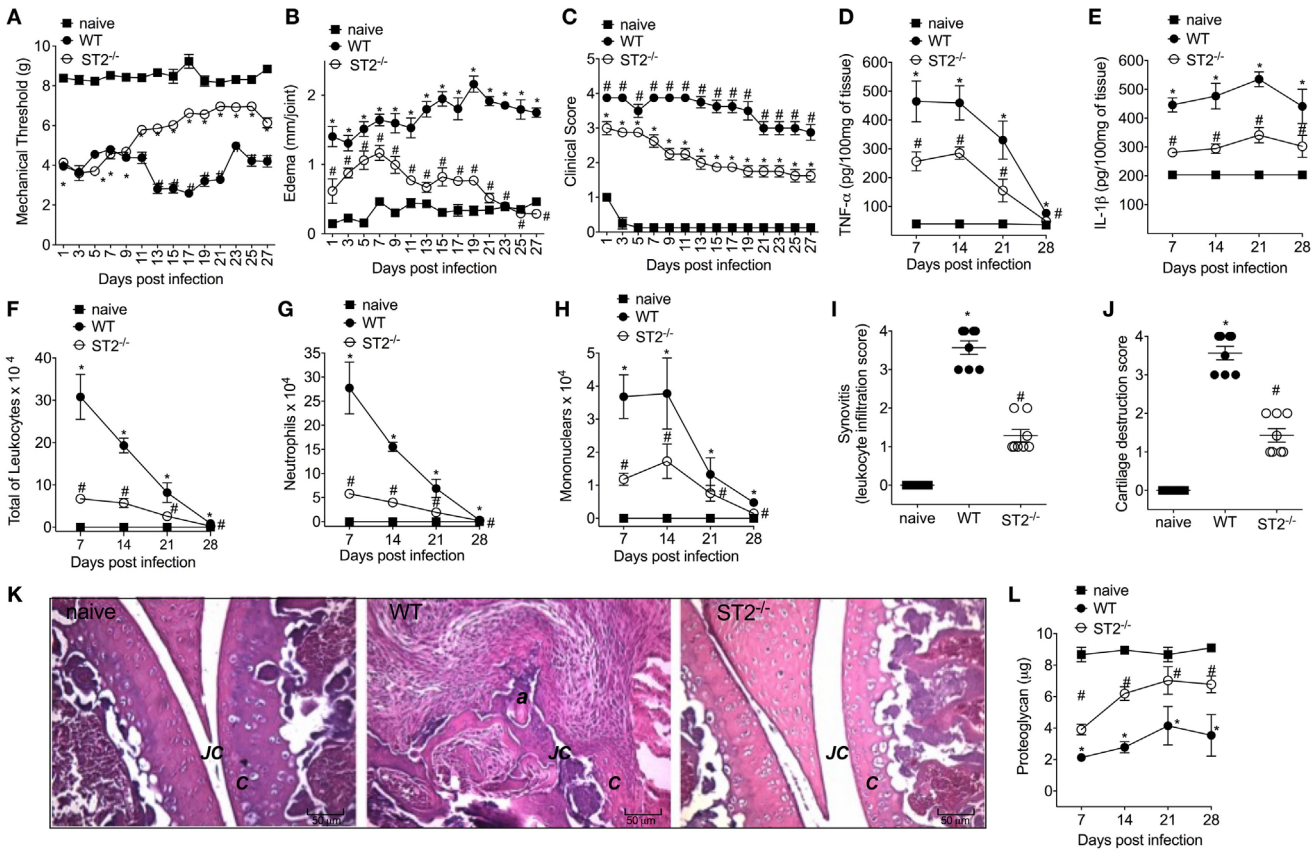


FIGURE 2 | ST2 deficiency ameliorates *Staphylococcus aureus*-induced septic arthritis. *S. aureus* or saline was injected in the femur-tibial joint of wild-type (WT) and ST2$^{-/-}$ mice. **(A)** Mechanical hyperalgesia, **(B)** articular edema, and **(C)** clinical score were evaluated over 27 days post-infection. Knee joints were collected and processed to determine the levels of **(D)** TNF-α and **(E)** IL-1β by ELISA determined at days 7–28 days post-infection. **(F)** Total leukocytes, **(G)** neutrophil, and **(H)** mononuclear recruitment to the knee joint were determined at 7–28 days post-infection. Knee joint samples were collected at the 28th day post-infection for histological analysis by hematoxylin/eosin stained slices to determine: **(I)** synovitis score (intensity: 1–4) and **(J)** cartilage destruction score (intensity: 1–4). **(K)** Representative images of knee joints at 28 post-infection in original magnification ×10. The letter ***a*** indicates a heavily inflamed joint with cartilage destruction and pannus formation. **(F)** Proteoglycan content in patella determined at 7–28 days post-infection. For inflammatory parameters and proteoglycan content: $n = 6$ per group per *in vivo* experiment, representative of two independent experiments. $^{*}P < 0.05$ vs naïve mice group, $^{\#}P < 0.05$ vs WT mice group **(A–H,L)**. One-way ANOVA followed by Tukey's test. For histological analysis: $n = 8$ per group per experiment, representative of two independent experiments. $^{*}P < 0.05$ vs naïve mice group, $^{\#}P < 0.05$ vs WT mice group **(I–K)**. Kruskal–Wallis test followed by Dunn's test. Abbreviations: ***C***, cartilage; ***JC,*** joint cavity.

inflammation since IL-33/ST2 signaling triggers inflammatory responses, or if ST2 deficiency would enhance the anti-microbial response due to boosting the immune response pattern necessary to combat *S. aureus* infection.

ST2 Deficiency Enhances Neutrophil and Macrophages Bactericidal Activity Against *S. aureus*

Inhibiting IL-33/ST2 signaling reduces inflammation induced by carrageenan and LPS, and in RA by targeting neutrophilic influx (14, 41, 43, 44). On the other hand, neutrophils have a fundamental role in controlling the bacterial load in infections. Indeed, the treatment with IL-33 improves the sepsis outcome due to inhibition of LPS-induced CXCR2 internalization, which maintains the neutrophil migration toward the infectious foci (19). To ascertain this issue and considering that mice lacking ST2 showed a protective phenotype against *S. aureus* local inflammation in septic arthritis (**Figure 2**), first, we evaluated if there was any difference in the bacterial load in WT and ST2$^{-/-}$ mice. Bacterial recovery showed that WT mice presented detectable *S. aureus* CFU in both the knee joint and the spleen (**Figures 3A,B**, respectively). On the other hand, ST2$^{-/-}$ mice presented reduced CFU number compared with WT mice as well as did not present *S. aureus* in the spleen, which indicates that the infection remained local in ST2$^{-/-}$, but not in WT mice (**Figure 3B**). Given neutrophils and macrophages are the initial defenders against *S. aureus* infection (45), we determined if ST2 deficiency would influence neutrophil and macrophage phagocytosis and killing of *S. aureus*. Using FITC-labeled *S. aureus*, we found that both neutrophils (**Figures 3C,D**) and macrophages (BMDMs) (**Figures 3E,F**) from WT mice presented reduced phagocytic capacity when compared with ST2$^{-/-}$ cells. In addition, neutrophils and BMDMs from WT mice also partially controlled bacterial growth, and, importantly, neutrophils and BMDMs from WT mice increased their bacterial killing after stimulation with recombinant IFN-γ (**Figures 3G,H**) in a manner that reached the capability of ST2$^{-/-}$ cells. In contrast, the IFN-γ treatment of neutrophils and BMDMs from ST2$^{-/-}$ mice did not further enhanced their bactericidal effect (**Figures 3G,H**), possibly because this pathway was already being enhanced by ST2 deficiency. Thus, these results show that impaired endogenous IL-33/ST2 signaling enhances neutrophil and macrophage bacterial killing with a profile that can be matched by IFN-γ treatment. Therefore, IFN-γ-related mechanisms were investigated in the following experiments (46).

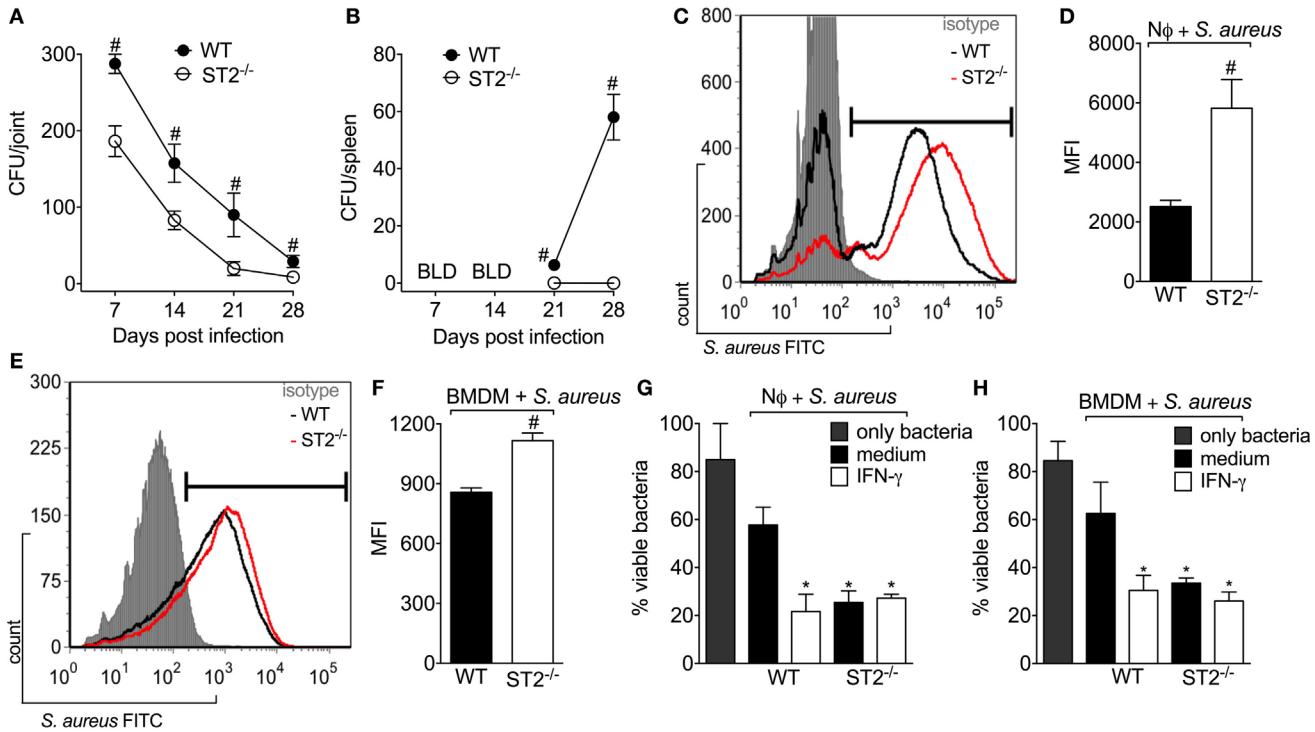


FIGURE 3 | ST2 deficiency enhances neutrophil and macrophages bactericidal activity against *Staphylococcus aureus. S. aureus* was injected in the femur-tibial joint of wild-type (WT) and ST2$^{-/-}$ mice. At indicated points (7–28 days post-infection), **(A)** knee joints and **(B)** spleen samples were collected and bacterial counts were determined on agar dishes. **(C,D)** FACS analysis of neutrophils (1 × 10^6) from WT and ST2$^{-/-}$ naïve mice incubated *in vitro* with *S. aureus* at a multiplicity of infection (MOI) of 3 to evaluate phagocytosis. **(E,F)** FACS analysis of naïve bone marrow-derived macrophages (BMDMs) (1 × 10^6) from WT and ST2$^{-/-}$ naïve mice incubated *in vitro* with *S. aureus* at a MOI of 3 to evaluate phagocytosis. Microbicidal activity of neutrophils **(G)** and BMDM **(H)** from WT and ST2$^{-/-}$ naïve mice preincubated with interferon-γ (IFN-γ) (100 IU/ml, 1 h) against *S. aureus*. All neutrophils were harvested from the bone marrow of mice. $N = 6$ wells per group per *in vitro* experiment, representative of two independent experiments. One-way ANOVA followed by Tukey's test. $^{\#}P < 0.05$ vs WT mice group **(A,B)**. Samples were pooled from 10 mice per group per *in vitro* experiment, representative of two independent experiments. One-way ANOVA followed by Tukey's test. $^{\#}P < 0.05$ vs WT neutrophils or BMDM group **(C–F)**. $^{*}P < 0.05$ vs WT group preincubated with only medium **(G,H)**.

ST2 Deficiency Enhances NO Production by Neutrophils and Macrophages and Reduces *S. aureus*-Induced Septic Arthritis

Since one of the IFN-γ mechanisms of bacterial killing is by increasing NO production in an iNOS-dependent manner (47) and that NO can also drive type 1 response (48–50), it was evaluated whether the protective phenotype of ST2 deficiency was related to an increase of IFN-γ/iNOS/NO signaling. First, it was measured the NO_2^- levels in macrophages and neutrophils, which is indicative of iNOS activity. We found that neutrophils from WT mice presented lower production of NO_2^- than neutrophils from ST2$^{-/-}$ mice (**Figure 4A**) as well as IFN-γ enhanced the NO_2^- production by WT neutrophils, but not ST2$^{-/-}$ neutrophils. In accordance, naïve macrophages (BMDMs) infected with *S. aureus* or peritoneal macrophages collected at 7–28 days post *S. aureus* joint infection from WT presented reduced levels of NO_2^- when compared with ST2$^{-/-}$ cells (**Figures 4B,C**, respectively). Considering neutrophils and macrophages

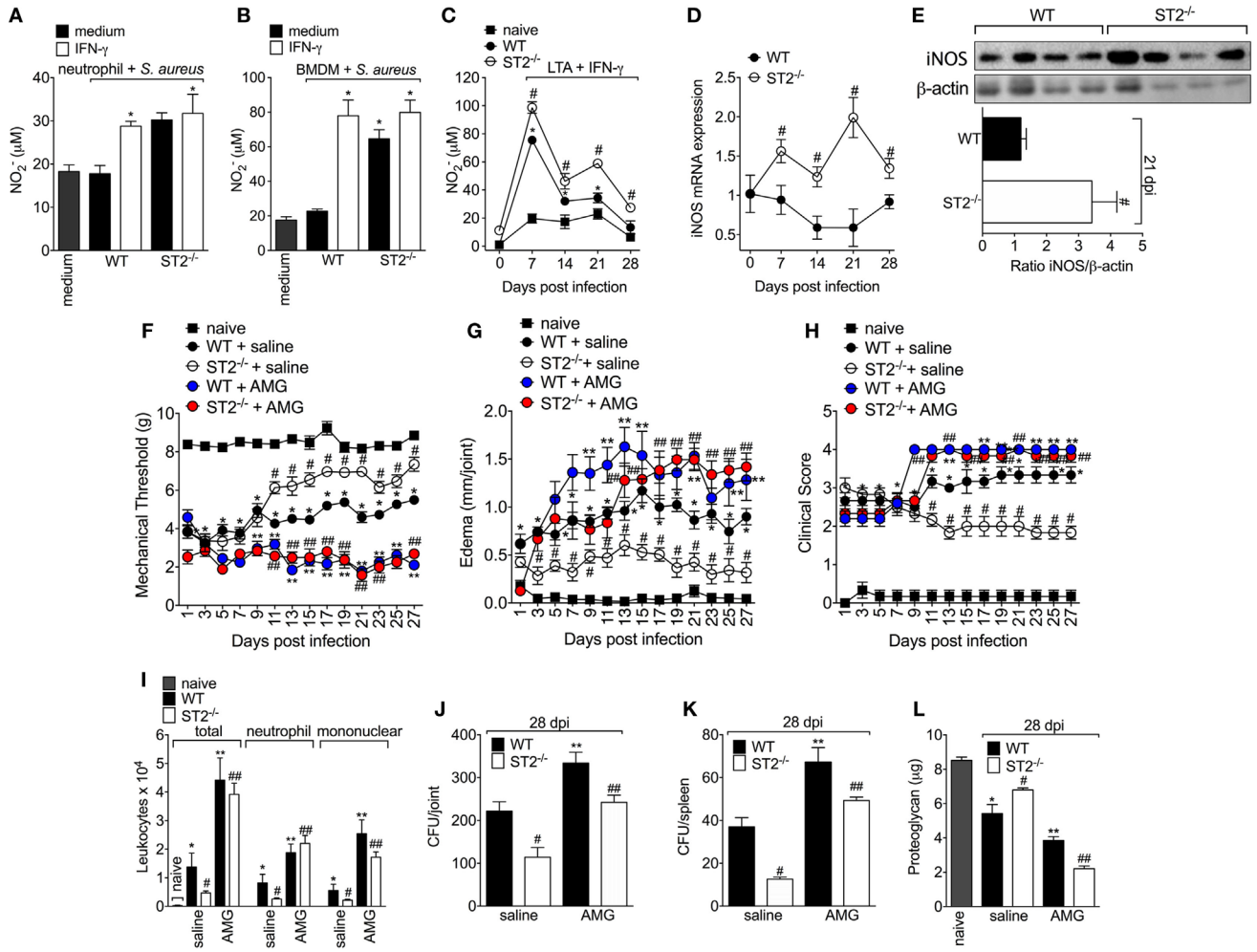


FIGURE 4 | ST2 deficiency enhances NO production by neutrophils and macrophages and reduces *Staphylococcus aureus*-induced septic arthritis. NO production was determined as nitrite concentration by Griess reagent in the culture supernatant of **(A)** neutrophils and **(B)** bone marrow-derived macrophages (BMDMs) cells from wild-type (WT) or ST2 $^{-/-}$ naïve mice preincubated *in vitro* with interferon-γ (IFN-γ) (100 IU/ml, 1 h) or medium, followed by incubation with *S. aureus*, or **(C)** in the culture supernatant of macrophages like-cells isolated from peritoneal cavity of WT or ST2$^{-/-}$ mice with staphylococcal arthritis and challenged with lipoteichoic acid (LTA) (10 μg/ml, a toll-like receptor 2 agonist) plus IFN-γ (100 UI/ml) for 48 h. *S. aureus* or saline (day 0) was injected in the femur-tibial joint of WT and ST2$^{-/-}$ mice and knee joint samples were collected and processed to determine: **(D)** the mRNA and **(E)** protein expression of iNOS at indicated time points post-infection by qPCR and Western Blot, respectively. WT and ST2$^{-/-}$ mice were treated with aminoguanidine (AMG, 30 mg/kg, s.c., 150 μl) or vehicle (saline, 150 μl) over 28 days after i.a. *S. aureus* [10^7 colony-forming unity (CFU)/10 μl/joint] injection: **(F)** mechanical hyperalgesia, **(G)** articular edema, and **(H)** clinical severity score were evaluated over 27 days post-bacterial infection. At the 28th day post-infection, **(I)** leukocyte recruitment to the articular cavity, **(J)** bacterial counts in knee joint cavity and **(K)** spleen, and **(L)** proteoglycan content in patella samples were determined. *N* = 6 per group per *in vivo* experiment or *N* = 4 per group for WB analysis and samples were pooled from 10 mice per *in vitro* experiment. **P* < 0.05 vs WT neutrophils or BMDM group preincubated with only medium, or vs mice naïve group; #*P* < 0.05 vs WT and ST2$^{-/-}$ neutrophils or BMDM group preincubated with only medium, or vs WT mice group **(A–E)**. **P* < 0.05 vs naïve mice group; #*P* < 0.05 vs ST2$^{-/-}$ + saline mice group vs WT + saline mice group; ***P* < 0.05 WT + AMG mice group vs WT + saline mice group; ##*P* < 0.05 ST2$^{-/-}$ + AMG mice group vs ST2$^{-/-}$ + saline mice group **(F–L)**. Representative of two independent experiments. One-way ANOVA followed by Tukey's test.

from WT mice presented reduced NO_2^- levels compared with ST2$^{-/-}$ cells, the iNOS expression was investigated in knee joint samples from WT and ST2$^{-/-}$ mice with staphylococcal arthritis. Corroborating, both iNOS mRNA expression and protein (day 21, peak of mRNA expression) were reduced in the knee joints from WT mice compared with ST2$^{-/-}$ mice (**Figures 4D,E**, respectively). Thus, to determine the contribution of iNOS to IL-33/ST2 signaling role in septic arthritis, WT and ST2$^{-/-}$mice with septic arthritis received daily treatment with AMG, a selective iNOS inhibitor. Treatment with AMG reverted the protective effect of ST2 deficiency and also worsened the phenotype of WT mice, as observed by increased hyperalgesia (reduced mechanical threshold, **Figure 4F**), edema (**Figure 4G**), and clinical score (**Figure 4H**), when compared with the group that receive only vehicle. Similarly, the treatment with AMG in ST2$^{-/-}$ mice increased leukocyte recruitment to the knee joint (**Figure 4I**), CFU count in both knee joint (**Figure 4J**) and spleen (**Figure 4K**), and proteoglycan degradation (**Figure 4L**) at the 28th day post-infection (**Figures 4I–L**). Altogether, these data indicate that ST2 receptor deficiency ameliorates *S. aureus*-induced septic arthritis by enhancing iNOS expression and thereby increasing NO levels, which is an important bactericidal mechanism and inductor of type 1 response.

ST2 Deficiency Enhances Type 1-Driven Immune Response Against *S. aureus* in Septic Arthritis

Proper Th1 response alongside with antibiotic therapy is fundamental to kill *S. aureus* (47, 51). Previous evidence shows that IL-33/ST2 signaling favors the expansion of type 2 cells; however, there is also evidence that IL-33 enhances type 1 immune responses (1, 4). Thus, to further address the mechanism underlying the outcome of ST2 deficiency in septic arthritis, it was next performed a flow cytometry gating CD4$^+$IFN-γ$^+$T cells and CD4$^+$IL-4$^+$T cells at the 7th and 14th day post-infection in LNs cells. These time points were chosen based on ST2$^{-/-}$phenotype observed during septic arthritis and on the fact that CFU count in the joint was higher at the 7th and 14th day post-infection. Flow cytometry data show that ST2 expression was essential to the development of a Th2 response in septic arthritis (Figures S2A,B in Supplementary Material). We showed that ST2$^{-/-}$ mice had higher number of gated CD4$^+$IFN-γ$^+$T cells than WT mice at days 7 and 14 post-infection (**Figures 5A,B**). In contrast, the *S. aureus* induced an increase of the percentage of gated CD4 + IL-4 + T cells at days 7 and 14 post-infection in WT mice, which was reduced by ST2 deficiency (Figures S2A,B in Supplementary Material). In line with FACS data, WT mice presented reduced amounts of IFN-γ and increased amounts of IL-4 in infected joints, which are opposed results compared to ST2$^{-/-}$ at all time points evaluated (7–28 days post-infection, **Figure 5C**; Figure S2C in Supplementary Material). This suggests that endogenous IL-33 is essential to driving type 2 response in septic arthritis which are mediated by IL-4. This axis favored the infection progression by counteracting type 1 response. In view of Th1 and Th2 response have long been balancing one another (52) and that there are similar evidences regarding the relationship between Th1 and Th17 (52, 53), we found a lower number of gated IL-17$^+$CD4$^+$T cells in LNs from ST2$^{-/-}$ mice compared with WT at 7th and 14th day post-infection (Figures S2D,E in Supplementary Material), which lined up with IL-17 amounts in infected joint tissue at all time points evaluated (7–28 days post-infection, Figure S2F in Supplementary Material), raising the possibility that ST2$^{-/-}$ mice use Th1 cells in lieu of Th17 cells to drive the immune response against *S. aureus*-induced septic arthritis. We also found that WT mice presented increased number of CD4$^+$ IL-10$^+$ T cells in LNs when compared with ST2$^{-/-}$ mice at the 7th day post-infection (Figures S2G,H in Supplementary Material). This high IL-10 production in the beginning of infection in WT mice possibly favored bacterial growth (54). At the 14th day post-infection, there was a decrease switch in the number of CD4$^+$ IL-10$^+$T$^+$ cells observed in WT and ST2$^{-/-}$ mice with an increase in ST2$^{-/-}$ mice (Figures S2G,H in Supplementary Material) and it lined up with the IL-10 amounts in infected joints at all times points evaluated (7–28 days post-infection, Figure S2I in Supplementary Material). Thus, increasing CD4$^+$IL-10$^+$T$^+$ cells and IL-10 production at later time points in the course of septic arthritis at which the infection was already controlled could be a mechanism to reduce joint inflammation in ST2$^{-/-}$ mice.

M1 macrophage polarization can be driven by microbial infection and secondarily drive the T helper (Th1) polarization under influence of IFN-γ. We observed that *S. aureus* induced the mRNA expression of IL-33 (**Figure 5D**) and ST2 (**Figure 5E**) as well as IL-33 (**Figure 5F**) production by BMDM. Therefore, the infectious agent induces IL-33 production, which is in line with an altered response in the absence of IL-33 receptor, ST2. IFN-γ is crucial for NO production and evidence demonstrates that BMDM produce IFN-γ and NO in response to *L. amazonensis* (55). In this sense, we found that *S. aureus*-induced IFN-γ mRNA (**Figure 5G**) and protein (**Figure 5H**) only in ST2$^{-/-}$ BMDM, but not in WT BMDM. This result explains why adding IFN-γ to the media could not increase the microbicide activity of ST2$^{-/-}$ BMDM (**Figure 4B**) and suggests that the effect of ST2 deficiency depends on inducing IFN-γ in BMDM in addition to increasing Th1 cell skewing. Corroborating this conclusion, anti-IFN-γ antibody treatment inhibited the enhanced iNOS mRNA expression (**Figure 5I**), iNOS levels (**Figure 5J**), NO_2^- production (**Figure 5K**), and bacterial killing (**Figure 5L**) of ST2$^{-/-}$ BMDMs compared with WT BMDMs in response to *S. aureus*. These results confirm that the protection against *S. aureus* conferred by ST2 deficiency depends on inducing IFN-γ production. It is likely that *S. aureus* stimulus induces IL-33 that acting *via* ST2 limits IFN-γ production by BMDM functioning as an endogenous regulator of anti-bacterial mechanisms, and upon ST2 deficiency, IFN-γ can be produced to unleash the full microbicide activity of BMDMs.

IFN-γ Contributes to the Resolution of Staphylococcal Arthritis

Considering the importance of IFN-γ in the bacterial clearance and that ST2 deficiency enhances IFN-γ$^+$CD4$^+$T cells skewing and IFN-γ-dependent killing activity of BMDM, it was then investigated whether mice lacking IFN-γ presented impaired

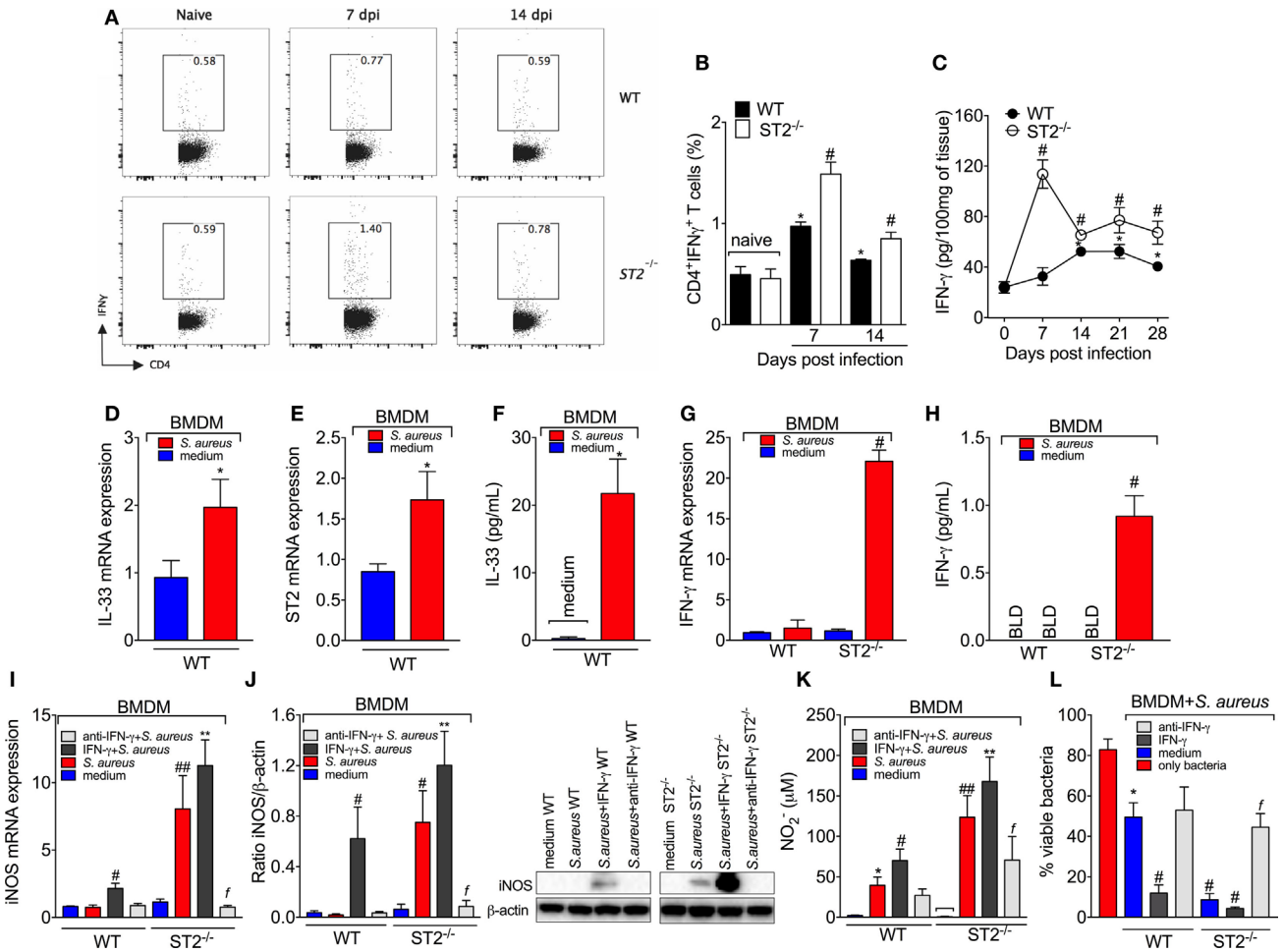


FIGURE 5 | ST2 deficiency enhances type 1-driven immune response against *Staphylococcus aureus* in septic arthritis *S. aureus* or saline was injected in in the femur-tibial joint of wild-type (WT) and ST2$^{-/-}$ mice. **(A)** Representative FACS plots and **(B)** the percentage of interferon-γ (IFN-γ)-producing CD4+T cells (CD4+IFNγ+T cells) from lymph node collected at day 7 and 14 post-infection and evaluated by flow cytometry, and **(C)** IFN-γ concentrations in the knee joints of WT and ST2$^{-/-}$ at 7–28 days post-infection determined by ELISA. bone marrow-derived macrophages (BMDMs) (1 × 10^6) from naïve WT mice were incubated *in vitro* with *S. aureus* at a multiplicity of infection (MOI) of 3 for 18 h to assess: **(D)** interleukin-33 (IL-33) mRNA and **(E)** ST2 mRNA expression by qPCR, **(F)** IL-33 levels by ELISA. BMDMs (1 × 10^6) from naïve WT and ST2$^{-/-}$ mice incubated *in vitro* with *S. aureus* at a MOI of 3 for 18 h to assess: **(G)** IFN-γ mRNA expression by qPCR and **(H)** IFN-γ levels by ELISA. BMDMs from naïve WT and ST2$^{-/-}$ mice preincubated 1 h *in vitro* with IFN-γ (100 IU/ml), anti-IFN-γ (10 μg/ml) or medium, followed by incubation with *S. aureus* at a MOI of 3 for 18 h to assess: **(I)** iNOS mRNA expression by qPCR, **(J)** iNOS protein expression by Western Blot, and **(K)** NO production determined as nitrite concentration by Griess reagent in the culture supernatant. **(L)** BMDMs (1 × 10^6) from naïve WT and ST2$^{-/-}$ mice preincubated 1 h *in vitro* with IFN-γ (100 IU/ml), anti-IFN-γ (10 μg/ml), or medium, followed by incubation with *S. aureus* at a MOI of 3 for 3 h to assess bactericidal capability of BMDMs by *Killing* assay. *N* = 5 per group per *in vivo* experiment. $^*P < 0.05$ vs WT naïve group **(B)** or vs day 0 of infection **(C)**, $^\#P < 0.05$ vs WT mice group **(B,C)**. Representative of two independent experiments. One-way ANOVA followed by Tukey's test. For *in vitro* experiments: Samples were pooled from 5 mice per group, N 5 wells per group per *in vitro* experiment. $^*P < 0.05$ vs WT medium group **(D–F, K)** or only bacteria group **(L)**. $^\#P < 0.05$ vs WT *S. aureus* group **(G–I)** or vs WT medium **(L)**, $^{\#\#}P < 0.05$ vs WT IFN-γ + *S. aureus* group and WT *S. aureus* group **(I,K)**, $^{**}P < 0.05$ vs ST2$^{-/-}$ *S. aureus* group and WT IFN-γ + *S. aureus* group **(I–K)**, $^fP < 0.05$ vs ST2$^{-/-}$ *S. aureus* group **(I–L)**. Representative of two independent experiments. One-way ANOVA followed by Tukey's test.

response in septic arthritis and whether this was related to IL-33 production. Mice lacking IFN-γ presented increased mechanical hyperalgesia and edema in all disease course (**Figures 6A,B**, respectively). The differences in clinical features were more prominent from day 13 post-infection as observed by an increase in clinical score in IFN-γ$^{-/-}$ when compared with WT mice (**Figure 6C**). IFN-γ$^{-/-}$ mice presented increased leukocyte recruitment inflammatory cell infiltrate with predominance of polymorphonuclear leukocytes over mononuclear cells (**Figures 6D–F**), remarkable cartilage destruction, and architecture as confirmed by proteoglycan content loss and histopathology (**Figures 6F–H**). The septic arthritis development pattern was thoroughly different comparing WT and IFN-γ$^{-/-}$ mice as observed by the knee joint edema and clinical score (**Figures 6B,C**). In fact, a lower bacterial killing was observed resulting in higher CFU counts in the joint and spleen of IFN-γ$^{-/-}$ mice compared with WT mice (**Figures 6I,J**). Ultimately, this profile observed in IFN-γ$^{-/-}$ mice was related to higher IL-33 production, and lower sST2 levels than WT mice (**Figures 6K,L**). All the parameters were observed up to or in the 28th day post-infection (**Figure 6**). Importantly, the same dose of *S. aureus* was used in WT C57BL/6 and WT Balb/c mice to induce septic arthritis. Both mouse strains were susceptible to infection;

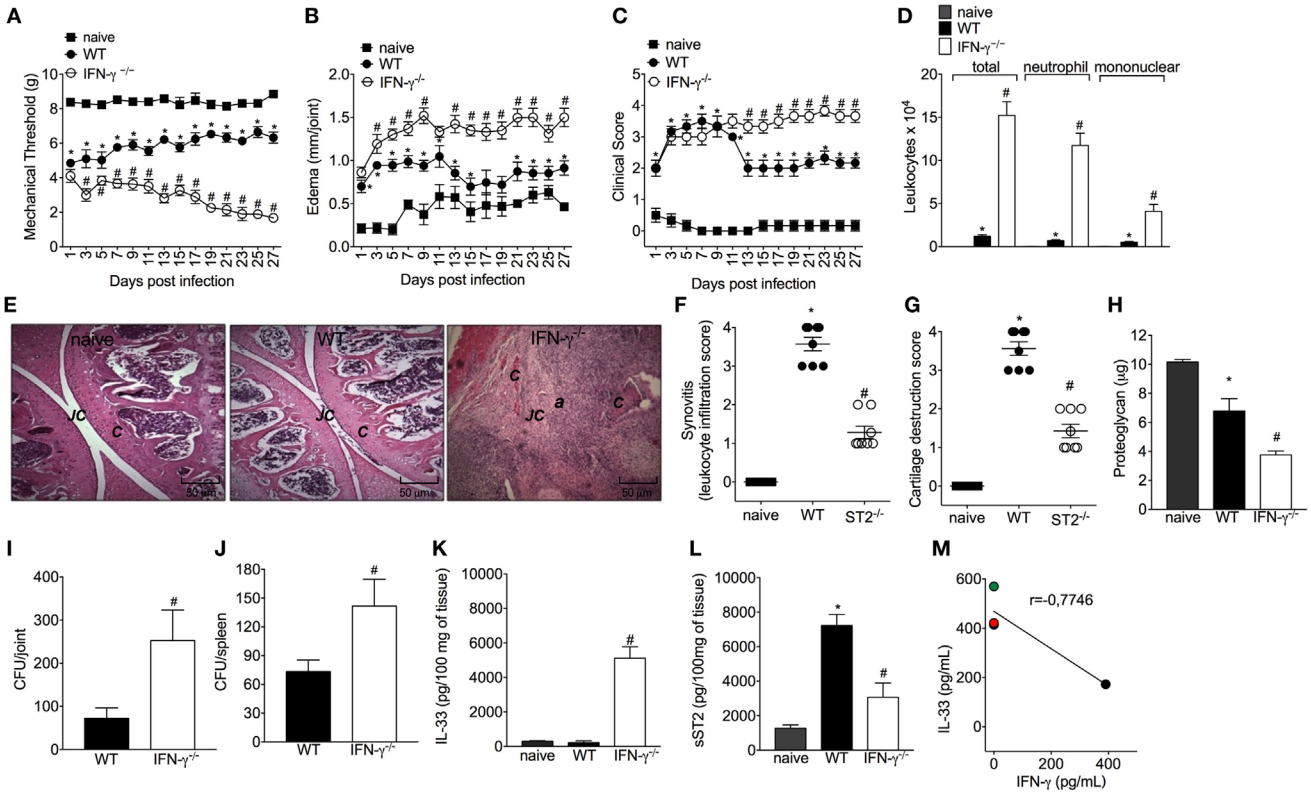


FIGURE 6 | Interferon-γ (IFN-γ) contributes to the resolution of staphylococcal arthritis. *Staphylococcus aureus* or saline was injected in in the femur-tibial joint of wild-type (WT) and IFN-$\gamma^{-/-}$ mice. **(A)** Mechanical hyperalgesia, **(B)** articular edema, and **(C)** clinical score were evaluated over 27 days post-infection. At 28-day post-infection, knee joint samples were collected at 28-day post-infection to determine: **(D)** leukocyte recruitment to the articular cavity, histopathological analysis of hematoxylin/eosin stained slices: **(E)** representative images of knee joints at 28 post-infection in original magnification ×10. The letter ***a*** indicates a heavily inflamed joint with cartilage destruction and pannus formation, **(F)** synovitis score (intensity: 1–4), and **(G)** cartilage destruction score (intensity: 1–4). At 28-day post-infection, **(H)** proteoglycan content in patellas, **(I)** bacterial counts in knee joint cavity, **(J)** and were determined, and **(K)** interleukin-33 (IL-33) and **(L)** sST2 concentrations in knee joints were determined by ELISA. Correlation analysis of IL-33 and IFN-γ levels determined by ELISA in synovial fluid samples from patients with septic arthritis. For inflammatory parameters and proteoglycan content: $n = 6$ per group per *in vivo* experiment, representative of two independent experiments. $^{*}P < 0.05$ vs naïve mice group, $^{\#}P < 0.05$ vs WT mice group **(A–D,H–L)**. One-way ANOVA followed by Tukey's test. For histological analysis: $n = 8$ per group per experiment, representative of two independent experiments. $^{*}P < 0.05$ vs naïve mice group, $^{\#}P < 0.05$ vs WT mice group **(E–G)**. Kruskal–Wallis test followed by Dunn's test. Spearman rank correlation test was used for the assessment of correlation **(M)**. Abbreviations: ***C***, cartilage; ***JC,*** joint cavity.

however, there was a clear difference in the disease intensity. The C57BL/6 mouse with pronounced type 1 and type 3 immune responses presented lessened disease severity that allowed observing the disease exacerbation in IFN-$\gamma^{-/-}$ mouse (**Figure 6**). The WT Balb/c with a more prominent type 2 profile could not inhibit bacterial growth. The ST2 deficiency enhanced the type 1 immune response resembling the WT C57BL/6 profile, indicate that IFN-γ deficiency impairs the immune response against *S. aureus*-induced septic arthritis similarly to Balb/c mice expressing ST2, which lines up with the data showing that IFN-γ deficiency increased IL-33 production. Thus, enhancing IFN-γ is an essential mechanism to solve septic arthritis and can be achieved by ST2 deficiency. Finally, we quantitated IFN-γ in synovial fluid samples of patients with septic arthritis and compared with the IL-33 levels observed in **Figure 1A**. An inverse relationship between the levels of IL-33 and IFN-γ was observed (**Figure 6M** and Figure S3 in Supplementary Material). Making a parallel of the data presented in this manuscript and **Figure 6M**, we would suggest that with higher levels of IL-33, lower levels of IFN-γ will be observed in the synovial fluid of septic arthritis patients, and changing the balance of this relationship may interfere with the disease outcome.

DISCUSSION

The present study demonstrates that the IL-33 receptor, ST2, contributes to the development of *S. aureus*-induced septic arthritis. This contribution is drastic in a manner that ST2 deficiency resulted in a better disease outcome. ST2 deficiency increased the Th1 skewing and induced IFN-γ production by BMDM that presented a boosted response with enhanced bacteria killing *via* NO. Septic arthritis presents severe and permanent joint sequelae (29, 31), and the present data suggests that ST2 deficiency ameliorates this infections disease.

The knees are the most common joints that are affected in septic arthritis in humans (27). The diagnosis of septic arthritis depends

on isolating the pathogen from aspirated synovial fluid from knee joints (29, 56). We observed that patients have significantly more IL-33 and no detectable concentrations of sST2 in their synovial fluid compared those of the osteoarthritis patients. In the mouse model of septic arthritis, substantial amounts of IL-33 as well as markedly reduced sST2 levels were observed in the knee joint. Thus, the mouse septic arthritis model replicates this IL-33/sST2 balance observed in septic arthritis patients. These clinical and experimental findings are consistent with the notion that the IL-33 levels are tightly regulated by sST2 availability (1, 4, 11, 57), suggesting that during *S. aureus* septic arthritis there is an enhanced availability and, potentially, activity of IL-33.

Infectious disease is the outcome of an intense crosstalk between invading pathogen and host defense armory (58). In septic arthritis, the synovial membrane inflammation leads to tissue destruction and dysfunction, resulting into significant painful condition and morbidity (28, 56, 59). In this context, the effect of ST2 deficiency in staphylococcal arthritis was evident since the first day after bacteria injection, inducing articular hyperalgesia, edema and a focal collection of immune cells that secret proinflammatory mediators such as TNF-α and IL-1β, which in turn contribute to the knee joint lesion, abscess and function loss (28). Indeed, IL-33 can orchestrate the influx of neutrophils and other immune cells subsidizing a dysfunctional joint inflammation in other arthritis models (13–15, 60). Conversely, using ST2 deficient mice, we observed a decrease in articular hyperalgesia, clinical score, and all inflammatory profile of staphylococcal arthritis over 28 days as result of a better control of infection, establishing a relationship between ST2 deficiency and intrinsic factors modulating microbicide mechanisms. The initial phenotype results could represent a reduction of inflammatory response since IL-33 induces hyperalgesia and edema (13, 41). Reduced inflammation would result in diminished killing of bacteria, which would be in line with evidence that in ovalbumin-induced airway inflammation IL-33 does not affect NO production from iNOS (61). However, this was not true in the present experimental condition since ST2 deficiency resulted in a better killing activity and control of staphylococcal arthritis avoiding the infection to become systemic. The ST2 deficiency allowed an enhancement of a Th1 cells activation of neutrophils and macrophages with IFN-γ with enhanced anti-bacterial activity. Unexpectedly, ST2 deficient macrophages produced IFN-γ for an autocrine induction of iNOS expression/NO production and killing activity, thus, suggesting that ST2 deficiency also favors an M1 macrophage phenotype that contributes to protect the host against *S. aureus* infection. Corroborating these data, an essential role for NO and other NO congeners in controlling septic arthritis was demonstrated (28, 62).

Extrapolating to others bacterial infections, IL-33/ST2 axis has apparent pleiotropic functions shaped by the local microenvironment (4, 19, 21, 38, 63, 64). Our findings appear to contradict the reports showing the beneficial role of IL-33 on bacterial infections, including cutaneous *S. aureus* infection (21, 63, 64). One fundamental explanation is that even before the recognition of IL-33, it was demonstrated that ST2 makes a negative-feedback control of IL-1RI and TLR signaling *via* sequestration of MyD88 and Mal through TIR domain (3, 65). Direct activation of TLRs in neutrophils, specifically TLR2 in staphylococcal infections, downregulates the expression of chemokine receptor CXCR2 that keeps their recruitment to infectious foci. Thus, once at infectious focus, neutrophils no longer need to continue migrating, but they must solve the infection by producing microbicide molecules such as NO (19, 66). Further, NO produced from iNOS also downregulates chemokine receptor expression (19, 67). Considering that IL-33 interferes with TLR signaling (19), it is reasonable to suppose that in ST2$^{-/-}$ mice with septic arthritis, TLR2 signaling will be boosted allowing neutrophils and macrophages to produce larger amounts of NO *via* iNOS to eliminate the bacteria. This signaling also links innate and Th1-biased adaptive response (65). Moreover, acute sepsis also presents differences with septic arthritis that contribute to understand the pleiotropic roles of IL-33. Acute sepsis is dependent on innate immune responses, although it triggers immune suppression in the patients that survive (38). On the other hand, septic arthritis is a disease with extremely aggressive acute effects, but also with a chronic temporal profile that allows the development of adaptive immune response as observed here in. The present data supports that ST2 deficiency will allow the development of Th1 adaptive immune response and a better control of infection *via* enhanced IFN-γ production and consequent increase microbicide activity of phagocytes.

NO has a selective enhancing effect on the induction and differentiation of Th1 but not Th2 cells (49, 68, 69). Th1 cells in a feedback loop amplify NO synthesis further *via* activation of M1 macrophages by IFN-γ and suppress IL-4 synthesis, leading to the production of large amounts of NO that is essential for killing pathogens (68). Importantly, a strong Th1 response is desirable for effective host defense against *S. aureus* (63). Conversely, a skewing toward a type 2 immune response is thought to contribute to impaired immune defenses in *S. aureus*-induced septic arthritis (70). Consistent with the inability of WT mice in controlling the infection, these mice showed Th2 profile in septic arthritis with high-IL-4/CD4^{+}T cell and low-IFN-γ/CD4^{+}T cell counts. In the absence of ST2, the polarization of lymphocytes and type 2 cytokines production was abolished allowing the skewing toward Th1 phenotype. The ST2 deficiency also inhibited Th17 expansion and promoted IL-10 production. These data are in agreement that IFN-γ production might overlap IL-17 release in septic arthritis (71) and that IL-10 was released in order to achieve a state of tissue homeostasis in chronic phase of septic arthritis (72).

Concluding, we find a novel endogenous role for the IL-33 receptor, ST2, in septic arthritis. Abrogating ST2 expression enhances the Th0 polarization toward a Th1 phenotype that *via* IFN-γ induces iNOS-derived NO production by macrophages and neutrophils improving the killing activity of these innate immune cells. Furthermore, ST2 deficient macrophages produce IFN-γ that boosts their bactericide armory. Therefore, ST2 deficiency is beneficial in *S. aureus*-induced septic arthritis.

ETHICS STATEMENT

Animal care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines, and approved by the Ethics Committee of the Londrina State University (OF.CIRC.CEUA, process number 20165/2009).

AUTHOR CONTRIBUTIONS

LS-F, JA-F, and WV conceived and designed the experiments. LS-F, ST, VF, DN, and KL performed the experiments. LS-F and FF pathological analysis. RO and PL-J patient's recruitment, collection of human samples. LS-F, ST, VF, DN, KL, and WV collection of data and analysis. JP, RC, SF, MT, TC, FL, FC, JA-F, and WV contributed reagents/materials/analysis tools. LS-F, VF, and WV writing-original draft. LS-F, RC, TC, FC, JA-F, and WV writing-review and editing. All authors contributed to manuscript revision, read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Giuliana Francisco, Ieda Schivo, Sérgio Rosa, and Tadeu Vieira for their technical assistance.

FUNDING

This work was supported by grants from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico); MCTI/SETI/Fundação Araucária (Ministério da Ciência, Tecnologia e Inovação/Secretaria da Ciência, Tecnologia, e Ensino Superior do Paraná/Fundação Araucária); PPSUS grant from Decit/SCTIE/MS (Departamento de Ciência e Tecnologia da Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Ministério da Saúde) intermediated by CNPq and support of Fundação Araucári; Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); INCT (National Institutes of Science and Technology)—MCTI/CNPq/CAPES/Fundação Araucária; São Paulo Research Foundation under grant agreements 2011/19670-0 (Thematic Project) and 2013/08216-2 (Center for Research in Inflammatory Disease); Financiadora de Estudos e Projetos and Secretaria de Estado da Ciência, Tecnologia e Ensino Superior do Paraná (FINEP/SETI-PR) and Central Multiusuária de Laboratórios de Pesquisa da UEL (CMLP). LS-F received a postdoctoral fellowship from CAPES.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00962/full#supplementary-material.

REFERENCES

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, 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. doi:10.1016/j.immuni.2005.09.015
2. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, 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):282–7. doi:10.1073/pnas.0606854104
3. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* (2005) 5(6):446–58. doi:10.1038/nri1630
4. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol* (2016) 16(11):676–89. doi:10.1038/nri.2016.95
5. Meisel C, Bonhagen K, Lohning M, Coyle AJ, Gutierrez-Ramos JC, Radbruch A, et al. Regulation and function of T1/ST2 expression on CD4+ T cells: induction of type 2 cytokine production by T1/ST2 cross-linking. *J Immunol* (2001) 166(5):3143–50. doi:10.4049/jimmunol.166.5.3143
6. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: the ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol* (2007) 179(4):2051–4. doi:10.4049/jimmunol.179.4.2051
7. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. 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):1503–13. doi:10.4049/jimmunol.1102832
8. Trajkovic V, Sweet MJ, Xu D. T1/ST2—an IL-1 receptor-like modulator of immune responses. *Cytokine Growth Factor Rev* (2004) 15(2–3):87–95. doi:10.1016/j.cytogfr.2004.02.004
9. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* (2000) 191(6):1069–76. doi:10.1084/jem.191.6.1069
10. Kropf P, Herath S, Klemenz R, Muller I. Signaling through the T1/ST2 molecule is not necessary for Th2 differentiation but is important for the regulation of type 1 responses in nonhealing *Leishmania major* infection. *Infect Immun* (2003) 71(4):1961–71. doi:10.1128/IAI.71.4.1961-1971.2003
11. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* (2010) 10(2):103–10. doi:10.1038/nri2692
12. Fattori V, Hohmann MSN, Rossaneis AC, Manchope MF, Alves-Filho JC, Cunha TM, et al. Targeting IL-33/St2 signaling: regulation of immune function and analgesia. *Expert Opin Ther Targets* (2017) 12:1141–52. doi:10.1080/14728222.2017.1398734
13. Verri WA Jr, Guerrero AT, Fukada SY, Valerio DA, Cunha TM, Xu D, et al. IL-33 mediates antigen-induced cutaneous and articular hypernociception in mice. *Proc Natl Acad Sci U S A* (2008) 105(7):2723–8. doi:10.1073/pnas.0712116105
14. Verri WA Jr, Souto FO, Vieira SM, Almeida SC, Fukada SY, Xu D, et al. IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. *Ann Rheum Dis* (2010) 69(9):1697–703. doi:10.1136/ard.2009.122655
15. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci U S A* (2008) 105(31):10913–8. doi:10.1073/pnas.0801898105
16. Palmer G, Talabot-Ayer D, Lamacchia C, Toy D, Seemayer CA, Viatte S, et al. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. *Arthritis Rheum* (2009) 60(3):738–49. doi:10.1002/art.24305
17. Matsuyama Y, Okazaki H, Tamemoto H, Kimura H, Kamata Y, Nagatani K, et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. *J Rheumatol* (2010) 37(1):18–25. doi:10.3899/jrheum.090492
18. Lee EJ, So MW, Hong S, Kim YG, Yoo B, Lee CK. Interleukin-33 acts as a transcriptional repressor and extracellular cytokine in fibroblast-like synoviocytes in patients with rheumatoid arthritis. *Cytokine* (2016) 77:35–43. doi:10.1016/j.cyto.2015.10.005
19. Alves-Filho JC, Sonego F, Souto FO, Freitas A, Verri WA Jr, Auxiliadora-Martins M, et al. Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nat Med* (2010) 16(6):708–12. doi:10.1038/nm.2156
20. Hazlett LD, McClellan SA, Barrett RP, Huang X, Zhang Y, Wu M, et al. IL-33 shifts macrophage polarization, promoting resistance against *Pseudomonas aeruginosa* keratitis. *Invest Ophthalmol Vis Sci* (2010) 51(3):1524–32. doi:10.1167/iovs.09-3983
21. Yin H, Li X, Hu S, Liu T, Yuan B, Ni Q, et al. IL-33 promotes *Staphylococcus aureus*-infected wound healing in mice. *Int Immunopharmacol* (2013) 17(2):432–8. doi:10.1016/j.intimp.2013.07.008
22. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol* (2008) 180(4):2443–9. doi:10.4049/jimmunol.180.4.2443

23. Hams E, Bermingham R, Wurlod FA, Hogan AE, O'Shea D, Preston RJ, et al. The helminth T2 RNase omega1 promotes metabolic homeostasis in an IL-33- and group 2 innate lymphoid cell-dependent mechanism. *FASEB J* (2016) 30(2):824–35. doi:10.1096/fj.15-277822
24. Jones LA, Roberts F, Nickdel MB, Brombacher F, McKenzie AN, Henriquez FL, et al. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with *Toxoplasma gondii*. *Eur J Immunol* (2010) 40(2):426–36. doi:10.1002/eji.200939705
25. Rostan O, Gangneux JP, Piquet-Pellorce C, Manuel C, McKenzie AN, Guiguen C, et al. The IL-33/ST2 axis is associated with human visceral leishmaniasis and suppresses Th1 responses in the livers of BALB/c mice infected with *Leishmania donovani*. *MBio* (2013) 4(5):e00383–13. doi:10.1128/mBio.00383-13
26. Khalid KE, Nascimento MSL, Sacramento LA, Costa DL, Lima-Junior DS, Carregaro V, et al. T1/ST2 deficient mice display protection against *Leishmania infantum* experimental infection. *Acta Trop* (2017) 172:1–6. doi:10.1016/j.actatropica.2017.04.011
27. Tarkowski A. Infection and musculoskeletal conditions: infectious arthritis. *Best Pract Res Clin Rheumatol* (2006) 20(6):1029–44. doi:10.1016/j.berh.2006.08.001
28. Staurengo-Ferrari L, Ruiz-Miyazawa KW, Pinho-Ribeiro FA, Domiciano TP, Fattori V, Mizokami SS, et al. The nitroxyl donor Angeli's salt ameliorates *Staphylococcus aureus*-induced septic arthritis in mice. *Free Radic Biol Med* (2017) 108:487–99. doi:10.1016/j.freeradbiomed.2017.04.016
29. Sharff KA, Richards EP, Townes JM. Clinical management of septic arthritis. *Curr Rheumatol Rep* (2013) 15(6):332. doi:10.1007/s11926-013-0332-4
30. Fattori V, Amaral FA, Verri WA Jr. Neutrophils and arthritis: role in disease and pharmacological perspectives. *Pharmacol Res* (2016) 112:84–98. doi:10.1016/j.phrs.2016.01.027
31. Shirtliff ME, Mader JT. Acute septic arthritis. *Clin Microbiol Rev* (2002) 15(4):527–44. doi:10.1128/CMR.15.4.527-544.2002
32. Caporali R, Caprioli M, Bobbio-Pallavicini F, Montecucco C. DMARDS and infections in rheumatoid arthritis. *Autoimmun Rev* (2008) 8(2):139–43. doi:10.1016/j.autrev.2008.05.001
33. Kaplan SL. Recent lessons for the management of bone and joint infections. *J Infect* (2014) 68(Suppl 1):S51–6. doi:10.1016/j.jinf.2013.09.014
34. Boff D, Oliveira VLS, Queiroz Junior CM, Silva TA, Allegretti M, Verri WA Jr, et al. CXCR2 is critical for bacterial control and development of joint damage and pain in *Staphylococcus aureus*-induced septic arthritis in mouse. *Eur J Immunol* (2017) 48(3):454–63. doi:10.1002/eji.201747198
35. Pinto LG, Cunha TM, Vieira SM, Lemos HP, Verri WA Jr, Cunha FQ, et al. IL-17 mediates articular hypernociception in antigen-induced arthritis in mice. *Pain* (2010) 148(2):247–56. doi:10.1016/j.pain.2009.11.006
36. Valerio DA, Ferreira FI, Cunha TM, Alves-Filho JC, Lima FO, De Oliveira JR, et al. Fructose-1,6-bisphosphate reduces inflammatory pain-like behaviour in mice: role of adenosine acting on A1 receptors. *Br J Pharmacol* (2009) 158(2):558–68. doi:10.1111/j.1476-5381.2009.00325.x
37. Zhu F, Zhou Y, Jian C, Zhang X. Role of JAK-STAT in signaling in maturation og phagosomes contaning *Staphylococcus aureus*. *Sci Rep* (2015) 5:14854. doi:10.1038/srep14854
38. Nascimento DC, Melo PH, Pineros AR, Ferreira RG, Colon DF, Donate PB, et al. IL-33 contributes to sepsis-induced long-term immunosuppression by expanding the regulatory T cell population. *Nat Commun* (2017) 8:14919. doi:10.1038/ncomms14919
39. Oh H, Siano B, Diamond S. Neutrophil isolation protocol. *J Vis Exp* (2008) (17):e745. doi:10.3791/745
40. Gutierrez FR, Lalu MM, Mariano FS, Milanezi CM, Cena J, Gerlach RF, et al. Increased activities of cardiac matrix metalloproteinases matrix metalloproteinase (MMP)-2 and MMP-9 are associated with mortality during the acute phase of experimental *Trypanosoma cruzi* infection. *J Infect Dis* (2008) 197(10):1468–76. doi:10.1086/587487
41. Zarpelon AC, Cunha TM, Alves-Filho JC, Pinto LG, Ferreira SH, McInnes IB, et al. IL-33/ST2 signalling contributes to carrageenin-induced innate inflammation and inflammatory pain: role of cytokines, endothelin-1 and prostaglandin E2. *Br J Pharmacol* (2013) 169(1):90–101. doi:10.1111/bph.12110
42. Walsh MC, Choi Y. Biology of the RANKL-RANK-OPG System in Immunity, Bone, and Beyond. *Front Immun* (2014) 5:511. doi:10.3389/fimmu.2014.00511
43. Espinassous Q, Garcia-de-Paco E, Garcia-Verdugo I, Synguelakis M, von Aulock S, Sallenave JM, et al. IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating lipopolysaccharide receptor complex. *J Immunol* (2009) 183(2):1446–55. doi:10.4049/jimmunol.0803067
44. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, 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. doi:10.1073/pnas.1003059107
45. Fournier B, Philpott DJ. Recognition of *Staphylococcus aureus* by the innate immune system. *Clin Microbiol Rev* (2005) 18(3):521–40. doi:10.1128/CMR.18.3.521-540.2005
46. Stuyt RJ, Kim SH, Reznikov LL, Fantuzzi G, Novick D, Rubinstein M, et al. Regulation of *Staphylococcus epidermidis*-induced IFN-gamma in whole human blood: the role of endogenous IL-18, IL-12, IL-1, and TNF. *Cytokine* (2003) 21(2):65–73. doi:10.1016/S1043-4666(02)00501-X
47. Hucke C, MacKenzie CR, Adjogble KD, Takikawa O, Daubener W. Nitric oxide-mediated regulation of gamma interferon-induced bacteriostasis: inhibition and degradation of human indoleamine 2,3-dioxygenase. *Infect Immun* (2004) 72(5):2723–30. doi:10.1128/IAI.72.5.2723-2730.2004
48. Huang FP, Niedbala W, Wei XQ, Xu D, Feng GJ, Robinson JH, et al. Nitric oxide regulates Th1 cell development through the inhibition of IL-12 synthesis by macrophages. *Eur J Immunol* (1998) 28(12):4062–70. doi:10.1002/(SICI)1521-4141(199812)28:12<4062::AID-IMMU4062>3.0.CO;2-K
49. Niedbala W, Cai B, Liew FY. Role of nitric oxide in the regulation of T cell functions. *Ann Rheum Dis* (2006) 65(Suppl 3):iii37–40. doi:10.1136/ard.2006.058446
50. Niedbala W, Besnard AG, Jiang HR, Alves-Filho JC, Fukada SY, Nascimento D, et al. Nitric oxide-induced regulatory T cells inhibit Th17 but not Th1 cell differentiation and function. *J Immunol* (2013) 191(1):164–70. doi:10.4049/jimmunol.1202580
51. Lin L, Ibrahim AS, Xu X, Farber JM, Avanesian V, Baquir B, et al. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. *PLoS Pathog* (2009) 5(12):e1000703. doi:10.1371/journal.ppat.1000703
52. Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. *Ann N Y Acad Sci* (2010) 1183:211–21. doi:10.1111/j.1749-6632.2009.05133.x
53. Luger D, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, et al. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J Exp Med* (2008) 205(4):799–810. doi:10.1084/jem.20071258
54. Leech JM, Lacey KA, Mulcahy ME, Medina E, McLoughlin RM. IL-10 plays opposing roles during *Staphylococcus aureus* systemic and localized infections. *J Immunol* (2017) 198(6):2352–65. doi:10.4049/jimmunol.1601018
55. Lima-Junior DS, Costa DL, Carregaro V, Cunha LD, Silva AL, Mineo TW, et al. Inflammasome-derived IL-1β production induces nitric oxide-mediated resistance to *Leishmania*. *Nat Med* (2013) 19(7):909–15. doi:10.1038/nm.3221
56. Mathew AJ, Ravindran V. Infections and arthritis. *Best Pract Res Clin Rheumatol* (2014) 28(6):935–59. doi:10.1016/j.berh.2015.04.009
57. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* (2007) 117(6):1538–49. doi:10.1172/JCI30634
58. Kaufmann SHE, Dorhoi A. Molecular determinants in phagocyte-bacteria interactions. *Immunity* (2016) 44(3):476–91. doi:10.1016/j.immuni.2016.02.014
59. Rowton J. Three cases of septic arthritis following a recent arthroscopic procedure. *BMJ Case Rep* (2013) 2013:bcr2012007507. doi:10.1136/bcr-2012-007507
60. Chen S, Chen B, Wen Z, Huang Z, Ye L. IL-33/ST2-mediated inflammation in macrophages is directly abrogated by IL-10 during rheumatoid arthritis. *Oncotarget* (2017) 8(20):32407–18. doi:10.18632/oncotarget.16299
61. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* (2009) 183(10):6469–77. doi:10.4049/jimmunol.0901575
62. McInnes IB, Leung B, Wei XQ, Gemmell CC, Liew FY. Septic arthritis following *Staphylococcus aureus* infection in mice lacking inducible nitric oxide synthase. *J Immunol* (1998) 160(1):308–15.

63. Li C, Li H, Jiang Z, Zhang T, Wang Y, Li Z, et al. Interleukin-33 increases antibacterial defense by activation of inducible nitric oxide synthase in skin. *PLoS Pathog* (2014) 10(2):e1003918. doi:10.1371/journal.ppat.1003918
64. Lan F, Yuan B, Liu T, Luo X, Huang P, Liu Y, et al. Interleukin-33 facilitates neutrophil recruitment and bacterial clearance in *S. aureus*-caused peritonitis. *Mol Immunol* (2016) 72:74–80. doi:10.1016/j.molimm.2016.03.004
65. Brint EK, Xu D, Liu H, Dunne A, McKenzie AN, O'Neill LA, et al. ST2 is an inhibitor of interleukin 1 receptor and toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol* (2004) 5(4):373–9. doi:10.1038/ni1050
66. Alves-Filho JC, Freitas A, Souto FO, Spiller F, Paula-Neto H, Silva JS, et al. Regulation of chemokine receptor by toll-like receptor 2 is critical to neutrophil migration and resistance to polymicrobial sepsis. *Proc Natl Acad Sci U S A* (2009) 106(10):4018–23. doi:10.1073/pnas.0900196106
67. Rios-Santos F, Alves-Filho JC, Souto FO, Spiller F, Freitas A, Lotufo CM, et al. Down-regulation of CXCR2 on neutrophils in severe sepsis is mediated by inducible nitric oxide synthase-derived nitric oxide. *Am J Respir Crit Care Med* (2007) 175(5):490–7. doi:10.1164/rccm.200601-103OC
68. Niedbala W, Wei XQ, Piedrafita D, Xu D, Liew FY. Effects of nitric oxide on the induction and differentiation of Th1 cells. *Eur J Immunol* (1999) 29(8):2498–505. doi:10.1002/(SICI)1521-4141(199908)29:08<2498::AID-IMMU2498>3.0.CO;2-M
69. Niedbala W, Wei XQ, Campbell C, Thomson D, Komai-Koma M, Liew FY. Nitric oxide preferentially induces type 1 T cell differentiation by selectively up-regulating IL-12 receptor beta 2 expression via cGMP. *Proc Natl Acad Sci U S A* (2002) 99(25):16186–91. doi:10.1073/pnas.252464599
70. Spellberg B, Edwards JE Jr. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis* (2001) 32(1):76–102. doi:10.1086/317537
71. Henningsson L, Jirholt P, Lindholm C, Eneljung T, Silverpil E, Iwakura Y, et al. Interleukin-17A during local and systemic *Staphylococcus aureus*-induced arthritis in mice. *Infect Immun* (2010) 78(9):3783–90. doi:10.1128/IAI.00385-10
72. Gjertsson I, Hultgren OH, Tarkowski A. Interleukin-10 ameliorates the outcome of *Staphylococcus aureus* arthritis by promoting bacterial clearance. *Clin Exp Immunol* (2002) 130(3):409–14. doi:10.1046/j.1365-2249.2002.01999.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Staurengo-Ferrari, Trevelin, Fattori, Nascimento, de Lima, Pelayo, Figueiredo, Casagrande, Fukada, Teixeira, Cunha, Liew, Oliveira, Louzada-Junior, Cunha, Alves-Filho and Verri. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 29 May 2018
doi: 10.3389/fimmu.2018.01179

Integrated Expression Profiles Analysis Reveals Correlations Between the IL-33/ST2 Axis and CD8+ T Cells, Regulatory T Cells, and Myeloid-Derived Suppressor Cells in Soft Tissue Sarcoma

Huoying Chen[1,2,3†], Yao Chen[2,4†], Hongbo Liu[1], Yi Que[2], Xing Zhang[2] and Fang Zheng[3,5]*

[1] Department of Laboratory Medicine, The Second Affiliated Hospital of Guilin Medical University, Guilin, China, [2] Melanoma and Sarcoma Medical Oncology Unit, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China, [3] Department of Immunology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4] Department of Radiotherapy, Affiliated Hospital of Guilin Medical University, Guilin, China, [5] Key Laboratory of Organ Transplantation, Ministry of Education, NHC Key Laboratory of Organ Transplantation, Chinese Academy of Medical Sciences, Wuhan, China

OPEN ACCESS

Edited by:
Rong Mu,
Peking University People's Hospital, China

Reviewed by:
Cinzia Fionda,
Sapienza Università di Roma, Italy
Damo Xu,
University of Glasgow, United Kingdom

***Correspondence:**
Fang Zheng
zhengfangtj@hust.edu.cn

[†]These authors have contributed equally to this work.

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *05 January 2018*
Accepted: *11 May 2018*
Published: *29 May 2018*

Citation:
Chen H, Chen Y, Liu H, Que Y, Zhang X and Zheng F (2018) Integrated Expression Profiles Analysis Reveals Correlations Between the IL-33/ST2 Axis and CD8+ T Cells, Regulatory T Cells, and Myeloid-Derived Suppressor Cells in Soft Tissue Sarcoma. Front. Immunol. 9:1179. doi: 10.3389/fimmu.2018.01179

Soft tissue sarcoma (STS) is a rare solid malignant cancer, and there are few effective treatment options for advanced disease. Cancer immunotherapy is a promising new strategy for STS treatment. IL-33 is a candidate cytokine for immunotherapy that can activate T lymphocytes and modulate antitumor immunity in some cancers. However, the expression and biological role of IL-33 in STS are poorly understood. In this study, we found that the expression of IL-33 and its receptor ST2 was decreased in STS using real-time PCR assays. By analyzing sarcoma data from The Cancer Genome Atlas, we found that higher transcriptional levels of IL-33 and ST2 were associated with a favorable outcome. There were positive correlations between the expression levels of ST2 and CD3E, CD4, CD8A, CD45RO, FOXP3, CD11B, CD33, and IFN-γ. Strong positive correlations between the expression of IFN-γ and CD3E and CD8A were also observed. Moreover, the expression levels of both IL-33 and ST2 were positively correlated with those of CD3E, CD8A, and chemokines that recruit CD8+ T cells, indicating that the IL-33/ST2 axis may play an important role in recruiting and promoting the immune response of type 1-polarized CD8+ T cells in STS. Meanwhile, we also found that the expression of IL-33 was negatively correlated with that of TGF-β1 and chemokines that recruit regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), indicating that the IL-33/ST2 axis may also contribute to antagonizing Tregs, MDSCs, and TGF-β1-mediated immunosuppression in STS. The correlations between the IL-33/ST2 axis and CD8+ T cells and IFN-γ, as well as Tregs, MDSCs, and TGF-β1 were validated by additional analyses using three other independent GEO datasets of sarcoma. Our results implicate the possible role of the IL-33/ST2 axis in modulating antitumor immunity in STS. IL-33 may not only serve as a useful prognostic biomarker for STS but also as a potential therapeutic target for STS immunotherapy and worth further investigation.

Keywords: IL-33, ST2, soft tissue sarcoma, IFN-γ, TGF-β1

INTRODUCTION

Soft tissue sarcoma (STS) is a rare solid malignancy derived from mesenchymal tissues that accounts for approximately 1% of all cancers in adult patients (1). The pathogenesis of STS is poorly understood, and there are few effective treatment options for advanced disease. At present, despite the combination of surgery, chemotherapy, radiotherapy, and other systemic treatment, the overall 5-year survival rate of STS patients is only 50–60% (2). Recently, immunotherapy has emerged as a promising new treatment for cancer. For example, blockade of immune checkpoints has shown remarkable success in the treatment of melanoma, lung cancer, and colorectal cancer (3–5). Immunotherapy also offers new strategies for STS treatment. However, many mechanisms responsible for the failure of antitumor immunity, including active immunosuppression by the tumor microenvironment and insufficient immune stimulatory signals, have not yet been fully elucidated in STS, which limits the development of immunotherapy for STS.

IL-33 is a nuclear cytokine from the IL-1 cytokine family, and its role in immune moderation has been widely studied (6, 7). IL-33 is constitutively expressed in epithelial barrier tissues and lymphoid organs and functions as an alarmin (6, 8, 9). At the site of inflammation and damage, IL-33 is rapidly released from producing cells and activates the downstream NF-κB and MAPK pathways via a heteromeric receptor that consists of ST2 (also known as IL1RL1) and IL-1R accessory protein (IL-1RAcP) (10), thereby regulating the transcription of a variety of chemokines and cytokines and recruiting local immune cells to the site of inflammation and injury (9). IL-33 has been well established as a pleiotropic cytokine that regulates T helper 2 (Th2) cells, Th17/1 cells, and regulatory T cells (Tregs)-mediated immune responses (11–14). Recently, evidence has also shown that IL-33 can play an antitumor role by promoting the immune response of natural killer cells (NKs) and $CD8^+$ T cells and enhancing IFN-γ production (15–17), which suggests that IL-33 is a potent cytokine for reversing the immunosuppressive tumor microenvironment and promoting antitumor immunity (16, 18). By analyzing publicly available tumor data from The Cancer Genome Atlas (TCGA), we found that IL-33 and ST2 mRNA is widely expressed in sarcoma, indicating that the IL-33/ST2 axis may play an important role in regulating antitumor immunity in STS. However, no reports currently define the role of the IL-33/ST2 axis in STS. Therefore, we analyzed the expression of IL-33/ST2 axis-related genes and clinical survival data of sarcoma from TCGA and GEO, hoping to provide clues as to whether IL-33 can modulate antitumor immunity and reverse the immunosuppressive tumor microenvironment in STS.

In the current study, we found that the mRNA expression of IL-33 and ST2 was decreased in STS. TCGA data analysis indicated that higher transcriptional levels of IL-33 and ST2 in STS were associated with a favorable outcome. By analyzing sarcoma data of TCGA and GEO, we found that the transcriptional levels of IL-33 and ST2 were positively correlated with those of CD3E, CD8A, IFN-γ, and chemokines that recruit $CD8^+$ T cells, indicating that the IL-33/ST2 axis may play an important role in promoting the recruitment of $CD8^+$ T cells and enhancing IFN-γ production in STS. We also found that the transcriptional level of IL-33 was negatively correlated with that of TGF-β1, an immunosuppressive cytokine, and chemokines that recruit Tregs and myeloid-derived suppressor cells (MDSCs), indicating that the IL-33/ST2 axis may also contribute to inhibiting the production of TGF-β1 and reducing the infiltration of Tregs and MDSCs in STS. Our results implicate the possible role of the IL-33/ST2 axis in the modulation of antitumor immunity in STS. IL-33 may serve as a useful prognostic biomarker for STS and a potential immunotherapeutic target for STS.

MATERIALS AND METHODS

Human Sarcoma Specimens

A total of 18 pairs of sarcoma and adjacent tissue specimens used for real-time PCR assays of IL-33 and ST2 expression were collected from Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China. Each biopsy specimen was immersed in RNAlater reagent overnight at 4°C and then preserved at −80°C until RNA extraction. Ethical approval was given by the Institutional Research Medical Ethics Committee of Sun Yat-sen University Cancer Center, and written informed consent was obtained from patients for the use of their clinical tissues in this study.

RNA Extraction, Reverse Transcription, and Real-Time PCR

Total RNA was extracted from sarcoma specimens using TRIZOL reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The RNA concentration and quantity were determined using a NanoDrop spectrophotometer (ND-1000, Thermo Scientific, USA). The first-strand cDNA was synthesized from 1 μg of total RNA using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The expression of IL-33 and ST2 was detected using real-time PCR according to the protocol supplied by the manufacturer (Bio-Rad, CA, USA), and amplification was monitored with iQTM SYBR Green Supermix (Bio-Rad, CA, USA) according to the manufacturer's instructions. The following primers were used for SYBR Green qPCR:

IL-33-forward: 5′-GTGACGGTGTTGATGGTAAGAT-3′
IL-33-reverse: 5′-AGCTCCACAGAGTGTTCCTTG-3′
ST2-forward: 5′-AGAAATCGTGTGTTTGCCTCA-3′
ST2-reverse: 5′-TCCAGTCCTATTGAATGTGGGA-3′
β-*actin*-forward: 5′-CGCGAGAAGATGACCCAGAT-3′
β-*actin*-reverse: 5′-GGGCATACCCCTCGTAGATG-3′

The expression data were normalized to the geometric mean of the housekeeping gene *β-actin* to control the variability in expression levels and calculated as $2^{-\Delta CT}$ [ΔC_T = (C_T of gene) − (C_T of *β-actin*)], where C_T represents the threshold cycle for each transcript.

TCGA Sarcoma Samples

Gene level 3 TCGA mRNA expression data were downloaded from the publicly accessible TCGA portal.[1] Informed consent

[1]https://portal.gdc.cancer.gov/ (Accessed: March 24, 2016).

was provided by patients participating in TCGA program based on the guidelines from the TCGA Ethics, Law and Policy Group. The mRNA data were normalized by the RSEM algorithm and included 261 sarcoma patient samples. TCGA survival data with matched mRNA expression data from sarcoma were downloaded from OncoLnc.[2] A heat map of the transcriptional expression data was generated with novel HemI software (Heatmap Illustrator, version 1.0) (19).

GEO Data Series of Sarcoma

The datasets of transcriptome profiling by microarray were searched on GEO Profiles[3] with the keywords "sarcoma *il33*" and limited the Organism to be "Homo sapiens." Finally, three microarray datasets of sarcoma (GSE2719, GSE6481, and GSE 967) were screened and downloaded for analysis. Summary of the three selected GEO data series is shown in **Table 1**.

Statistics

All of the statistical analyses were performed using SPSS standard version 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). A paired *t*-test was used when two paired measurements were analyzed. The correlation between gene expression levels was analyzed by Pearson correlation test. Survival analysis was performed by Kaplan–Meier and Log-rank test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Higher Expression Levels of IL-33 and ST2 Are Associated With a Favorable Prognosis in STS

To evaluate the expression of IL-33 and ST2 in STS, we examined 18 pairs of sarcoma and adjacent normal tissue specimens for IL-33 and ST2 expression using real-time PCR assays. We found that the expression levels of IL-33 and ST2 were decreased in tumor tissues compared with adjacent normal tissues (**Figure 1A**). Furthermore, we analyzed transcriptome sequencing and survival data from sarcoma from the TCGA and found that both IL-33 and ST2 expression were associated with the prognosis of sarcomas. Patients with higher transcriptional levels of IL-33 and ST2 have a more favorable prognosis (**Figure 1B**). These results indicate that IL-33 and ST2 are involved in the progression of STS and may serve as useful prognostic biomarkers for STS.

[2]http://www.oncolnc.org/ (Accessed: November 30, 2016).

[3]https://www.ncbi.nlm.nih.gov/geoprofiles/ (Accessed: February 10, 2017).

TABLE 1 | Summary of the three GEO data series used in this study.

Data set	Microarray platform	Sarcoma samples	Publication
GSE2719	GPL96	39	*Cancer Res* 2005; PMID: 5994966 *J Surg Res* 2006; PMID: 16603191
GSE6481	GPL96	105	*Mod Pathol* 2007; PMID: 17464315
GSE967	GPL91	23	*Int J Cancer* 2004; PMID: 15146558

IL-33 and ST2 Are Positively Correlated With the Expression of Different Immune Cell Subpopulation-Specific Genes in STS

We analyzed the immunological components in the tumor microenvironment of STS and found that the components of the antitumor immune response in STS were mainly CD3E/CD4/CD8A-labeled T cells, CD57-labeled NK cells, and CD45RO-labeled memory T cells that can secrete IFN-γ. The components that inhibited the immune response were primarily FOXP3-labeled Tregs and CD11B/CD33-labeled MDSCs, as well as IL-10, IL-6, TGF-β1, and other immunosuppressive cytokines (**Figure 2A**). In particular, the expression of TGF-β1 was very high. It is likely that TGF-β1 is the major immunosuppressive cytokine in the tumor microenvironment of STS. Meanwhile, the expression levels of IL-17A, IL-2, and the Th2 cytokines IL-4, IL-5, and IL-13 were almost undetectable (**Figure 2A**), indicating that the Th17 and Th2 immune responses were almost ineffective in STS. It has been reported that Th1, $CD8^+$ T cells, NKs, Tregs, and MDSCs can express ST2 (7, 9, 16, 20), and IL-33 can directly regulate the immune function of these cells through ST2. To determine in which of the above cells the IL-33/ST2 axis may play a role in STS, we performed a series of correlation analyses. By analyzing TCGA data, we found that there were positive correlations between the expression of ST2 and CD3E, CD4, CD8A, and CD45RO (**Figure 2B**). Weak correlations between the expression of IL-33 and CD3E, CD8A, and CD45RO were also observed (**Figure 2C**). There were also positive correlations between the expression of ST2 and FOXP3, CD11B, and CD33 (**Figure 2D**). The correlations between the expression of ST2 and CD4, CD8A, and CD33, and between IL-33 and CD8A and CD45RO were then validated by analysis using GSE2719 or GSE 967 GEO datasets of sarcoma (**Figures 2B–D**). However, there was no correlation between the expression of ST2 and CD57 or between IL-33 and CD57 (data not shown). Taken together, these results indicate that the IL-33/ST2 axis may regulate the immune function of Th1, $CD8^+$ T cells, and memory T cells, as well as Tregs and MDSCs in the tumor microenvironment of STS.

ST2 Is Positively Correlated With the Expression of IFN-γ in STS

IFN-γ is an important cytokine in antitumor responses (16). We further explored whether the IL-33/ST2 axis is associated with the production of IFN-γ in STS. Correlation analyses using sarcoma data of TCGA showed that the expression of IFN-γ was positively correlated with that of CD4 and CD45RO, but strong positive correlations were observed with CD3E and CD8A expression (**Figure 3A**). This suggests that CD3E/CD8A-labeled T cells may be the major cells that secrete IFN-γ in STS. The correlations between the expression of IFN-γ and CD3E, CD8A, and CD45RO were then validated by analysis using GSE2719 or GSE967 datasets of sarcoma (**Figure 3A**). Moreover, we found that the expression of ST2 was positively correlated with that of IFN-γ (**Figure 3B**). Positive correlation between the expression of IFN-γ and IL-33 was also observed by analysis using GSE2719 dataset (**Figure 3B**). Combined with the results indicating that the expression of ST2 is positively correlated with that of CD3E,

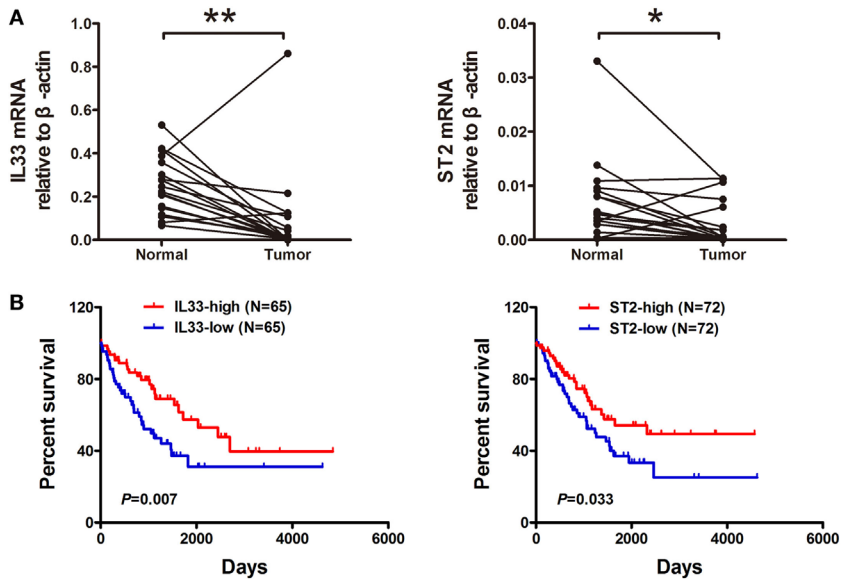


FIGURE 1 | Expression and prognostic significance of IL-33 and ST2 in soft tissue sarcoma. **(A)** The expression of IL-33 and ST2 in tumor tissues was lower than those in adjacent normal tissues. A total of 18 pairs of sarcoma and adjacent normal tissue specimens were collected and used for real-time PCR assays for IL-33 and ST2 expression. The expression data were normalized to the geometric mean of the housekeeping gene β-actin. **P* < 0.05, ***P* < 0.01 using paired *t*-test. **(B)** Higher transcriptional levels of IL-33 and ST2 were associated with a better outcome. Transcriptional sequencing and survival data from patients with soft tissue sarcoma were obtained from the publicly accessible The Cancer Genome Atlas portal, and overall survival analysis was evaluated by Kaplan–Meier and Log-rank test.

CD4, CD8A, and CD45RO (**Figure 2A**), it is easy to deduce that the IL-33/ST2 axis may promote T cells, especially $CD8^+$ T cells, to produce IFN-γ in STS.

IL-33 and ST2 Are Positively Correlated With the Expression of Chemokines That Recruit $CD8^+$ T Cells in STS

It has been reported that the chemokine CCL5 and its receptor CCR5, as well as CXCL9, CXCL10, CXCL11, and their receptor CXCR3, are involved in recruiting $CD8^+$ T cells in the tumor microenvironment (21–23). Here, correlation analyses of TCGA data showed that the expression levels of CCL5 and CCR5, as well as CXCL9, CXCL10, CXCL11, and CXCR3, had strong correlations with those of CD3E and CD8A (**Figure 4A**). The correlations were then validated by analysis using GSE2719 and GSE6481 datasets of sarcoma (**Figure 4A**). Moreover, the expression levels of both ST2 and IL-33 were found to be positively correlated with those of CCL5, CCR5, CXCL9, CXCL10, CXCL11, and CXCR3 (**Figure 4B**). These results suggest that the IL-33/ST2 axis may recruit $CD8^+$ T cells into cancer lesions by promoting the release of multiple chemokines in the tumor microenvironment of STS.

IL-33 Is Negatively Correlated With the Expression of TGF-β1 and Chemokines That Recruit Tregs and MDSCs in STS

To determine whether the IL-33/ST2 axis is also involved in the regulation of immunosuppressive cells and cytokines, we performed a series of correlation analyses. It has been reported that the chemokine CCL20 and its receptor CCR6 recruit Tregs into the inflammation site (24, 25), while CXCL5 and its receptor CXCR2 recruit MDSCs and promote TGF-β1 secretion (26, 27). Here, by analyzing TCGA data, we found that the expression levels of CCL20 and CCR6 were positively correlated with those of CD4 and FOXP3 (**Figure 5A**), while CXCL5 and CXCR2 levels were positively correlated with those of CD11B and CD33 in STS (**Figure 5B**). As shown in **Figure 2A**, TGF-β1 is highly expressed in STS. We found that there was a weak positive correlation between the expression of TGFB1 and FOXP3 but obvious positive correlations between the expression of TGFB1 and CD11B and CD33 (**Figure 5C**), indicating that CD11B/CD33-expressing MDSCs may be the main cells, which secrete TGF-β1. Furthermore, positive correlations were observed between the expression of TGFB1 and CCL20, CXCL5, and CXCR2 (**Figure 5D**). At the same time, we found that IL-33 was negatively correlated with the expression of the chemokines CCL20 and CXCL5, as well as with TGF-β1 (**Figures 5E,F**). The negative correlations between the expression of IL-33 and CCL20 and TGFB1 were then validated by analysis using GSE6481 or GSE967 datasets of sarcoma (**Figures 5E,F**). These results suggest that the IL-33/ST2 axis may also inhibit the production of TGF-β1 and reduce the infiltration of Tregs and MDSCs by inhibiting the expression of chemokines such as CCL20 and CXCL5 in the tumor microenvironment of STS, thus contribute to antagonizing Tregs, MDSCs, and TGF-β1-mediated immunosuppression.

DISCUSSION

Cancer immunotherapy opens up new avenues of treatment for many types of cancers, including STS. At present, a number of

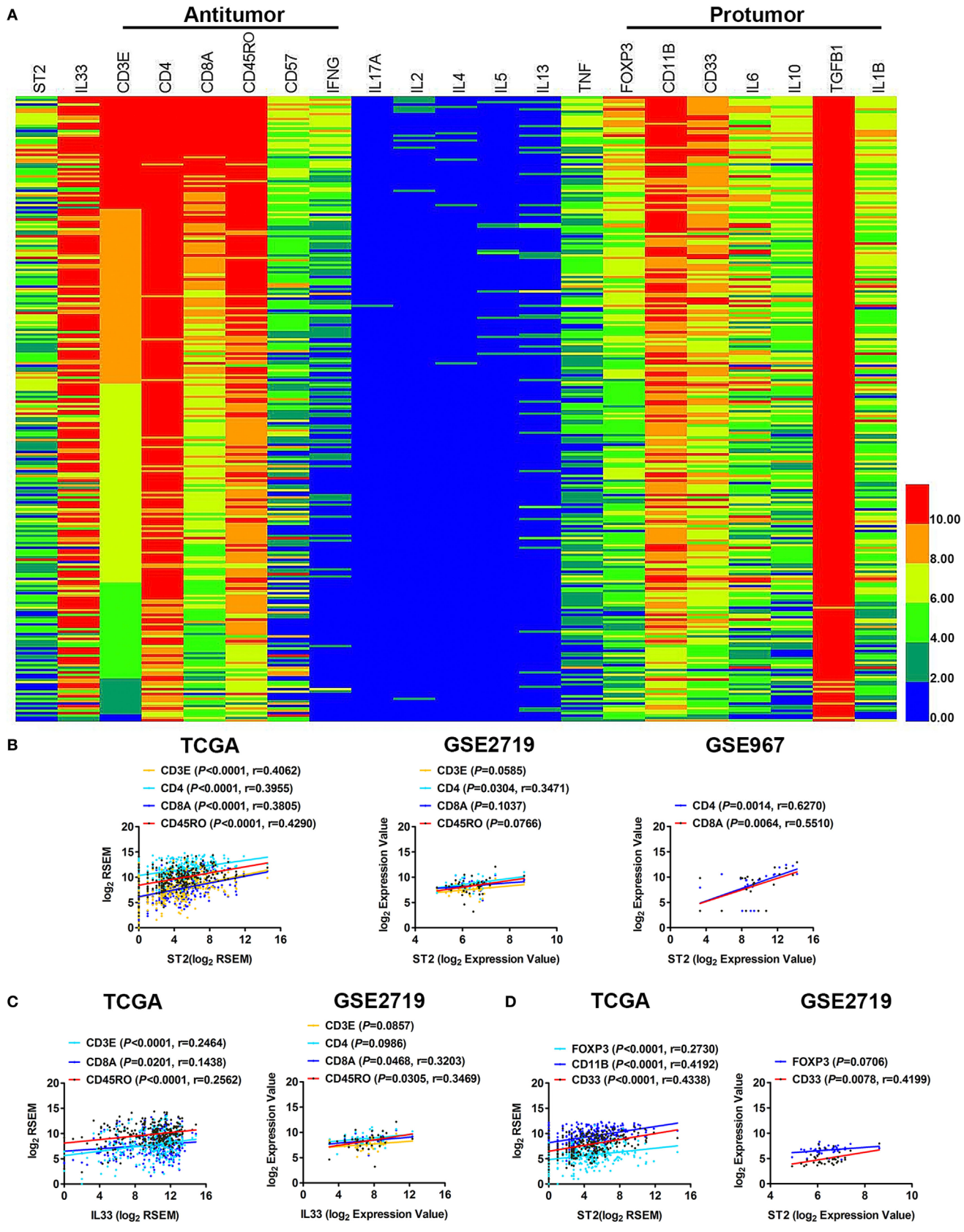


FIGURE 2 | Continued

FIGURE 2 | Correlation between the expression of ST2 and different immune cell subpopulation-specific genes in soft tissue sarcoma (STS). **(A)** The transcriptional expression of antitumor and pro-tumor immune components in the tumor microenvironment of STS. The transcriptome sequencing data (sample number = 261) were obtained from the publicly accessible The Cancer Genome Atlas (TCGA) portal, and the heat map was generated with novel HemI software (Heatmap Illustrator, version 1.0). CD3E and CD4 were used to label T cells; CD8A labeled cytotoxic T lymphocytes; CD45RO labeled memory T cells; CD57 labeled activated T cells and NK cells; FOXP3 labeled Tregs; and CD11B and CD33 labeled myeloid-derived suppressor cells. These data are in descending order according to the level of CD3E expression. Highly expressed samples are in red, and samples with lower expression are in blue. **(B)** Positive correlations between the transcriptional levels of ST2 and CD3E, CD4, CD8A, and CD45RO by analyzing sarcoma data of TCGA, GSE2719 (sample number = 39) and GSE967 (sample number = 23). **(C)** Positive correlations between the transcriptional levels of IL-33 and CD3E, CD8A, and CD45RO. **(D)** Positive correlations between the transcriptional levels of ST2 and FOXP3, CD11B, and CD33. The correlation between gene expression levels was analyzed by Pearson correlation test.

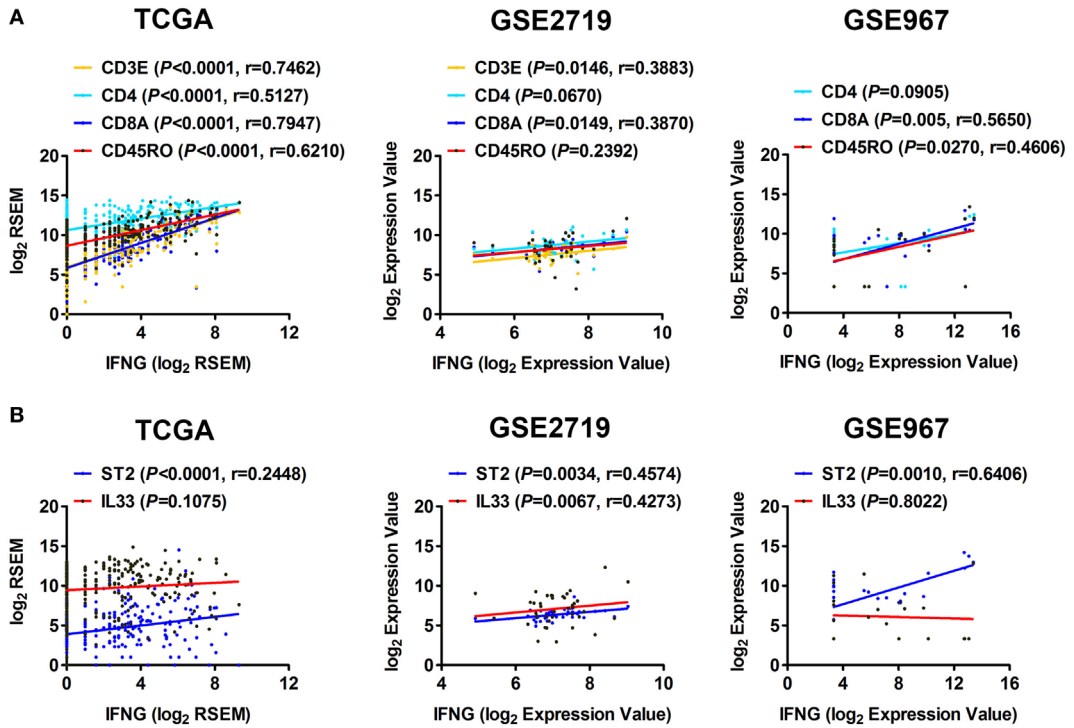


FIGURE 3 | Correlation between the expression of ST2 and IFN-γ in soft tissue sarcoma. **(A)** Positive correlations between the transcriptional levels of IFN-γ and CD3E, CD4, CD8A, and CD45RO by analyzing sarcoma data of The Cancer Genome Atlas, GSE2719, and GSE967. **(B)** Positive correlations between the transcriptional levels of IFN-γ and ST2, IL-33. The correlation between gene expression levels was analyzed by Pearson correlation test.

immunotherapy clinical trials for STS are ongoing, including treatment with anti-PD-1 antibody (28) and adoptive transfer of T cells targeting NY-ESO-1 antigen (29). However, despite some achievements in cancer immunotherapy, patients with STS have a low response rate. In a Phase I clinical trial using a SYT-SSX peptide vaccine in patients with synovial sarcoma, peptide-specific CD8 cytotoxic T lymphocytes were successfully induced in 4 of 6 patients, but suppression of tumor progression only occurred in one patient (30). In another small clinical trial investigating the clinical activity of the anti-CTLA4 antibody Ipilimumab in patients with synovial sarcoma expressing NY-ESO-1 antigens, only 1 of the 6 patients showed remission (31).

The major obstacle to immunotherapy is immunosuppression in the tumor microenvironment. Immunosuppression includes the accumulation of Tregs and MDSCs as well as the production of various immunosuppressive cytokines in the tumor microenvironment (32). The type I immune responses mediated by Th1, $CD8^+$ T, NK, NKT, and γδ T cells that produce IFN-γ are considered to be the basis of antitumor immune responses (33). However, immunosuppression in the tumor microenvironment results in these immune cells being in a dysfunctional immune state, which eventually leads to the immune escape of tumor cells (32, 34). Therefore, reversing immunosuppression in the tumor microenvironment is a crucial step toward the success of cancer immunotherapy.

Cytokines have been shown to activate immune responses and have antitumor effects. A variety of cytokines have been approved as drugs for the treatment of cancer patients, including IL-2, IFN-γ, and GM-CSF (35, 36). Recently, studies have shown that IL-33 also has antitumor activity in some cancers, such as melanoma and breast cancer (15–17). IL-33 could be released by damaged or dead tumor cells and act on immune cells that

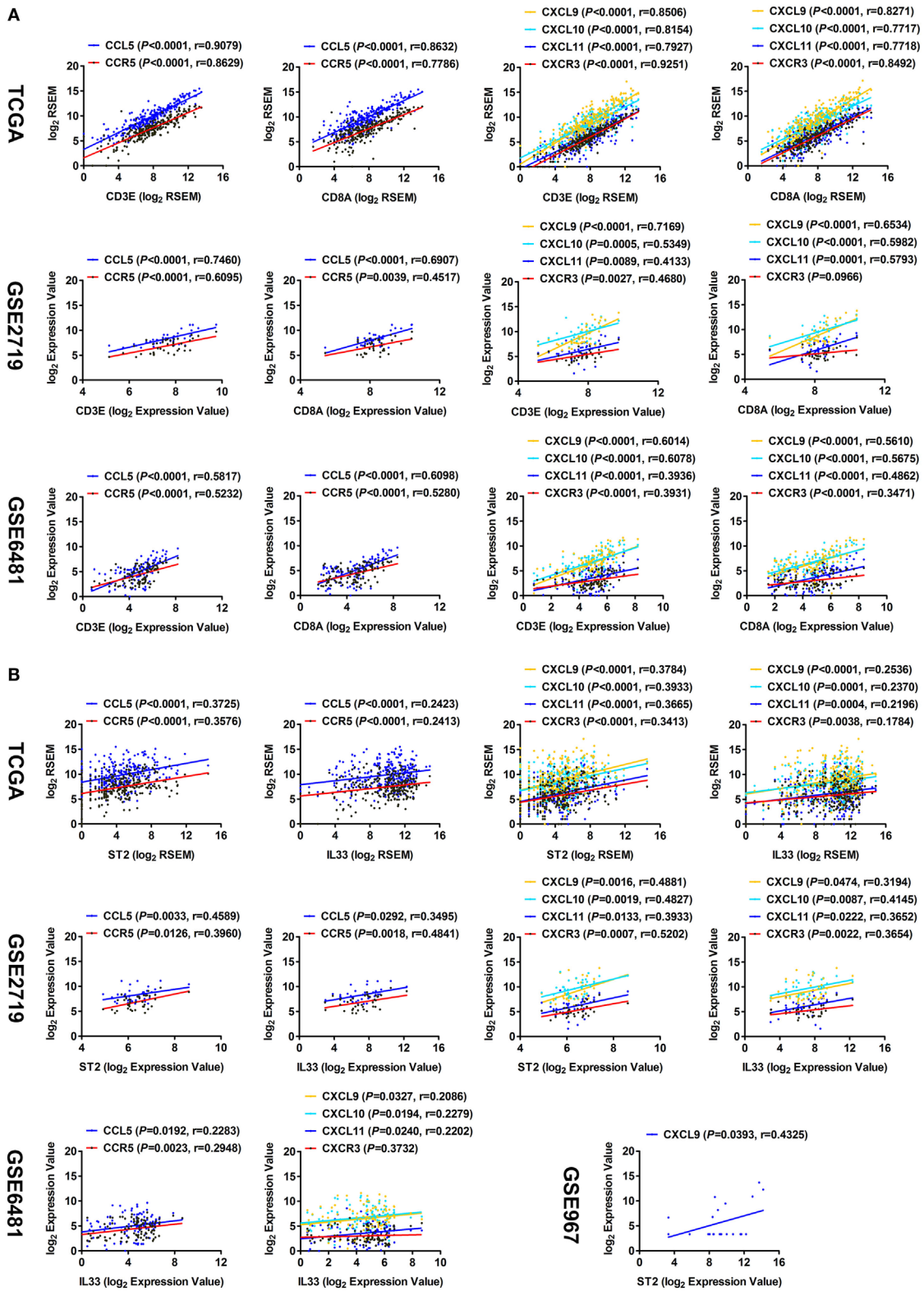


FIGURE 4 | Correlation between the expression of ST2/IL-33 and chemokines that recruit CD8$^+$ T cells in soft tissue sarcoma. **(A)** Positive correlations between the transcriptional levels of CD3E/CD8A and CCL5 and its receptor CCR5, and between CD3E/CD8A and CXCL9/10/11 and their receptor CXCR3 by analyzing sarcoma data of The Cancer Genome Atlas (TCGA), GSE2719, and GSE6481 (sample number = 105). **(B)** Positive correlations between the transcriptional levels of ST2/IL-33 and CCL5 and its receptor CCR5, and between ST2/IL-33 and CXCL9/10/11 and their receptor CXCR3 by analyzing sarcoma data of TCGA, GSE2719, GSE6481, and GSE967. The correlation between gene expression levels was analyzed by Pearson correlation test.

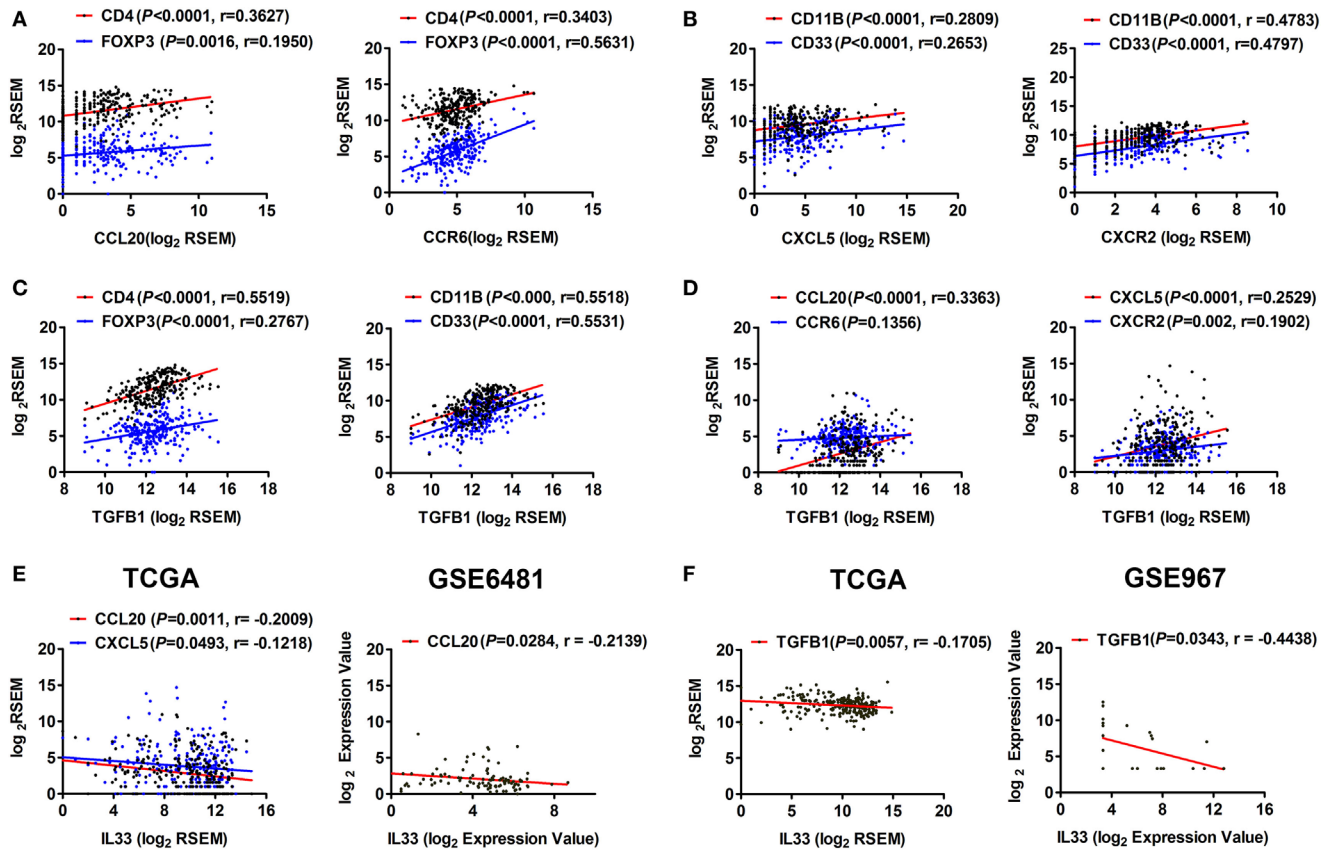


FIGURE 5 | Correlation between the expression of IL-33 and TGF-β1, chemokines that recruit Tregs and myeloid-derived suppressor cells in soft tissue sarcoma. **(A)** Positive correlations between the transcriptional levels of CCL20 and its receptor CCR6 and CD4/FOXP3. **(B)** Positive correlations between the transcriptional levels of CXCL5 and its receptor CXCR2 and CD11B/CD33. **(C)** Positive correlations between the transcriptional levels of TGFB1 and CD4/FOXP3 and CD11B/CD33. **(D)** Positive correlations between the transcriptional levels of TGFB1 and CCL20, CXCL5, and CXCR2. Above analyses were performed using data from The Cancer Genome Atlas (TCGA). **(E)** Negative correlations between the transcriptional levels of IL-33 and the chemokines CCL20 and CXCL5 by analyzing sarcoma data of TCGA and GSE6481. **(F)** Negative correlation between the transcriptional levels of IL-33 and TGFB1 by analyzing sarcoma data of TCGA and GSE967. The correlation between gene expression levels was analyzed by Pearson correlation test.

express ST2 in a paracrine or autocrine manner (37). In this study, we found that the expression of IL-33 and ST2 were decreased in STS. Furthermore, we found that both IL-33 and ST2 expression were associated with the prognosis of sarcomas; patients with higher transcriptional levels of IL-33 and ST2 have a more favorable prognosis. These results indicate that IL-33 may be released from sarcoma cells and regulate the antitumor immunity during the progression of STS.

It has been reported that the antitumor effect of IL-33 is mainly achieved by promoting the production of IFN-γ by NK and $CD8^+$ T cells (15–17). In addition, IL-33 can also promote NK and NKT cells to produce IFN-γ (13, 38). IL-33 is also able to recruit large amounts of type 2 innate lymphoid cells to the tumor lesions and inhibit tumor growth (39). Thus, IL-33 is a potent pleiotropic cytokine that reverses immunosuppression in the tumor microenvironment. Here, we found that the expression level of IFN-γ was positively correlated with those of CD4 and CD45RO, but strong positive correlations were observed with the levels of CD3E and CD8A, indicating that CD3E/CD8A-labeled T cells may be the major cells that secrete IFN-γ in STS. Additionally, there was a positive correlation between the expression of ST2 and IFN-γ. Combined with the results indicating that the expression levels of both ST2 and IL-33 were positively correlated with those of CD3E, CD8A, and CD45RO, it is easy to deduce that the IL-33/ST2 axis may promote T cells, especially $CD8^+$ T cells, to produce IFN-γ in STS. Furthermore, both the expression of ST2 and IL-33 were found to be positively correlated with that of CCL5 and its receptor CCR5, as well as with the expression of CXCL9, CXCL10, CXCL11, and their receptor CXCR3. All of these chemokines have been reported to recruit $CD8^+$ T cells to the site of inflammation and participate in antitumor immune responses (21–23). Taken together, these results suggest that the IL-33/ST2 axis may enhance the antitumor immunity by promoting the recruitment recruitment of type 1-polarized CD8+ T cells into tumor lesions in STS.

However, it has also been reported that IL-33 can promote the accumulation of $ST2^+$ Tregs in tumor lesions and exhibits an immunosuppressive effect (16); IL-33 can also promote MDSCs to

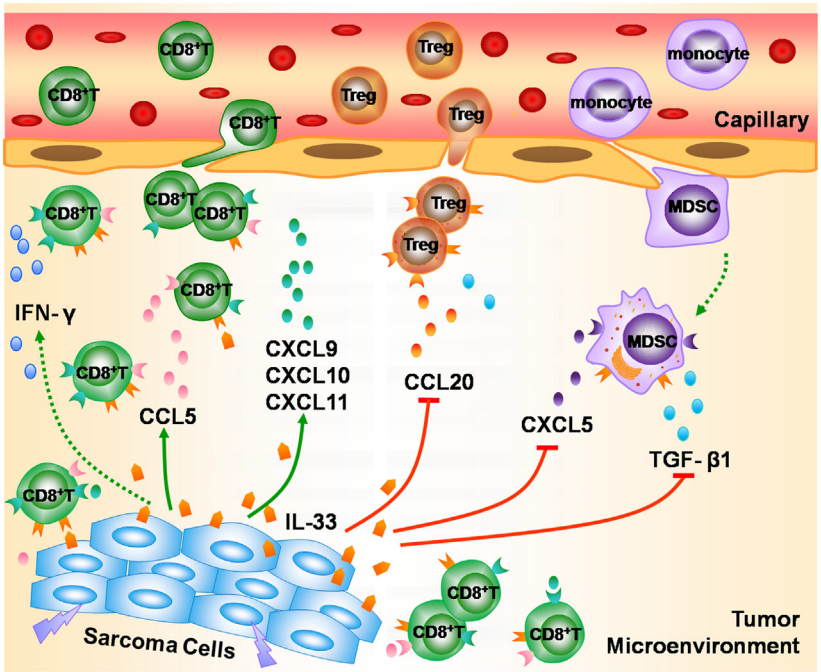


FIGURE 6 | The possible role of the IL-33/ST2 axis in modulating antitumor immunity in soft tissue sarcoma (STS). During the development of STS, IL-33 is released from damaged or dead sarcoma cells into the tumor microenvironment, and enhances the recruitment of $CD8^+$ T cells into tumor lesion by promoting the release of multiple chemokines such as CCL5, CXCL9, CXCL10, and CXCL11, and promotes $CD8^+$ T cells to produce IFN-γ, thereby playing an important role in the modulation of antitumor immunity in STS. Meanwhile, IL-33/ST2 axis reduces the infiltration of Tregs and myeloid-derived suppressor cells *via* inhibiting the production of chemokines CCL20 and CXCL5, and suppresses the secretion of TGF-β1, and thus contributes to antagonizing the immunosuppression in STS.

accumulate in the tumor and secrete a large amount of immunosuppressive cytokines, such as TGF-β1, resulting in tumor metastasis (40). In this study, we found that there were positive correlations between the expression of ST2 and FOXP3, CD11B, and CD33, indicating that the IL-33/ST2 axis may also regulate the function of Tregs and MDSCs in the tumor microenvironment of STS. TGF-β1 is highly expressed in STS; it is likely that TGF-β1 is the major immunosuppressive cytokine in the STS tumor microenvironment. We also found that there were obvious positive correlations between the expression levels of TGFB1 and CD11B and CD33, indicating that CD11B/CD33-labeled MDSCs may be the main cells that secrete TGF-β1 in STS. It has been reported that the CCL20-CCR6 axis recruits Tregs (24, 25) and the CXCL5-CXCR2 axis can recruit MDSCs (26, 27) into the inflammation site. Here, we found that the expression levels of CCL20 and CCR6 were positively correlated with those of FOXP3, while CXCL5 and CXCR2 were positively correlated with CD11B and CD33. Furthermore, positive correlations were observed between the expression levels of TGFB1 and CCL20 and CXCL5. The above results indicate that TGF-β1 may play a major immunosuppressive role in STS. However, we found that IL-33 was negatively correlated with the expression of the chemokines CCL20 and CXCL5, as well as with TGF-β1, in STS, suggesting that the IL-33/ST2 axis may reverse immunosuppression mainly by reducing the infiltration of Tregs and MDSCs and inhibiting the production of TGF-β1 in the STS tumor microenvironment. The regulatory effect of IL-33 on TGF-β1 is controversial. Some studies have shown that IL-33 induces the production of TGF-β by eosinophils and M2 macrophages (41, 42), and some studies have reported that treatment with anti-IL-33 antibody or sST2 in allergic asthma did not change the level of TGF-β1 (43). Whether IL-33 inhibits TGF-β1 production in the STS microenvironment and the mechanism involved remain to be confirmed.

In summary, we found that the mRNA expression of IL-33 and ST2 was decreased in STS. TCGA data analysis indicated that higher transcriptional levels of IL-33 and ST2 in STS were associated with a favorable outcome. Integrated analysis of TCGA and GEO sarcoma datasets implicated the possible role of the IL-33/ST2 axis in STS. IL-33/ST2 may play an important role in the modulation of antitumor immunity in STS by promoting the recruitment of $CD8^+$ T cells and enhancing IFN-γ production, as well as by antagonizing Tregs, MDSCs, and TGF-β1-mediated immunosuppression (**Figure 6**). Our study suggests that IL-33 may not only serve as a useful prognostic biomarker for STS but also as a potential immunotherapeutic target for STS. However, further *in vivo* and *in vitro* experiments are required to validate the possible antitumor effect of the IL-33/ST2 axis on STS.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of ICH-QCP guidelines, Institutional Research Medical Ethics Committee of Sun Yat-sen University Cancer Center. The protocal was approved by the Institutional Research Medical

Ethics Committee of Sun Yat-sen University Cancer Center. The written informed consent was obtained from patients for the use of their clinical tissues in this study.

AUTHOR CONTRIBUTIONS

Initiation and study design: FZ; clinical samples contribution: HC, YQ, and XZ; performed experiments: HC; statistical analyses: HC, HL, and YC; supervision of research: FZ; writing of the first draft of the manuscript: HC and YC. All authors contributed to the writing and editing of the current manuscript and approved the final manuscript.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 31470852, Grant No. 31670876 and Grant No. 81772863) and the Basic Professional Ability Improvement Project for Young and Middle-aged Teachers of Colleges and Universities in Guangxi (Grant No.2018KY0411).

REFERENCES

1. Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CD, Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978–2001: an analysis of 26,758 cases. *Int J Cancer* (2006) 119(12):2922–30. doi:10.1002/ijc.22239
2. Vincenzi B, Frezza AM, Santini D, Tonini G. New therapies in soft tissue sarcoma. *Expert Opin Emerg Drugs* (2010) 15(2):237–48. doi:10.1517/14728211003592108
3. Callahan MK, Kluger H, Postow MA, Segal NH, Lesokhin A, Atkins MB, et al. Nivolumab plus ipilimumab in patients with advanced melanoma: updated survival, response, and safety data in a phase I dose-escalation study. *J Clin Oncol* (2017):JCO2017722850. doi:10.1200/JCO.2017.72.2850
4. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2017) 377(14):1345–56. doi:10.1056/NEJMoa1709684
5. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* (2012) 366(26):2455–65. doi:10.1056/NEJMoa1200694
6. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, 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. doi:10.1016/j.immuni.2005.09.015
7. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol* (2016) 16(11):676–89. doi:10.1038/nri.2016.95
8. Mirchandani AS, Salmond RJ, Liew FY. Interleukin-33 and the function of innate lymphoid cells. *Trends Immunol* (2012) 33(8):389–96. doi:10.1016/j.it.2012.04.005
9. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity* (2015) 42(6):1005–19. doi:10.1016/j.immuni.2015.06.006
10. Lingel A, Weiss TM, Niebuhr M, Pan B, Appleton BA, Wiesmann C, 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):1398–410. doi:10.1016/j.str.2009.08.009
11. Matta BM, Lott JM, Mathews LR, Liu Q, Rosborough BR, Blazar BR, 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):4010–20. doi:10.4049/jimmunol.1400481
12. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* (2010) 10(2):103–10. doi:10.1038/nri2692
13. Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int Immunol* (2008) 20(8):1019–30. doi:10.1093/intimm/dxn060
14. Jiang HR, Milovanovic M, Allan D, Niedbala W, Besnard AG, Fukada SY, 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):1804–14. doi:10.1002/eji.201141947
15. Gao K, Li X, Zhang L, Bai L, Dong W, Shi G, et al. Transgenic expression of IL-33 activates CD8(+) T cells and NK cells and inhibits tumor growth and metastasis in mice. *Cancer Lett* (2013) 335(2):463–71. doi:10.1016/j.canlet.2013.03.002
16. Gao X, Wang X, Yang Q, Zhao X, Wen W, Li G, et al. Tumoral expression of IL-33 inhibits tumor growth and modifies the tumor microenvironment through CD8+ T and NK cells. *J Immunol* (2015) 194(1):438–45. doi:10.4049/jimmunol.1401344
17. Lucarini V, Zicchedda G, Macchia I, La Sorsa V, Peschiaroli F, Buccione C, et al. IL-33 restricts tumor growth and inhibits pulmonary metastasis in melanoma-bearing mice through eosinophils. *Oncoimmunology* (2017) 6(6):e1317420. doi:10.1080/2162402X.2017.1317420
18. Villarreal DO, Wise MC, Walters JN, Reuschel EL, Choi MJ, Obeng-Adjei N, et al. Alarmin IL-33 acts as an immunoadjuvant to enhance antigen-specific tumor immunity. *Cancer Res* (2014) 74(6):1789–800. doi:10.1158/0008-5472.CAN-13-2729
19. Deng W, Wang Y, Liu Z, Cheng H, Xue Y. HemI: a toolkit for illustrating heatmaps. *PLoS One* (2014) 9(11):e111988. doi:10.1371/journal.pone.0111988
20. Peine M, Marek RM, Lohning M. IL-33 in T cell differentiation, function, and immune homeostasis. *Trends Immunol* (2016) 37(5):321–33. doi:10.1016/j.it.2016.03.007
21. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* (2013) 14(10):1014–22. doi:10.1038/ni.2703
22. Liu J, Li F, Ping Y, Wang L, Chen X, Wang D, et al. Local production of the chemokines CCL5 and CXCL10 attracts CD8+ T lymphocytes into esophageal squamous cell carcinoma. *Oncotarget* (2015) 6(28):24978–89. doi:10.18632/oncotarget.4617
23. Domingos-Pereira S, Hojeij R, Reggi E, Derre L, Chevalier MF, Romero P, et al. Local *Salmonella* immunostimulation recruits vaccine-specific CD8 T cells and increases regression of bladder tumor. *Oncoimmunology* (2015) 4(7):e1016697. doi:10.1080/2162402X.2015.1016697
24. Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, et al. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol* (2008) 181(12):8391–401. doi:10.4049/jimmunol.181.12.8391
25. Cook KW, Letley DP, Ingram RJ, Staples E, Skjoldmose H, Atherton JC, et al. CCL20/CCR6-mediated migration of regulatory T cells to the *Helicobacter pylori*-infected human gastric mucosa. *Gut* (2014) 63(10):1550–9. doi:10.1136/gutjnl-2013-306253
26. Toh B, Wang X, Keeble J, Sim WJ, Khoo K, Wong WC, et al. Mesenchymal transition and dissemination of cancer cells is driven by myeloid-derived suppressor cells infiltrating the primary tumor. *PLoS Biol* (2011) 9(9):e1001162. doi:10.1371/journal.pbio.1001162
27. Wang G, Lu X, Dey P, Deng P, Wu CC, Jiang S, et al. Targeting YAP-dependent MDSC infiltration impairs tumor progression. *Cancer Discov* (2016) 6(1):80–95. doi:10.1158/2159-8290.CD-15-0224
28. ClinicalTrials. *SARC028: A Phase II Study of the Anti-PD1 Antibody Pembrolizumab (MK-3475) in Patients With Advanced Sarcomas*. Bethesda: U.S. National Library of Medicine (2014). Available from: https://clinicaltrials.gov/ct2/show/NCT02301039?term=PD1 (Accessed: November 17, 2017).
29. ClinicalTrials. *Study of TBI-1301 (NY-ESO-1 T Cell Receptor Gene Transduced Autologous T Lymphocytes) in Patients With Synovial Sarcoma*. Bethesda: U.S. National Library of Medicine (2017). Available from: https://clinicaltrials.gov/ct2/show/NCT03250325?term=NY-ESO-1 (Accessed: November 17, 2017).
30. Kawaguchi S, Wada T, Ida K, Sato Y, Nagoya S, Tsukahara T, et al. Phase I vaccination trial of SYT-SSX junction peptide in patients with disseminated synovial sarcoma. *J Transl Med* (2005) 3(1):1. doi:10.1186/1479-5876-3-31

31. Maki RG, Jungbluth AA, Gnjatic S, Schwartz GK, D'Adamo DR, Keohan ML, et al. A pilot study of anti-CTLA4 antibody ipilimumab in patients with synovial sarcoma. *Sarcoma* (2013) 2013:168145. doi:10.1155/2013/168145
32. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* (2005) 5(4):263–74. doi:10.1038/nrc1586
33. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* (2006) 313(5795):1960–4. doi:10.1126/science.1129139
34. Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol* (2006) 6(4):295–307. doi:10.1038/nri1806
35. Lee S, Margolin K. Cytokines in cancer immunotherapy. *Cancers (Basel)* (2011) 3(4):3856–93. doi:10.3390/cancers3043856
36. Young PA, Morrison SL, Timmerman JM. Antibody-cytokine fusion proteins for treatment of cancer: engineering cytokines for improved efficacy and safety. *Semin Oncol* (2014) 41(5):623–36. doi:10.1053/j.seminoncol.2014.08.002
37. Chen H, Sun Y, Lai L, Wu H, Xiao Y, Ming B, et al. Interleukin-33 is released in spinal cord and suppresses experimental autoimmune encephalomyelitis in mice. *Neuroscience* (2015) 308:157–68. doi:10.1016/j.neuroscience.2015.09.019
38. Bourgeois E, Van LP, Samson M, Diem S, Barra A, Roga S, et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol* (2009) 39(4):1046–55. doi:10.1002/eji.200838575
39. Kim J, Kim W, Moon UJ, Kim HJ, Choi HJ, Sin JI, et al. Intratumorally establishing type 2 innate lymphoid cells blocks tumor growth. *J Immunol* (2016) 196(5):2410–23. doi:10.4049/jimmunol.1501730
40. Jovanovic IP, Pejnovic NN, Radosavljevic GD, Pantic JM, Milovanovic MZ, Arsenijevic NN, et al. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. *Int J Cancer* (2014) 134(7):1669–82. doi:10.1002/ijc.28481
41. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. *J Immunol* (2010) 185(6):3472–80. doi:10.4049/jimmunol.1000730
42. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, et al. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J Allergy Clin Immunol* (2014) 134(6):1422–32.e11. doi:10.1016/j.jaci.2014.05.011
43. Lee HY, Rhee CK, Kang JY, Byun JH, Choi JY, Kim SJ, et al. Blockade of IL-33/ST2 ameliorates airway inflammation in a murine model of allergic asthma. *Exp Lung Res* (2014) 40(2):66–76. doi:10.3109/01902148.2013.870261

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Chen, Chen, Liu, Que, Zhang and Zheng. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 03 August 2018
doi: 10.3389/fimmu.2018.01775

Genetic Regulation of the Thymic Stromal Lymphopoietin (TSLP)/TSLP Receptor (TSLPR) Gene Expression and Influence of Epistatic Interactions Between IL-33 and the TSLP/TSLPR Axis on Risk of Coronary Artery Disease

Shao-Fang Nie[1,2†], Ling-Feng Zha[1,2,3†], Qian Fan[1,2,4†], Yu-Hua Liao[1,2], Hong-Song Zhang[1,2], Qian-Wen Chen[1,2], Fan Wang[5], Ting-Ting Tang[1,2], Ni Xia[1,2], Cheng-Qi Xu[6], Jiao-Yue Zhang[7], Yu-Zhi Lu[1,2], Zhi-Peng Zeng[1,2], Jiao Jiao[1,2], Yuan-Yuan Li[1,2], Tian Xie[1,2], Wen-Juan Zhang[8], Dan Wang[6], Chu-Chu Wang[6], Jing-Jing Fa[6], Hong-Bo Xiong[6], Jian Ye[6], Qing Yang[6], Peng-Yun Wang[6], Sheng-Hua Tian[7], Qiu-Lun Lv[9], Qing-Xian Li[10], Jin Qian[11], Bin Li[12], Gang Wu[13], Yan-Xia Wu[14], Yan Yang[1,2], Xiang-Ping Yang[15], Yu Hu[16], Qing K. Wang[6], Xiang Cheng[1,2] and Xin Tu[6]**

[1] Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Key Laboratory for Biological Targeted Therapy of Education Ministry and Hubei Province, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3] Innovation Institute, Huazhong University of Science and Technology, Wuhan, China, [4] Department of Cardiology, Zhongnan Hospital of Wuhan University, Wuhan, China, [5] Department of Molecular Cardiology, Cleveland Clinic Lerner Research Institute, Cleveland, OH, United States, [6] Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Center for Human Genome Research, Cardio-X Institute, Huazhong University of Science and Technology, Wuhan, China, [7] Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [8] Department of Geriatrics, the Central Hospital of Wuhan, Tongji Medica College, Huazhong University of Science and Technology, Wuhan, China, [9] Section of Molecule Medicine, Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, [10] Jining Medical College Affiliated Hospital, Jining, China, [11] Suizhou Central Hospital, Suizhou, China, [12] Xiangyang Central Hospital, Xiangyang, China, [13] Renmin Hospital of Wuhan University, Wuhan, China, [14] Wuhan No. 1 Hospital, Wuhan, China, [15] School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [16] Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

The thymic stromal lymphopoietin (TSLP)/TSLP receptor (TSLPR) axis is involved in multiple inflammatory immune diseases, including coronary artery disease (CAD). To explore the causal relationship between this axis and CAD, we performed a three-stage case-control association analysis with 3,628 CAD cases and 3,776 controls using common variants in the genes *TSLP*, *interleukin 7 receptor* (*IL7R*), and *TSLPR*. Three common variants in the TSLP/TSLPR axis were significantly associated with CAD in a Chinese Han population [rs3806933^{T} in *TSLP*, $P_{adj} = 4.35 \times 10^{-5}$, odds ratio (OR) = 1.18; rs6897932^{T} in *IL7R*, $P_{adj} = 1.13 \times 10^{-7}$, OR = 1.31; g.19646A>G^{A} in *TSLPR*, $P_{adj} = 2.04 \times 10^{-6}$, OR = 1.20]. Reporter gene analysis demonstrated that rs3806933 and rs6897932 could influence *TSLP* and *IL7R* expression, respectively. Furthermore, the "T" allele of rs3806933 might increase plasma TSLP levels ($R^2 = 0.175$, $P < 0.01$). In a stepwise procedure, the risk for

OPEN ACCESS

Edited by:
Jose Carlos Alves-Filho, Universidade de São Paulo, Brazil

Reviewed by:
Marinos Kallikourdis, Humanitas Università, Italy
Maria Rosaria Coscia, Istituto di biochimica delle proteine (IBP), Italy
Gilberto Vargas Alarcón, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico

***Correspondence:**
Xiang Cheng
nathancx@mail.hust.edu.cn;
Xin Tu
xtu@hust.edu.cn

[†]These authors have contributed equally to this work.

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *21 March 2018*
Accepted: *18 July 2018*
Published: *03 August 2018*

Citation:
Nie S-F, Zha L-F, Fan Q, Liao Y-H, Zhang H-S, Chen Q-W, Wang F, Tang T-T, Xia N, Xu C-Q, Zhang J-Y, Lu Y-Z, Zeng Z-P, Jiao J, Li Y-Y, Xie T, Zhang W-J, Wang D, Wang C-C, Fa J-J, Xiong H-B, Ye J, Yang Q, Wang P-Y, Tian S-H, Lv Q-L, Li Q-X, Qian J, Li B, Wu G, Wu Y-X, Yang Y, Yang X-P, Hu Y, Wang QK, Cheng X and Tu X (2018) Genetic Regulation of the Thymic Stromal Lymphopoietin (TSLP)/TSLP Receptor (TSLPR) Gene Expression and Influence of Epistatic Interactions Between IL-33 and the TSLP/TSLPR Axis on Risk of Coronary Artery Disease. Front. Immunol. 9:1775. doi: 10.3389/fimmu.2018.01775

CAD increased by nearly fivefold compared with the maximum effect of any single variant ($P_{adj} = 6.99 \times 10^{-4}$, OR = 4.85). In addition, the epistatic interaction between *TSLP* and *IL33* produced a nearly threefold increase in the risk of CAD in the combined model of rs3806933^{TT}-rs7025417^{TT} ($P_{adj} = 3.67 \times 10^{-4}$, OR = 2.98). Our study illustrates that the TSLP/TSLPR axis might be involved in the pathogenesis of CAD through upregulation of mRNA or protein expression of the referenced genes and might have additive effects on the CAD risk when combined with IL-33 signaling.

Keywords: thymic stromal lymphopoietin/TSLP receptor, IL-33, epistatic, coronary artery disease, genetic regulation

INTRODUCTION

Genetic factors have been demonstrated to be equally important as environmental factors in the pathogenesis of coronary artery disease (CAD). Based on family and twin studies, researchers have estimated that the heritability of CAD is between 40 and 60% (1). Much progress has been made in this area. Currently, about 95 common risk variants of CAD have been detected by genome-wide association studies (GWASs), and a total of 202 independent signals in 109 risk loci have been discovered using genome-wide complex trait analysis software, which together explain more than 28% of the estimated heritability of CAD (2–5). The remaining unexplained heritability may be due to many reasons, including that the diverse characteristics of the commercial chips used in GWASs, the problem of genome-wide statistical significance level, epistatic interactions (epistasis), epigenetic influences, etc. (6, 7). Therefore, identification of causal genes or SNPs for complex diseases such as CAD by functional studies has become a hotspot for research worldwide.

The thymic stromal lymphopoietin (TSLP)/TSLP receptor (TSLPR) axis plays an important role in the regulation of a broad spectrum of inflammatory immune response-related diseases, including asthma and CAD. The key proteins of this axis are as follows: TSLP, a four-helix bundle cytokine targeting a variety of inflammatory immune cells, such as dendritic cells (DCs), B cells, mast cells, regulatory T cells, and $CD4^+$ and $CD8^+$ T cells (8–12); and the TSLPR complex, which consists of the IL-7 receptor alpha (IL-7Rα) and the unique TSLPR subunit (TSLPR; also known as CRLF2) (13–15). Under the stimulation of oxidized low-density lipoprotein (ox-LDL), human umbilical vein endothelial cells and vascular smooth muscle cells release large amounts of TSLP, which might activate DCs and then accelerate the development of atherosclerosis (16). TSLPR may also be expressed on human platelets and plays a role in the activation of platelets in acute coronary syndrome (17). Most recently, Li et al. identified that variants in *TSLP* might be involved in the development of asthma *via* regulation of the expression of *TSLP* (18). These results indicated that it is likely that causal SNPs or genes in the TSLP/TSLPR axis for CAD also exist. However, previous reports that addressed the role of the TSLP/TSLPR axis in CAD and atherosclerosis have yielded inconsistent results. In *in vitro* experiments, Lin et al. and Zhao et al. reported that TSLP can induce Th17 cell differentiation (16, 19). However, until now, the effects of Th17 cells and IL-17 on atherosclerosis remain unclear (20). In *in vivo* experiments with $ApoE^{-/-}$ mice, Yu et al. found that the aortic root of mice treated with TSLP and TSLP-expressing DCs developed fewer atherosclerotic plaques than did control mice, suggesting a protective role for TSLP in CAD progression (21). However, more recently, Wu et al. reported that fewer arterial lesions developed in TSLPR-chain deficient ApoE-double knockout mice (ApoE-TSLPR DKO) than that did in ApoE knockout mice, indicating that the TSLP/TSLPR axis might promote the development of CAD (22).

Therefore, we performed the following studies to uncover the potential causal genes for the influence of the TSLP/TSLPR axis in the pathogenesis of CAD: (1) selecting all the tag SNPs covering the key genes of the TSLP/TSLPR axis; (2) performing a three-stage case-control genetic association study for CAD based on Chinese Han population with a large sample size; the selected tag SNPs were tested in stage 1 discovery study and then the positive associations from stage 1 discovery study were further tested in stage 2 validation study and stage 3 replication study; (3) testing correlations between the significant variants for CAD and the expression levels of their genes, by a reporter gene analysis or a circulation level study; and (4) constructing the interaction model and detecting the effect size of the causal variants in the contribution to the development of CAD.

Though common variants themselves may have very low effects on the pathogenesis of diseases, the epistasis (epistatic gene interaction) between common variants in the genes regulated by each other might have quite different functions in populations with different genetic backgrounds, thereby leading to a quite different effect size or direction on the risk of the diseases. In 2013, we reported that variants in the IL-33 signaling pathway might influence the development of CAD by regulating the expression levels of key genes of the pathway (23). The Framingham Heart Study identified that five missense variants in the *IL1RL1* gene, which encodes the receptor of IL-33, might influence the circulation level of soluble ST2 (sST2). The exact mechanism might be that the missense variants in *IL1RL1* regulate the expression of

Abbreviations: TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor; IL7R, interleukin 7 receptor; CAD, coronary artery disease; GWAS, genome-wide association studies; Tregs, regulatory T cells; DCs, dendritic cells; IL-7Rα, IL-7 receptor alpha; ApoE KO, ApoE knockout mice; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; GRS, genetic risk score.

IL33 and the interaction between IL-33 and its receptor ST2L, and then significantly increase the circulating level of sST2 (24). Other studies found that the TSLP and IL-33 signaling pathways might interact with each other in Th2 cell-mediated inflammatory responses (25–27).

These results indicated that variations in genes in the IL-33 and TSLP signaling pathways might contribute to epistatic interactions and then affect the develop of conditions such as CAD. In the present study, we investigated the potential epistatic interaction between *IL33* and *TSLP* in the pathogenesis of CAD.

MATERIALS AND METHODS

Study Populations

The total sample size was 7,404, with 3,628 CAD cases and 3,776 controls (GeneID database) (23, 28–30). The discovery cohort in stage 1 was composed of 1,345 cases and 1,156 controls (total of 2,501 subjects). The validation cohort in stage 2 was composed of 1,347 cases and 1,156 controls (total of 2,503 subjects). The replication cohort in stage 3 was composed of 936 cases and 1,464 controls (total of 2,400 subjects). The inclusion and exclusion criteria for the CAD patients and controls were described previously (23, 28–30).

This study followed the guidelines set forth by the Declaration of Helsinki and passed the review of the Ethics Committee of Tongji Medical College, and Huazhong University of Science and Technology. All study participants have signed a written informed consent form.

Genetic Analysis

DNA samples were obtained using a Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). A High-Resolution Melt system with Rotor-gene 6000 software (Corbett Life Science) was used for genotyping, which was validated by direct DNA sequencing analysis (23, 28–30). Two positive controls for each genotype were included in each run. For each SNP, a total of 48 cases and controls were randomly selected for verification of genotyping results using direct DNA sequencing analysis. Two principals were applied to select the tag SNPs (23, 28–30): (1) the threshold of r^2 or D' was more than 0.8 between the SNPs [Haploview (v.4.2), based on HapMap CHB and JPT datasets for *TSLP* and interleukin 7 receptor (*IL7R*) (v.3, release 2) or on direct sequencing data for *TSLPR*]; (2) the threshold of the minor allele frequency was greater than 0.05. Tag SNPs, such as rs3806933 and rs2289276 in *TSLP* and rs1494558, rs1494555, rs7737000, and rs6897932 in *IL7R* were selected (**Figures 1A,B**). For *TSLPR*, we detected the common variants by direct DNA sequencing of all the exons, the 500 bp of the 5′ flanking region and the intron–exon junctions of the gene in 50 CAD cases and 50 healthy controls. Among the identified 18 variants (Table S1 in Supplementary Material), 4 variants (rs150166261, rs36133495, rs36177645, and g.19646A>G) in *TSLPR* were selected for the association analysis (**Figure 1C**).

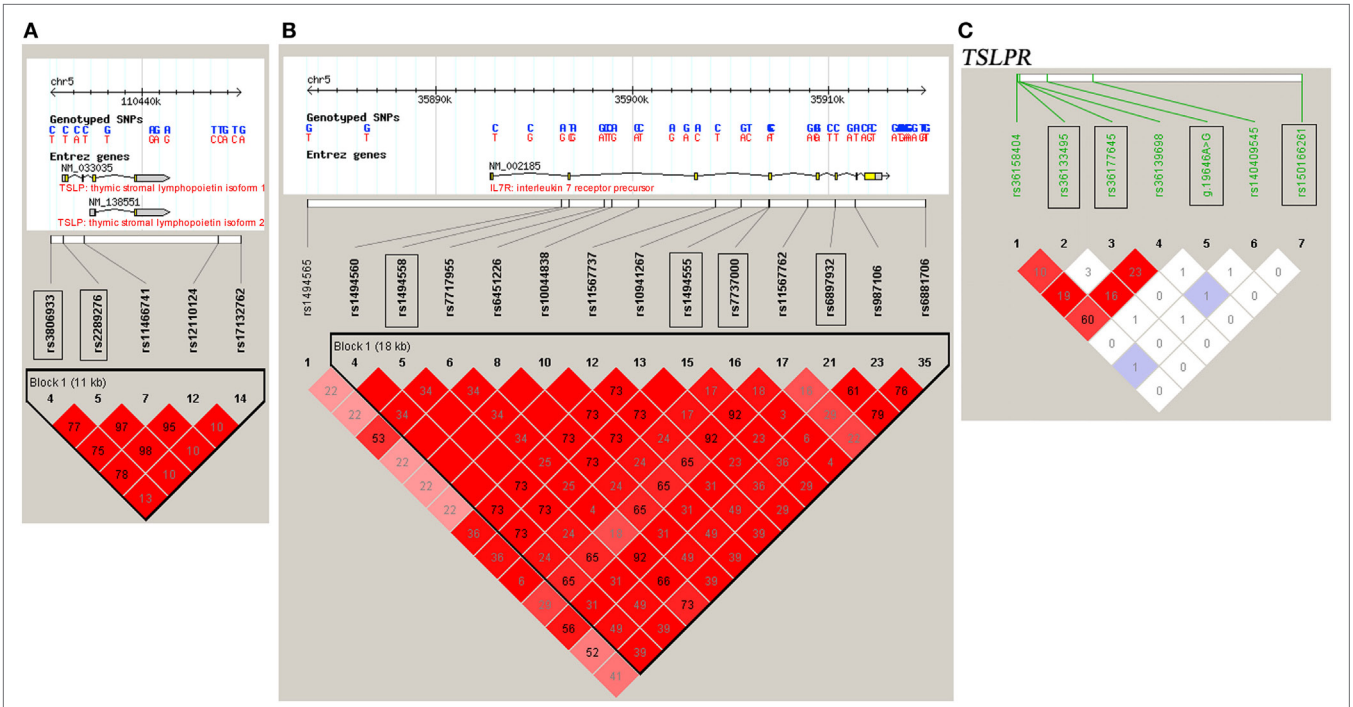


FIGURE 1 | Linkage disequilibrium (LD) blocks of thymic stromal lymphopoietin (*TSLP*) and interleukin 7 receptor (*IL7R*) and TSLP receptor (*TSLPR*). The LD blocks of **(A)** *TSLP* and **(B)** *IL7R* were constructed based on the HapMap CHB and JPT datasets, and the LD blocks of **(C)** *TSLPR* were constructed based on the data obtained by direct DNA sequencing of all the exons, the 500 bp of the 5′ flanking region and the intron–exon junctions of the gene *TSLPR* in 50 coronary artery disease cases and 50 healthy controls. The selected tag SNPs were marked with rectangular boxes. Each diamond represents the LD degree between the SNPs. The color indicates the D' (a redder color represents a higher D') and the numbers within the diamonds are the r^2 values.

Reporter Gene Assay

We performed the reporter gene assay to investigate whether the variants could regulate their gene expression. For the promoter variant rs3806933 in *TSLP*, two plasmids of TSLP-C and TSLP-T were generated with the pGL3-basic vector (23). In addition, 500 ng of each plasmid, along with 50 ng of a pRL-TK vector (Promega), were transfected into the human embryonic kidney 293 (American Type Culture Collection) cell line using Lipofectamine™ 2000 reagent (Invitrogen). Then, we measured the firefly luciferase activity using a Dual Luciferase Reporter Assay Kit (Promega) at 48 h after transfection. Three independent experiments were carried out with the empty pGL3-basic vector, and each was carried out in triplicate. For rs6897932 in *IL7R* and g.19646A>G in *TSLPR*, the reporter gene assay experiments were carried out with the pGL3-control vector (Promega) in the human HELA cell line, and three independent experiments were performed.

Plasma Level of TSLP in CAD Patients

A total of 336 CAD patients were selected from the replication cohort for analysis of the association between rs3806933 genotypes and the plasma level of TSLP. The plasma samples were frozen at –80°C and tested in less than 2 months for TSLP concentration with an enzyme-linked immunosorbent assay kit (eBioscience Inc.).

Genetic Risk Score (GRS) Analysis

Genetic risk scores were analyzed using a Cox regression model (SPSS version 17.0, SPSS, Inc., Chicago, IL, USA), adjusting for the conventional risk factors of CAD to obtain survival forecasts and calculate the C indices. GRS1 comprised SNP rs3806933 of *TSLP*; GRS2 comprised SNP rs7025417 of *IL33*; and GRS3 comprised the both SNPs rs3806933 of *TSLP* and rs7025417 of *IL33*.

Statistical Analysis

All the control cohorts were examined using the Hardy–Weinberg equilibrium (HWE) test (PLINK version 1.06). The allelic and genotypic association analyses were performed using chi-square tests with Pearson's 2 × 2 and 2 × 3 contingency tables (SPSS version 17.0). Under the genotypic model, the interaction analysis was carried out by a logistic association analysis with the conventional risk factors for CAD as covariates (23). Statistical power analyses were performed with a free power and sample size calculation program (PS v.3.0.12) for single gene association analysis and a free software QANTO (QANTO V.1.2.4) for interaction analysis (23). The difference in the luciferase activity among the reference plasmids was analyzed using the ANOVA test. The correlations among the rs3806933 genotypes and TSLP plasma concentrations were studied by linear regression analysis and the ANOVA test. The terminology of $P < 0.01$ in the text, figures, and figure legends indicates statistical significance.

RESULTS

Population Characteristics

The CAD patients were more likely to have hypertension and diabetes mellitus than the control subjects. Moreover, in all studied populations, the age, body mass index (BMI), total cholesterol, and low-density lipoprotein cholesterol levels were much higher in CAD patients than in controls, and the high-density lipoprotein cholesterol level was significantly lower in CAD patients than in controls (**Table 1**).

The statistic power for all the variants selected in this study was more than 80% with an effect size of 1.2 (HapMap CHB + JPT data). The statistical power was also more than 70% for the two interaction analyses (rs3806933^T in *TSLP* and rs6897932^T in *IL7R*, rs3806933^T in *TSLP* and rs7025417^T in *IL33* under the genotypic model) (23).

Association Analysis Between Variants in TSLP, IL7R, and TSLPR and CAD

All selected variants passed the HWE test ($P > 0.001$). In stage 1, the allelic frequencies of rs3806933^T in *TSLP*, rs6897932^T in *IL7R*,

TABLE 1 | Clinical characteristics of the studied Chinese Han population.

Characteristics	Stage 1-discovery		*P*	Stage 2-validation		*P*	Stage 3-replication		*P*	Combined		*P*
	CAD	Control		CAD	Control		CAD	Control		CAD	Control	
Subject numbers	1,345	1,156	–	1,347	1,156	–	936	1,464	–	3,628	3,776	–
Age (years)	67.8 ± 11.4	59.2 ± 9.94	0.00	63.1 ± 11.8	61.0 ± 10.6	0.00	60.7 ± 10.4	58.1 ± 11.6	0.00	64.2 ± 11.7	59.3 ± 10.9	0.00
Gender (male, %)	58.8	61.9	0.11	73.6	53.9	0.00	54.2	66.7	0.00	63.1	61.3	0.12
Smoking (%)	23.0	22.1	0.59	31.3	14.1	0.00	36.1	25.4	0.00	29.4	20.9	0.00
BMI (kg/m^2)	24.4 ± 0.35	24.0 ± 1.92	0.00	24.4 ± 1.28	23.9 ± 1.25	0.00	25.0 ± 2.17	24.3 ± 0.68	0.00	24.6 ± 1.39	24.1 ± 1.35	0.00
Hypertension (%)	75.2	54.4	0.00	68.0	22.5	0.00	61.4	42.1	0.00	69.0	39.9	0.00
DM (%)	29.4	24.0	0.00	22.6	8.13	0.00	15.0	7.58	0.00	23.2	12.8	0.00
Tch (mmol/L)	4.39 ± 1.01	4.28 ± 0.74	0.00	4.51 ± 1.05	4.23 ± 0.76	0.00	4.60 ± 1.19	4.39 ± 0.24	0.00	4.49 ± 1.07	4.31 ± 0.61	0.00
TG (mmol/L)	1.56 ± 1.07	1.50 ± 0.65	0.14	1.78 ± 1.04	1.48 ± 0.67	0.00	1.82 ± 1.30	1.51 ± 0.50	0.00	1.71 ± 1.13	1.50 ± 0.60	0.00
HDL-c (mmol/L)	1.11 ± 0.27	1.18 ± 0.22	0.00	1.12 ± 0.26	1.18 ± 0.20	0.00	1.19 ± 0.36	1.26 ± 0.07	0.00	1.13 ± 0.29	1.21 ± 0.18	0.00
LDL-c (mmol/L)	2.62 ± 0.83	2.46 ± 0.64	0.00	2.64 ± 0.80	2.42 ± 0.66	0.00	2.59 ± 0.67	2.41 ± 0.25	0.00	2.62 ± 0.78	2.43 ± 0.53	0.00

The data are presented as mean ± SD or percentages.

CAD, coronary artery disease; BMI, body mass index; DM, diabetes mellitus; Tch, total cholesterol; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

and g.19646A>G^{A} in *TSLPR* were significantly different between cases and controls [rs3806933^{T} in *TSLP*, $P_{adj} = 3.01 \times 10^{-2}$, odds ratio (OR) = 1.17, 95% confidence interval (CI): 1.02–1.34; rs6897932^{T} in *IL7R*, $P_{adj} = 9.64 \times 10^{-3}$, OR = 1.26, 95%CI: 1.06–1.51; g.19646A>G^{A} in *TSLPR*, $P_{adj} = 2.78 \times 10^{-3}$, OR = 1.22, 95%CI: 1.07–1.39] after adjustment for the conventional risk factors (**Table 2**), however, those results did not pass the Bonferroni correction. In stage 2, the rs3806933^{T} allele of *TSLP*, rs6897932^{T} allele of *IL7R*, and g.19646A>G^{A} allele of *TSLPR* also conferred significant risk for CAD (rs3806933^{T} in *TSLP*, $P_{adj} = 7.51 \times 10^{-4}$, OR = 1.30, 95%CI: 1.11–1.50; rs6897932^{T} in *IL7R*, $P_{adj} = 5.13 \times 10^{-4}$, OR = 1.40, 95%CI: 1.16–1.69; g.19646A>G^{A} in *TSLPR*, $P_{adj} = 1.52 \times 10^{-2}$, OR = 1.19, 95%CI: 1.03–1.36) in the validation cohort, which passed Bonferroni correction. In stage 3, the associations between the three variants and CAD were confirmed in the replication cohorts (rs3806933^{T} in *TSLP*, $P_{adj} = 8.89 \times 10^{-3}$, OR = 1.20, 95%CI: 1.05–1.37; rs6897932^{T} in *IL7R*, $P_{adj} = 1.71 \times 10^{-3}$, OR = 1.33, 95%CI: 1.11–1.60; g.19646A>G^{A} in *TSLPR*, $P_{adj} = 6.17 \times 10^{-3}$, OR = 1.20, 95%CI: 1.05–1.37; **Table 2**), which also passed Bonferroni correction.

Combining the three cohorts together, a meta-analysis with 3,628 CAD cases and 3,776 controls was performed. In the combined cohorts: 3,339 CAD cases and 3,569 controls were genotyped successfully for rs3806933; 3,330 CAD cases and 3,761 controls were genotyped successfully for rs6897932; 3,628 CAD cases and 3,776 controls were genotyped successfully for g.19646A>G. The above three variants all were significantly associated with CAD in the combined cohort (rs3806933^{T} in *TSLP*, $P_{adj} = 4.35 \times 10^{-5}$, OR = 1.18, 95%CI: 1.09–1.27; rs6897932^{T} in *IL7R*, $P_{adj} = 1.13 \times 10^{-7}$, OR = 1.31, 95%CI: 1.19–1.45; g.19646A>G^{A} in *TSLPR*, $P_{adj} = 2.04 \times 10^{-6}$, OR = 1.20, 95%CI: 1.11–1.29) after adjustment for covariates of CAD, which passed Bonferroni correction (**Table 2**). Significant genotypic association results for the three variants above (rs3806933^{T} in *TSLP*, rs6897932^{T} in *IL7R*, g.19646A>G^{A} in *TSLPR*) were also identified for CAD in the combined cohort under an additive model after applying Bonferroni correction (Table S2 in Supplementary Material).

The allelic association results between rs2289276 in *TSLP*, rs7737000, rs1494555, and rs1494558 in *IL7R*, and rs150166261, rs36133495, and rs36177645 in *TSLPR* and CAD were not significant with adjusted *P* values of more than 0.05 in the first stage (Table S3 in Supplementary Material). The genotypic association results also were not significant with adjusted *P* values of more than 0.05 under any one of the three models (additive, dominant, and recessive) for these variants (Table S4 in Supplementary Material). Therefore, we discarded these variants in the next two stages.

Reporter Gene Analysis

Rs3806933 was located in the promoter region of *TSLP* which might regulate the expression of *TSLP*. Using luciferase assay, we found that compared to the empty pGL3-basic vector group, the luciferase activity increased in both the construct TSLP-T and construct TSLP-C groups, and the luciferase activity increased more for the construct TSLP-T than for TSLP-C ($P < 0.01$; **Figure 2A**). These results indicated that rs3806933^{T} might increase the expression of *TSLP*. Rs6897932, located within the alternatively spliced exon 6 of *IL7R*, had a functional effect on gene expression. Luciferase activity in the construct IL7R-T and construct IL7R-C group were decreased than that in the empty pGL3-control vector group, and plasmid with the risk allele of rs6897932 in *IL7R* (IL7R-T) showed considerably lower luciferase activity than the plasmid IL7R-C ($P < 0.01$; **Figure 2B**), which suggested that rs6897932^{T} might decrease the expression of *IL7R*. These results were validated in another two independent experiments. In addition, we did not find significantly different luciferase activity between the two plasmids for g.19646A>G in *TSLPR* (TSLPR-G and TSLPR-A; $P \geq 0.05$; data not shown). It was concluded that the allele changes of rs3806933 in *TSLP* and rs6897932 in *IL7R* could both regulate the expression of their genes, whereas g.19646A>G in *TSLPR* might not.

TABLE 2 | Allelic association analysis of rs3806933 in *TSLP*, rs6897932 in *IL7R*, and g.19646A>G in *TSLPR* with CAD in the studied Chinese Han population.

Gene, SNP (Allele)	Population	*N*		Frequency		P_{hwe}	P_{obs}	P_{adj}	OR (95%CI)
		Cases	Controls	Cases	Controls				
TSLP, rs3806933^{T}	Discovery	1,207	1,104	0.354	0.317	0.673	8.27×10^{-3}	3.01×10^{-2}	1.17 (1.02–1.34)
	Validation	1,213	1,104	0.353	0.316	0.710	7.50×10^{-3}	7.51×10^{-4}	1.30 (1.11–1.50)
	Replication	919	1,361	0.392	0.344	0.332	9.78×10^{-4}	8.89×10^{-3}	1.20 (1.05–1.37)
	Combined	3,339	3,569	0.364	0.327	0.273	4.69×10^{-6}	4.35×10^{-5}	1.18 (1.09–1.27)
IL7R, rs6897932^{T}	Discovery	1,219	1,149	0.188	0.149	0.577	3.47×10^{-4}	9.64×10^{-3}	1.26 (1.06–1.51)
	Validation	1,223	1,150	0.188	0.150	0.598	3.58×10^{-4}	5.13×10^{-4}	1.40 (1.16–1.69)
	Replication	888	1,462	0.169	0.131	0.682	3.25×10^{-4}	1.71×10^{-3}	1.33 (1.11–1.60)
	Combined	3,330	3,761	0.183	0.142	0.367	3.80×10^{-11}	1.13×10^{-7}	1.31 (1.19–1.45)
TSLPR, g.19646A>G^{A}	Discovery	1,345	1,156	0.405	0.361	0.169	1.41×10^{-3}	2.78×10^{-3}	1.22 (1.07–1.39)
	Validation	1,347	1,156	0.405	0.362	0.167	2.06×10^{-3}	1.52×10^{-2}	1.19 (1.03–1.36)
	Replication	936	1,464	0.413	0.368	0.336	1.89×10^{-3}	6.17×10^{-3}	1.20 (1.05–1.37)
	Combined	3,628	3,776	0.407	0.364	0.359	8.57×10^{-8}	2.04×10^{-6}	1.20 (1.11–1.29)

P_{hwe}, P value from Hardy–Weinberg equilibrium tests; P_{obs}, observed P value; P_{adj}, P value adjusted by covariates; OR, odds ratio after adjustment; TSLP, thymic stromal lymphopoietin; IL7R, interleukin 7 receptor; TSLPR, TSLP receptor; CAD, coronary artery disease; CI, confidence interval.

In the combined cohorts: 3,339 CAD cases and 3,569 controls were genotyped successfully for rs3806933; 3,330 CAD cases and 3,761 controls were genotyped successfully for rs6897932; 3,628 CAD cases and 3,776 controls were genotyped successfully for g.19646A>G.

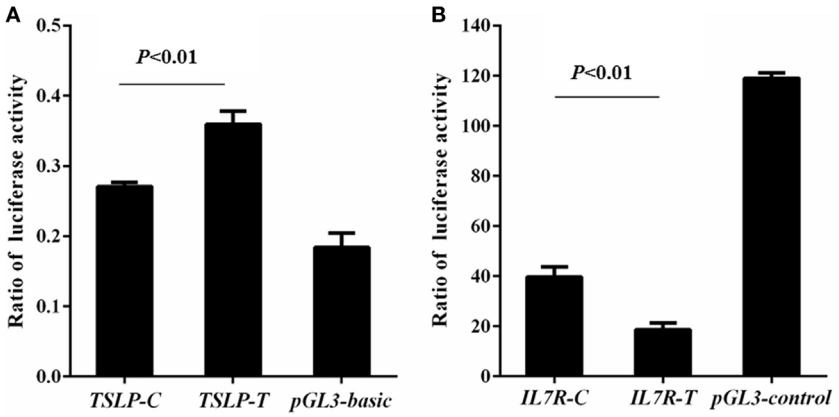


FIGURE 2 | Reporter gene analysis for thymic stromal lymphopoietin (*TSLP*) **(A)** and interleukin 7 receptor (*IL7R*) **(B)**. Luciferase activity was tested by means of cellular extracts. Mean ± SD of the relative luciferase activity is shown. The difference in the luciferase activity among the reference plasmids was analyzed using the ANOVA test. The terminology of $P < 0.01$ indicates statistical significance.

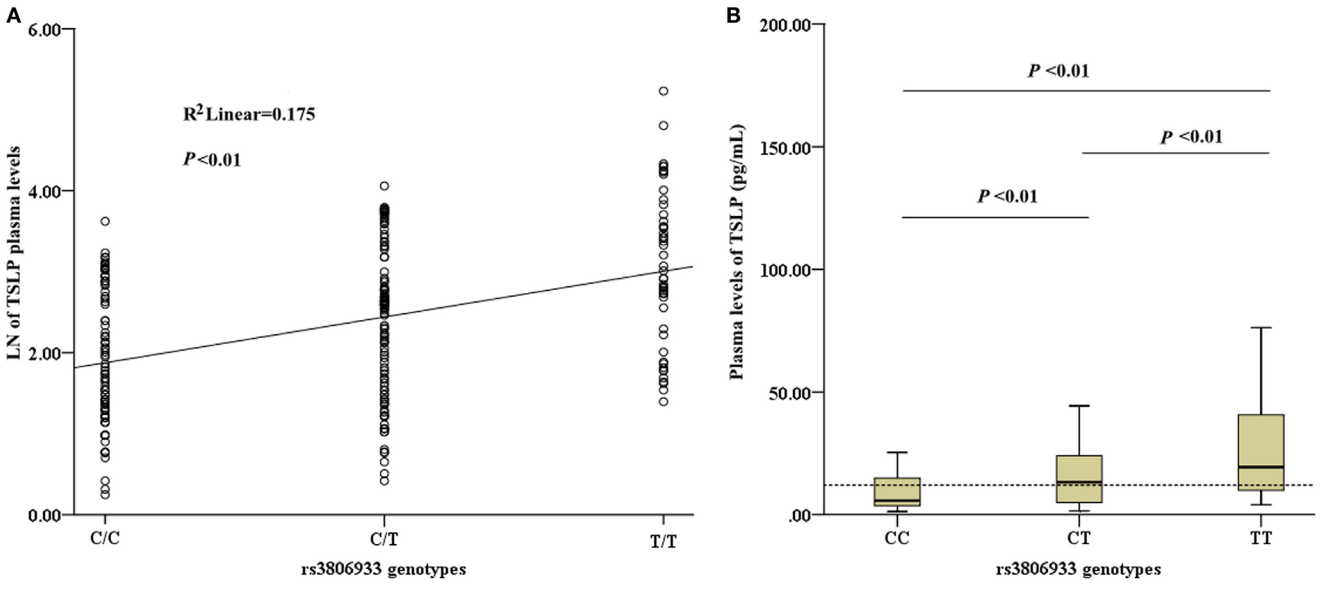


FIGURE 3 | Analysis of circulating thymic stromal lymphopoietin (TSLP) level. **(A)** Association analysis between the circulating level of TSLP and the rs3806933 genotypes in a linear regression model; **(B)** Comparisons among every pair of the three genotypes, *via* the ANOVA test. The terminology of $P < 0.01$ indicates statistical significance. A broken line indicates the median value of 12.03 pg/mL of TSLP levels that were detectable in coronary artery disease patients. The extremes in the Box Graph analysis of panel **(B)** have been removed to avoid the interference.

Association of rs3806933 Genotypes With Plasma TSLP Concentration

Among the 336 CAD patients, the detection rate of plasma TSLP concentrations was 70.5% (median, 12.03 pg/mL; range, 1.28–187.04 pg/mL). All the detectable samples were included in this study. Linear regression analysis revealed a significant association between the rs3806933 genotypes and the plasma TSLP levels in the subjects with a detectable TSLP level ($n = 237$, $R^2 = 0.175$, $P < 0.01$; **Figure 3A**). This trend was confirmed by the ANOVA test for the contrast among each pair of the three genotypes ($P < 0.01$; **Figure 3B**). These results indicated that the circulating level of TSLP could be regulated by the variant rs3806933 in *TSLP* and as the number of risk allele "T" of rs3806933 increased, the circulating level of TSLP increased.

Interaction Association Analysis Between rs3806933 in *TSLP* and rs6897932 in *IL7R* in the Combined Chinese Han Population

Because rs3806933 in *TSLP* and rs6897932 in *IL7R* have been suggested to be functional variants by previous studies (31–33), we performed an interaction analysis between these two variants under the genotypic model in the combined Chinese Han

population. In the combined cohort, 2,879 CAD cases and 3,249 controls were genotyped successfully for both rs3806933 and rs6897932. The interaction analysis showed a considerably lower *P* value (5.97×10^{-10}) in association with CAD than the single variants did (**Table 3**). The combination genotype of "rs3806933_TT/rs6897932_TT" with the largest effect size provided a nearly fivefold increase in the risk for CAD ($P_{adj} = 6.99 \times 10^{-4}$, OR = 4.85, 95%CI: 1.95–12.2; **Table 3**).

Interaction Association Analysis Between rs3806933 in *TSLP* and rs7025417 in *IL33* in the Combined Chinese Han Population

Thymic stromal lymphopoietin and IL-33 might interact with each other in immune inflammatory diseases. However, in CAD, this has not been studied yet. It was interesting that in 2013 we found the IL-33–ST2L pathway is causally involved in the development of CAD (23). Considering that most of the same samples were used in the two studies, we further performed an interaction analysis between rs3806933 in *TSLP* and rs7025417 in *IL33* under the genotypic model in the same population. In the combined cohort, 1,736 CAD cases and 1,093 controls were genotyped successfully for both rs3806933 and rs7025417. The interaction analysis of the two SNPs (rs3806933 in *TSLP* and rs7025417 in *IL33*) indicated significant associations with a *P* value of 3.67×10^{-4} for the type of rs3806933_TT/rs7025417_TT, which provided a nearly threefold increase in the risk of CAD (OR = 2.98, 95%CI: 1.63–5.43; **Table 4**; **Figure 4**).

GRS Analysis

In addition, we analyzed the GRSs based on the SNPs in *TSLP* and *IL33* (GRS3, rs3806933 and rs7025417) or only an SNP in the single gene (GRS1, rs3806933 in *TSLP*; GRS2, rs7025417 in *IL33*) by a Cox regression model. Our results showed that, after adjusting for the conventional risk factors of CAD, GRS3 increased the C index by 3.1% as compared to GRS1 and by 0.3% as compared to GRS2 (**Table 5**).

DISCUSSION

In this study, we discovered that rs3806933 in *TSLP*, rs6897932 in *IL7R*, and g.19646A>G in *TSLPR* were significantly associated with the pathogenesis of CAD in the Chinese Han population. In addition, we confirmed that rs3806933 in *TSLP* and rs6897932 in *IL7R* could influence the expression of their genes as evidenced by a reporter gene analysis or protein circulation level study. We also found that the interaction between rs3806933 in *TSLP* and rs6897932 in *IL7R* contributed to CAD with the highest risk effect. Interestingly, the significant association result from the

TABLE 3 | Interaction analysis between rs3806933 in thymic stromal lymphopoietin and rs6897932 in interleukin 7 receptor under the genotypic model in the combined population.

Population (*n*, case/control)	Types	Case (%)	Control (%)	P_{adj}	Odds ratio (95% confidence interval)
Combined (2,879/3,249)	TT/TT	24 (0.83)	8 (0.25)	6.99×10^{-4}	4.85 (1.95–12.2)
	TT/CT	129 (4.48)	92 (2.83)	6.06×10^{-5}	1.88 (1.38–2.57)
	TT/CC	280 (9.73)	253 (7.79)	4.07×10^{-4}	1.47 (1.19–1.81)
	CT/TT	48 (1.67)	30 (0.92)	6.94×10^{-3}	2.03 (1.21–3.39)
	CT/CT	319 (11.1)	346 (10.6)	3.32×10^{-2}	1.24 (1.02–1.50)
	CT/CC	862 (29.9)	1,029 (31.7)	2.92×10^{-2}	0.86 (0.74–0.98)
	CC/TT	41 (1.42)	32 (0.98)	3.50×10^{-2}	1.73 (1.04–2.87)
	CC/CT	350 (12.2)	338 (10.4)	2.68×10^{-4}	1.43 (1.18–1.74)
	CC/CC	826 (28.7)	1,121 (34.5)	–	1
	ADD/ADD	–	–	5.97×10^{-10}	–

P_{adj}, P value after adjustment for covariates, such as age, gender, smoking, body mass index, hypertension, diabetes mellitus, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol; Additive model, rs3806933_CC/CT/TT, rs6897932_CC/CT/TT.

TABLE 4 | Interaction analysis between rs3806933 in thymic stromal lymphopoietin (*TSLP*) and rs7025417 in *IL33* under the genotypic model in the combined population.

Population (*n*, case/control)	Types	Case (%)	Control (%)	P_{adj}	OR (95% CI)
Combined (1,736/1,093)	TT/TT	83 (4.78)	22 (2.01)	3.67×10^{-4}	2.98 (1.63–5.43)
	TT/CT	132 (7.60)	61 (5.58)	1.17×10^{-2}	1.82 (1.14–2.89)
	TT/CC	40 (2.30)	27 (2.47)	6.52×10^{-1}	0.86 (0.45–1.66)
	CT/TT	232 (13.4)	106 (9.70)	9.29×10^{-3}	1.71 (1.14–2.56)
	CT/CT	373 (21.5)	283 (25.9)	4.74×10^{-1}	0.88 (0.62–1.25)
	CT/CC	111 (6.39)	79 (7.23)	1.59×10^{-1}	0.72 (0.46–1.14)
	CC/TT	222 (12.8)	104 (9.52)	3.25×10^{-3}	1.84 (1.23–2.76)
	CC/CT	426 (24.5)	306 (28.0)	2.67×10^{-1}	0.82 (0.58–1.17)
	CC/CC	117 (6.74)	105 (9.61)	–	1
	ADD/ADD	–	–	1.98×10^{-3}	–

P_{adj}, P value adjusted for age, gender, body mass index, hypertension, diabetes mellitus, smoking, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol; OR, odds ratio after the adjustment; CI, confidence interval. Additive model, rs3806933_CC/CT/TT, rs7025417_CC/CT/TT.

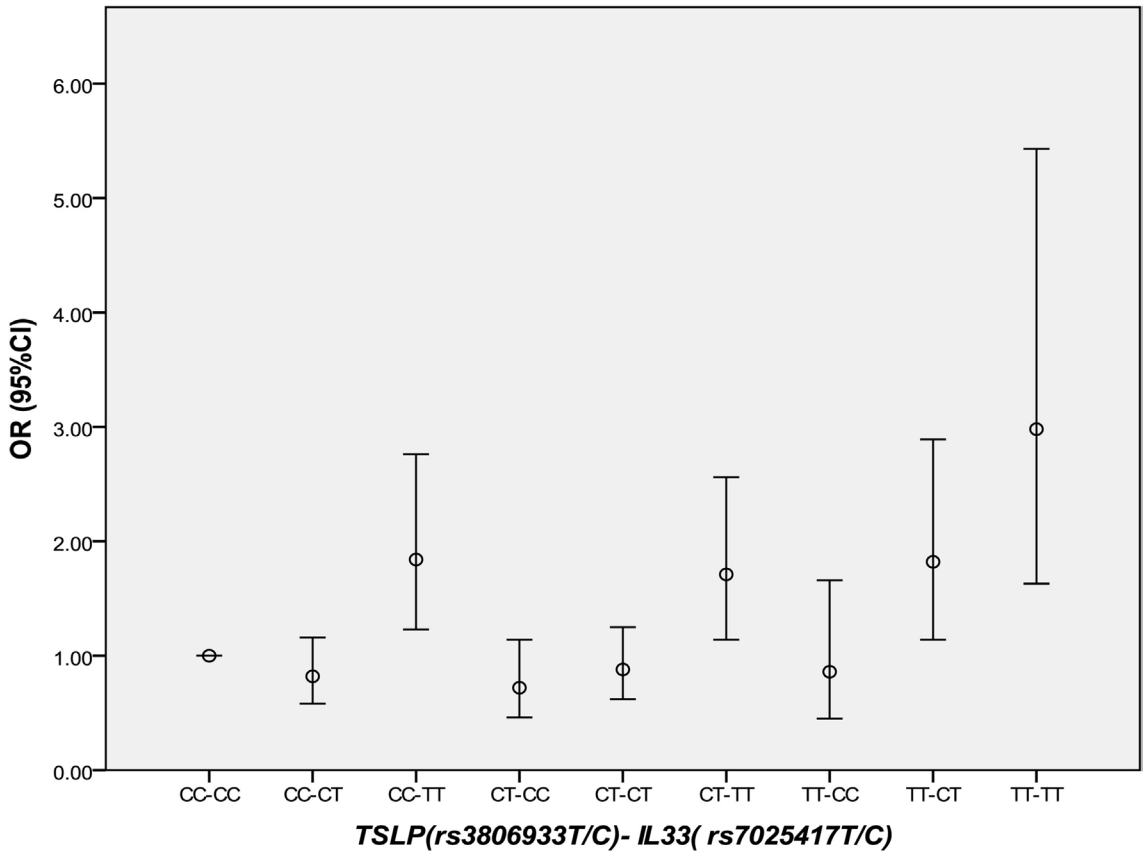


FIGURE 4 | Comparison of odds ratio (OR) values for the interaction analysis between rs3806933 in thymic stromal lymphopoietin (*TSLP*) and rs7025417 in *IL33*. OR values with a 95% confidence interval (CI) for the combinations under the genotypic model.

TABLE 5 | Comparison of the GRSs for prediction of CAD in the combined population.

GRS	C index (95%CI)	*P*	Comparison with GRS1 C index change	Comparison with GRS2 C index change
GRS1 (*TSLP*)	0.526 (0.50–0.55)	1.99×10^{-2}	NA	–0.028
GRS2 (*IL33*)	0.554 (0.53–0.58)	1.06×10^{-6}	0.028	NA
GRS3 (*TSLP-IL33*)	0.557 (0.54–0.58)	2.55×10^{-7}	0.031	0.003

GRS, genetic risk score; GRS1, genetic risk score based on rs3806933 in TSLP; GRS2, genetic risk score based on rs7025417 in IL33; GRS3, genetic risk score based on rs3806933 in TSLP and rs7025417 in IL33; TSLP, thymic stromal lymphopoietin; CAD, coronary artery disease; CI, confidence interval.

interaction analysis between rs3806933 in *TSLP* and rs7025417 in *IL33* indicated that the two genes of *TSLP* and *IL33* might confer an epistatic effect in the development of CAD.

In 1994, Friend et al. first discovered the murine TSLP as a new growth factor that could regulate the B and T lineage cells (34). In 2001, Reche et al. scanned the human genome database and identified its human homolog TSLP (35). Although the human and murine TSLP share only 43% amino acid homology, they have similar biological functions (9, 36). Multiple inflammatory cells, such as DCs and mast cell lines, express TSLP; TSLP can also impact a wide range of inflammatory cell lines, such as basophils, eosinophils, $CD4^+$, $CD8^+$, NK T cells, and B cells (37–39). Studies have reported that TSLP played important roles in inflammatory diseases, including asthma and Behçet's disease (40, 41). The functional TSLP receptor (TSLPR subunit and IL-7Rα) is mainly expressed on monocytes and DCs, and occasionally on lymphocytes (33, 42). These studies demonstrated that the TSLP/TSLPR axis is involved in the immune system, which prompted us to investigate the role of this axis in CAD.

In our study, we discovered three genetic risk variants (rs3806933 in *TSLP* and rs6897932 in *IL7R* and g.19646A>G in *TSLPR*) for CAD. Furthermore, reporter gene analysis showed that rs3806933 in *TSLP* and rs6897932 in *IL7R* could regulate the mRNA expression of their respective genes. In addition, the circulating level study also confirmed that the variant rs3806933 in *TSLP* could influence the circulating level of TSLP protein. These results indicate that variants in the TSLP/TSLPR axis might affect the risk of CAD through upregulating mRNA or protein

expression, and the variants or key genes are likely to be causal risk factors for CAD.

In 2000, Pandey et al. and Park et al. demonstrated that IL-7Rα, in combination with TSLPR, can increase the binding affinity of TSLP for its receptors, which might promote the downstream signaling of TSLP, thus improving its biological efficacy (14, 15). In 2007, Gregory et al. and Lundmark et al. both independently demonstrated that rs6897932 in *IL7R* could increase the ratio of membrane-bound IL-7Rα to soluble IL-7Rα by decreasing the amount of soluble IL-7Rα protein (32, 33). In 2009, Harada et al. demonstrated that rs3806933, which is in the promoter region of *TSLP*, can influence the binding activity of the transcription factor activating protein-1 and thereby regulate the expression of TSLP (31). In the present study, we confirmed the functional roles of rs3806933 in *TSLP* and rs6897932 in *IL7R* by reporter gene analysis and a circulating level study, which indicated that the two variants could regulate the expression of their respective genes. As the effect of the common single variant is too small to be of clinical significance, we performed the gene–gene interaction analysis and found that the OR values for the combined types of rs3806933 in *TSLP* and rs6897932 in *IL7R* were much higher than those for the individual types for the associations with CAD. Therefore, we hypothesized that the two variants (rs3806933 in *TSLP* and rs6897932 in *IL7R*) might interact with each other in a biologically relevant way and the crosstalk between TSLP and IL-7Rα might greatly enhance the risk of CAD.

A previous CAD GWAS meta-analysis showed that rs3806933 in *TSLP* is moderately associated with CAD and rs6897932 in *IL7R* is not associated with CAD in the European ancestry (43). Here, we found that rs3806933 in *TSLP* and rs6897932 in *IL7R* might be the specific cis-expression quantitative trait loci (eQTLs) for CAD in our Chinese Han population. This inconsistency between the results may be explained by the heterogeneity of the eQTL in different diseases or populations with different ancestries (44). Further functional studies for CAD in the Chinese Han population are warranted to confirm our results.

In classical Mendelian genetics, some variants may influence the effects of other variants, such as "stopping" or "standing above," and are defined as epistatic (45). Though they exist in different pathways, these variants might interact with each other in some diseases, in which their pathways are both involved. In general, the specific composition of the alleles with an epistatic effect is determined specifically by the disease's phenotype, and accumulating evidence (45) has demonstrated the epistatic effect of these related variants in complex diseases.

In 2013, we reported that variants in the IL-33 signaling pathway might influence the development of CAD by regulating the expression of key genes of the pathway and then influenced the risk of CAD (23). IL-33 was a strong inducer of Th2 responses and TSLP also engaged in the process of Th2 inflammation (46). In recent years, more and more studies have indicated that the TSLP and IL-33 signaling pathways might interact with each other in Th2 cell-mediated inflammatory responses (25–27). In 2015, Li et al. found that the promoter variant rs992969 in *IL33* could increase the gene protein expression and eosinophil counts in human bronchial epithelial biopsy (BEC) and then influence the risk of asthma (18). In the same year, Liao et al. studied the cross-regulation between the IL-33 and TSLP signaling pathways in human nasal epithelial cells and discovered that IL-33 could induce the expression of TSLP/TSLPR/IL-7Rα, and conversely, TSLP could induce the expression of IL-33 receptors, such as ST2L, thereby upregulating the IL-33-induced TSLP expression (47). The positive feedback loop between IL-33 and TSLP and their receptors might facilitate the Th2-skewed inflammation in eosinophilic chronic rhinosinusitis with nasal polyps (47). However, in atherosclerosis, the role of Th2 inflammation was still confused yet: when targeted deletion of Th2 cytokine, IL-5, atherosclerosis progression was accelerated, which proposed an athero-protective role of Th2 inflammation (48); when targeted deletion of Th2 cytokine, IL-4, the ApoE$^{-/-}$ mice and LDLR$^{-/-}$ mice developed less severe atherosclerotic, which implied a proatherogenic function of Th2 inflammation (49, 50). In this study, we not only demonstrated that variants in the TSLP/TSLPR axis regulated the expression of the key genes and influenced the risk of CAD but also discovered that the effect of the interaction between variants in *TSLP* and *IL33* was much higher than those of the single genes. Considering our findings together, we hypothesized that variants in the TSLP/TSLPR axis might regulate the expression of the key genes in the pathways as well as the cytokines and their receptors involved in the development of CAD by a positive feedback effect, which could also be called an epistatic effect. This positive feedback loop among IL-33, TSLP, and their receptors might increase CAD risk through facilitating the Th2-skewed inflammation. Further functional studies are needed to confirm our hypothesis.

In conclusion, this study indicated that the TSLP/TSLPR axis might affect the CAD risk through upregulating the reference genes' mRNA expression or protein secretion, and the TSLP and IL-33 signaling pathways might have an epistatic effect on the risk of CAD. These results might provide a novel point of view for the prevention and treatment of CAD based on targeting the mechanisms of the epistatic effect between the related cytokines.

DATA AVAILABILITY

We declare that all the data supporting the findings of this study are available within the article and the Supplementary Information files and can be obtained from the corresponding authors upon reasonable request.

ETHICS STATEMENT

This study followed the guidelines set forth by the Declaration of Helsinki and passed the review of the Ethics Committee of Tongji Medical College, and Huazhong University of Science and Technology. All study participants signed a written informed consent form.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: XT and XC. Performed the experiments and analyzed the data: S-FN, L-FZ,

and QF. Contributed reagents/materials/analysis tools: S-FN, L-FZ, QF, Y-HL, H-SZ, Q-WC, FW, T-TT, NX, C-QX, J-YZ, Y-ZL, W-JZ, Z-PZ, JJ, Y-YL, TX, DW, C-CW, J-JF, H-BX, JY, QY, P-YW, S-HT, Q-LL, Q-XL, JQ, BL, GW, Y-XW, YY, X-PY, YH, QW, XC, and XT. Wrote the paper: S-FN. All authors reviewed the manuscript.

ACKNOWLEDGMENTS

We are very grateful to all participations and supporters of this study and to all members of the GeneID team and laboratory of CX for their assistance.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China [No. 81525003, 81720108005 and 91639301 to XC; 91439109 and 81270163 to XT; 81500186 to S-FN; 81400364 to NX; 81200177 and 81670361 to T-TT].

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.01775/full#supplementary-material.

REFERENCES

1. Vinkhuyzen AA, Wray NR, Yang J, Goddard ME, Visscher PM. Estimation and partition of heritability in human populations using whole-genome analysis methods. *Annu Rev Genet* (2013) 47:75–95. doi:10.1146/annurev-genet-111212-133258
2. Klarin D, Zhu QM, Emdin CA, Chaffin M, Horner S, McMillan BJ, et al. Genetic analysis in UK Biobank links insulin resistance and transendothelial migration pathways to coronary artery disease. *Nat Genet* (2017) 49:1392–7. doi:10.1038/ng.3914
3. Howson JMM, Zhao W, Barnes DR, Ho WK, Young R, Paul DS, et al. Fifteen new risk loci for coronary artery disease highlight arterial-wall-specific mechanisms. *Nat Genet* (2017) 49:1113–9. doi:10.1038/ng.3874
4. Nelson CP, Goel A, Butterworth AS, Kanoni S, Webb TR, Marouli E, et al. Association analyses based on false discovery rate implicate new loci for coronary artery disease. *Nat Genet* (2017) 49:1385–91. doi:10.1038/ng.3913
5. McPherson R, Tybjaerg-Hansen A. Genetics of coronary artery disease. *Circ Res* (2016) 118:564–78. doi:10.1161/CIRCRESAHA.115.306566
6. Visscher PM, Brown MA, McCarthy MI, Yang J. Five years of GWAS discovery. *Am J Hum Genet* (2012) 90:7–24. doi:10.1016/j.ajhg.2011.11.029
7. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature* (2009) 461:747–53. doi:10.1038/nature08494
8. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* (2010) 11:289–93. doi:10.1038/ni.1852
9. Miazgowicz MM, Headley MB, Larson RP, Ziegler SF. Thymic stromal lymphopoietin and the pathophysiology of atopic disease. *Expert Rev Clin Immunol* (2009) 5:547–56. doi:10.1586/eci.09.45
10. Astrakhan A, Omori M, Nguyen T, Becker-Herman S, Iseki M, Aye T, et al. Local increase in thymic stromal lymphopoietin induces systemic alterations in B cell development. *Nat Immunol* (2007) 8:522–31. doi:10.1038/ni1452
11. Ziegler SF, Liu YJ. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. *Nat Immunol* (2006) 7:709–14. doi:10.1038/ni1360
12. Watanabe N, Hanabuchi S, Soumelis V, Yuan W, Ho S, de Waal Malefyt R, et al. Human thymic stromal lymphopoietin promotes dendritic cell-mediated CD4+ T cell homeostatic expansion. *Nat Immunol* (2004) 5:426–34. doi:10.1038/ni1048
13. Quentmeier H, Drexler HG, Fleckenstein D, Zaborski M, Armstrong A, Sims JE, et al. Cloning of human thymic stromal lymphopoietin (TSLP) and signaling mechanisms leading to proliferation. *Leukemia* (2001) 15:1286–92. doi:10.1038/sj.leu.2402175
14. Pandey A, Ozaki K, Baumann H, Levin SD, Puel A, Farr AG, et al. Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. *Nat Immunol* (2000) 1:59–64. doi:10.1038/76923
15. Park LS, Martin U, Garka K, Gliniak B, Di Santo JP, Muller W, et al. Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: formation of a functional heteromeric complex requires interleukin 7 receptor. *J Exp Med* (2000) 192:659–70. doi:10.1084/jem.192.5.659
16. Lin J, Chang W, Dong J, Zhang F, Mohabeer N, Kushwaha KK, et al. Thymic stromal lymphopoietin over-expressed in human atherosclerosis: potential role in Th17 differentiation. *Cell Physiol Biochem* (2013) 31:305–18. doi:10.1159/000343369
17. Wang B, Peng Y, Dong J, Lin J, Wu C, Su Y, et al. Human platelets express functional thymic stromal lymphopoietin receptors: a potential role in platelet activation in acute coronary syndrome. *Cell Physiol Biochem* (2013) 32:1741–50. doi:10.1159/000356608
18. Li X, Hastie AT, Hawkins GA, Moore WC, Ampleford EJ, Milosevic J, et al. eQTL of bronchial epithelial cells and bronchial alveolar lavage deciphers GWAS-identified asthma genes. *Allergy* (2015) 70:1309–18. doi:10.1111/all.12683
19. Zhao H, Li M, Wang L, Su Y, Fang H, Lin J, et al. Angiotensin II induces TSLP via an AT1 receptor/NF-KappaB pathway, promoting Th17 differentiation. *Cell Physiol Biochem* (2012) 30:1383–97. doi:10.1159/000343327
20. Taleb S, Tedgui A, Mallat Z. IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles. *Arterioscler Thromb Vasc Biol* (2015) 35:258–64. doi:10.1161/ATVBAHA.114.303567
21. Yu K, Zhu P, Dong Q, Zhong Y, Zhu Z, Lin Y, et al. Thymic stromal lymphopoietin attenuates the development of atherosclerosis in ApoE-/- mice. *J Am Heart Assoc* (2013) 2:e000391. doi:10.1161/JAHA.113.000391
22. Wu C, He S, Peng Y, Kushwaha KK, Lin J, Dong J, et al. TSLPR deficiency attenuates atherosclerotic lesion development associated with the inhibition of TH17 cells and the promotion of regulator T cells in ApoE-deficient mice. *J Mol Cell Cardiol* (2014) 76:33–45. doi:10.1016/j.yjmcc
23. Tu X, Nie S, Liao Y, Zhang H, Fan Q, Xu C, et al. The IL-33-ST2L pathway is associated with coronary artery disease in a Chinese Han population. *Am J Hum Genet* (2013) 93:652–60. doi:10.1016/j.ajhg.2013.08.009
24. Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X, et al. Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. *J Clin Invest* (2013) 9:3967–82. doi:10.1172/JCI65570
25. Akasaki S, Matsushita K, Kato Y, Fukuoka A, Iwasaki N, Nakahira M, et al. Murine allergicrhinitis and nasal Th2 activation are mediated via TSLP- and IL-33-signaling pathways. *Int Immunol* (2016) 28:65–76. doi:10.1093/intimm/dxv055
26. Iijima K, Kobayashi T, Hara K, Kephart GM, Ziegler SF, McKenzie AN, et al. IL-33 and thymic stromal lymphopoietin mediate immune pathology in response to chronic airborne allergen exposure. *J Immunol* (2014) 193:1549–59. doi:10.4049/jimmunol
27. Sugita J, Asada Y, Ishida W, Iwamoto S, Sudo K, Suto H, et al. Contributions of interleukin-33 and TSLP in a papain-soaked contact lens-induced mouse conjunctival inflammation model. *Immun Inflamm Dis* (2017) 5:515–25. doi:10.1002/iid3.189
28. Wang F, Xu C, He Q, Cai J, Li X, Wang D, et al. Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population. *Nat Genet* (2011) 43:345–9. doi:10.1038/ng.783
29. Cheng X, Shi L, Nie S, Wang F, Li X, Xu C, et al. The same chromosome 9p21.3 locus is associated with type 2 diabetes and coronary artery disease in a Chinese Han population. *Diabetes* (2011) 60:680–4. doi:10.2337/db10-0185
30. Fan Q, Nie S, Li S, Liao Y, Zhang H, Zha L, et al. Analysis of the genetic association between IL27 variants and coronary artery disease in a Chinese Han population. *Sci Rep* (2016) 6:25782. doi:10.1038/srep25782

31. Harada M, Hirota T, Jodo AI, Doi S, Kameda M, Fujita K, et al. Functional analysis of the thymic stromal lymphopoietin variants in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* (2009) 40:368–74. doi:10.1165/rcmb.2008-0041OC
32. Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A, et al. Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat Genet* (2007) 39:1083–91. doi:10.1038/ng2103
33. Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallström E, Khademi M, et al. Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. *Nat Genet* (2007) 39:1108–13. doi:10.1038/ng2106
34. Friend SL, Hosier S, Nelson A, Foxworthe D, Williams DE, Farr A, et al. A thymic stromal cell line supports in vitro development of surface IgM+ B cells and produces a novel growth factor affecting B and T lineage cells. *Exp Hematol* (1994) 22:321–8.
35. Reche PA, Soumelis V, Gorman DM, Clifford T, Liu Mr, Travis M, et al. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J Immunol* (2001) 167:336–43. doi:10.4049/jimmunol.167.1.336
36. Sims JE, Williams DE, Morrissey PJ, Garka K, Foxworthe D, Price V, et al. Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *J Exp Med* (2000) 192:671–80. doi:10.1084/jem.192.5.671
37. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* (2002) 3:673–80. doi:10.1038/ni805
38. Kashyap M, Rochman Y, Spolski R, Samsel L, Leonard WJ. Thymic stromal lymphopoietin is produced by dendritic cells. *J Immunol* (2011) 187:1207–11. doi:10.4049/jimmunol.1100355
39. Kuan EL, Ziegler SF. Thymic stromal lymphopoietin and cancer. *J Immunol* (2014) 193:4283–8. doi:10.4049/jimmunol.1400864
40. Burkard-Mandel L, O'Neill R, Colligan S, Seshadri M, Abrams SI. Tumor-derived thymic stromal lymphopoietin enhances lung metastasis through an alveolar macrophage-dependent mechanism. *Oncoimmunology* (2018) 7(5):e1419115. doi:10.1080/2162402X.2017.1419115
41. Lopalco G, Lucherini OM, Lopalco A, Venerito V, Fabiani C, Frediani B, et al. Cytokine signatures in mucocutaneous and ocular Behçet's disease. *Front Immunol* (2017) 8(8):200. doi:10.3389/fimmu.2017.00200
42. Zhang W, Wang J, Wang Q, Chen G, Zhang J, Chen T, et al. Identification of a novel type I cytokine receptor CRL2 preferentially expressed by human dendritic cells and activated monocytes. *Biochem Biophys Res Commun* (2001) 281:878–83. doi:10.1006/bbrc.2001.4432
43. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* (2013) 45:25–33. doi:10.1038/ng.2480
44. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* (2013) 45:1238–43. doi:10.1038/ng.2756
45. Phillips PC. Epistasis – the essential role of gene interactions in the structure and evolution of genetic systems. *Nat Rev Genet* (2008) 9:855–67. doi:10.1038/nrg245
46. Sun L, Chen C, Wu J, Dai C, Wu X. TSLP-activated dendritic cells induce T helper type 2 inflammation in *Aspergillus fumigatus* keratitis. *Exp Eye Res* (2018) 171:120–30. doi:10.1016/j.exer.2018.03.014
47. Liao B, Cao PP, Zeng M, Zhen Z, Wang H, Zhang YN, et al. Interaction of thymic stromal lymphopoietin, IL-33, and their receptors in epithelial cells in eosinophilic chronic rhinosinusitis with nasal polyps. *Allergy* (2015) 70:1169–80. doi:10.1111/all.12667
48. Binder CJ, Hartvigsen K, Chang MK, Miller M, Broide D, Palinski W, et al. IL-5 links adaptive and natural immunity specifc for epitopes of oxidized LDL and protects from atherosclerosis. *J Clin Invest* (2004) 114:427–37. doi:10.1172/JCI20479
49. Davenport P, Tipping PG. The role of interleukin-4 and interleukin-12 in the progression of atherosclerosis in apolipoprotein E-deficient mice. *Am J Pathol* (2003) 163:1117–25. doi:10.1016/S0002-9440(10)63471-2
50. King VL, Szilvassy SJ, Daugherty A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor-/- mice. *Arterioscler Thromb Vasc Biol* (2002) 22:456–61. doi:10.1161/hq0302.104905

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Nie, Zha, Fan, Liao, Zhang, Chen, Wang, Tang, Xia, Xu, Zhang, Lu, Zeng, Jiao, Li, Xie, Zhang, Wang, Wang, Fa, Xiong, Ye, Yang, Wang, Tian, Lv, Li, Qian, Li, Wu, Wu, Yang, Yang, Hu, Wang, Cheng and Tu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 16 August 2018
doi: 10.3389/fimmu.2018.01895

Interleukin 33 Selectively Augments Rhinovirus-Induced Type 2 Immune Responses in Asthmatic but not Healthy People

Lisa M. Jurak[1†], Yang Xi[1†], Megan Landgraf[1], Melanie L. Carroll[1], Liisa Murray[1] and John W. Upham[1,2]*

[1] Lung and Allergy Research Centre, Diamantina Institute, University of Queensland, Woolloongabba, QLD, Australia, [2] Department of Respiratory Medicine, Princess Alexandra Hospital, Brisbane, QLD, Australia

OPEN ACCESS

Edited by:
Fang-Ping Huang, University of Hong Kong, Hong Kong

Reviewed by:
Yusei Ohshima, University of Fukui, Japan
Mohan Maddur, Pfizer, United States
John F. Alcorn, University of Pittsburgh, United States

***Correspondence:**
John W. Upham
j.upham@uq.edu.au

[†]*These authors have contributed equally to this work.*

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *21 May 2018*
Accepted: *31 July 2018*
Published: *16 August 2018*

Citation:
Jurak LM, Xi Y, Landgraf M, Carroll ML, Murray L and Upham JW (2018) Interleukin 33 Selectively Augments Rhinovirus-Induced Type 2 Immune Responses in Asthmatic but not Healthy People. Front. Immunol. 9:1895. doi: 10.3389/fimmu.2018.01895

Interleukin- 33 (IL-33) is an epithelial-derived cytokine that initiates type 2 immune responses to allergens, though whether IL-33 has the ability to modify responses to respiratory viral infections remains unclear. This study aimed to investigate the effects of IL-33 on rhinovirus (RV)-induced immune responses by circulating leukocytes from people with allergic asthma, and how this response may differ from non-allergic controls. Our experimental approach involved co-exposing peripheral blood mononuclear cells to IL-33 and RV in order to model how the functions of virus-responsive lymphocytes could be modified after recruitment to an airway environment enriched in IL-33. In the current study, IL-33 enhanced RV-induced IL-5 and IL-13 release by cells from people with allergic asthma, but had no effect on IL-5 and IL-13 production by cells from healthy donors. In asthmatic individuals, IL-33 also enhanced mRNA and surface protein expression of ST2 (the IL-33 receptor IL1RL1), while soluble ST2 concentrations were low. In contrast, IL-33 had no effect on mRNA and surface expression of ST2 in healthy individuals. In people with allergic asthma, RV-activated ST2$^+$ innate lymphoid cells (ST2$^+$ILC) were the predominant source of IL-33 augmented IL-13 release. In contrast, RV-activated natural killer cells (NK cells) were the predominant source of IL-33 augmented IFNγ release in healthy individuals. This suggests that the effects of IL-33 on the cellular immune response to RV differ between asthmatic and healthy individuals. These findings provide a mechanism by which RV infections and IL-33 might interact in asthmatic individuals to exacerbate type 2 immune responses and allergic airway inflammation.

Keywords: asthma, IL-33, rhinovirus, type 2 immunity, ST2, innate lymphoid cells

INTRODUCTION

Acute exacerbations make a major contribution to the burden of asthma, with respiratory viral infections, in particular, rhinoviruses (RV), triggering most exacerbations. However, the underlying mechanisms by which RV trigger serious asthma exacerbations are not well understood. Even though RV is usually confined to epithelial cells lining the upper respiratory tract and may

sometimes involve the lower respiratory tract, there is strong evidence that systemic immune function plays an important role in host defense against RV infections. High titers of specific neutralizing antibodies are protective against experimental RV infections (1). Moreover, the capacity of circulating T-cells to proliferate and produce IFNγ and IL-10 after RV stimulation *in vitro* is inversely associated with viral load during an RV infection (2). More recently, a large birth cohort study has shown that RV-activated blood mononuclear cells exhibit varying cytokine response patterns *in vitro* that are associated with different clinical outcomes (3).

Viruses typically trigger a type 1 (Th1) immune response in most individuals, yet, they are a common trigger of asthma exacerbations, a disease in which type 2 (Th2) immune responses are often prominent, with type 2 innate lymphoid cells (ILC2), allergen activated Th2 cells, mast cells, and eosinophils all playing key roles. Increased capacity for type 2 cytokine production in asthmatic individuals is linked to more severe virus-induced asthma symptoms and airway eosinophilia following experimental RV challenge (4). Moreover, immune responses to RV in asthma are somewhat Th1 deficient and may be skewed toward a Th2 response (5, 6).

Interleukin- 33 (IL-33) is a strong inducer of Th2 immune responses. Recent studies in mice and humans have implicated the importance of IL-33 in the development of Th2 inflammation during RV infection (6, 7), thus providing a possible mechanistic link between viral infections and eosinophilic inflammation. Asthma is associated with variants in the genes encoding IL-33 and its receptor ST2 (also known as ST2L and IL1RL1) (8, 9). The IL-33 receptor itself comprises two proteins ST2 and IL-1RacP. IL-33 binds to ST2 causing a conformational change that enables it to interact with IL-1RacP (10). Epithelial cells and macrophages release IL-33, which activate Th2 cells, ILC2 cells, and other immune cells *via* ST2, thereby amplifying type 2 immune responses (11). However, ST2 can also exist as a soluble receptor (sST2), which has the ability to neutralize the effects of IL-33 (12). Although, viral infections such as influenza can induce IL-33 release in murine lungs (13), it is not clear whether IL-33 can modify cellular responses directed against respiratory viruses.

A study of experimental RV infection in asthma showed that virus-induced IL-33 release correlated with high IL-5 and IL-13 concentrations in the airway (6). However, RV infection induced similar IL-33 release in asthmatic and control subjects, suggesting that virus-induced IL-33 release *per se* may not be sufficient to discriminate between asthmatic and healthy responses to RV (6). More recently, Werder et al. (14) showed that during RV infection of mice with allergic airways disease, RV has the ability to amplify inflammation through IL-33 (14). Whether IL-33 can modify cellular responses to respiratory viruses in humans is unknown.

Thus, the objective of the current study was to examine the effects of IL-33 on RV-induced cellular immune responses *in vitro* and to determine whether this differs in asthmatic and healthy individuals. Our approach sought to model how virus-responsive lymphocyte function could be modified after recruitment to an airway environment enriched in IL-33.

MATERIALS AND METHODS

Patients

The Metro South Human Research Ethics Committee approved this study (Ethics approval number 2008000037) and written informed consent was obtained from all participants. A detail questionnaire documented respiratory symptoms, prior diagnoses, and medication use. Healthy subjects had no symptoms or prior diagnosis of respiratory disease. Asthma diagnoses had been confirmed by a physician, and symptoms had occurred within the last 12 months. Current smokers and those with respiratory infection within the preceding 4 weeks were excluded. Allergic sensitization was assessed by skin prick test using a panel of common aeroallergens (grass pollens, house dust mite, domestic animals, and molds). Those asthmatics taking inhaled corticosteroids were asked to withhold these for 24 h prior to blood collection.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood mononuclear cells were isolated from venous blood and cultured as described (15). PBMCs were exposed to recombinant IL-33 (Preprotech) or media for 6 h prior to addition of RV strain 16 (multiplicity of infection = 1). Cultures were incubated at 37°C with 5% CO_2 for 5 days (a time dominated by antigen specific recall responses). On the basis of initial dose response experiments, IL-33 was used at 10 ng/mL for all subsequent experiments.

Quantitative Real-Time PCR

Total RNA was extracted from cell pellets obtained from 5-day cultures, using RNeasy micro RNA kit (Qiagen, Hilden, Germany). The ratio of absorbance at 260 and 280 nm of RNA was measured using Nanodrop (Thermo Scientific, Waltham, MA, USA). RNA was immediately converted to complimentary DNA (cDNA) using cDNA synthesis Kit (Bioline, Alexandria, NSW, Australia) and cycling conditions as per the manufactures instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) contained three volumes of SYBER green master mix (Bioline, Alexandria, NSW, Australia), two volumes of cDNA, and 0.6 volumes of standard primers specific for membrane bound ST2 (Forward primer: 5′-*GGAAAAAACGCAAACCTA*-3′, Reverse primer: 5′-*GGCCTCAATCCAGAACATTTT*-3′) and IL-1RAcP (Forward primer: 5′-*CTGAGGATCTCAAGCGCAGCTA*-3′, Reverse primer: 5′-*AGCAGGACTGTGGCTCCAAAAC*-3′) as indicated. The qRT-PCR cycle consisted of an initial incubation at 95°C for 2 min followed by a subsequent 40 cycles of denaturation and annealing at 95°C for 5 s and 60°C for 10 s. Each reaction was conducted in duplicate, using a Roche light cycler 480 real-time PCR system (Roche, North Ryde, Australia). Analysis was conducted using light cycler 480 software 1.5.0. Genes of interest were calculated in relation to the reference gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the delta–delta Ct method. Gene expression was normalized to a corresponding control sample for each donor.

ELISA

Cell free supernatants were collected and stored at −20°C until required. The concentration of IL-5, IL 13, IFNγ (BD Biosciences, Franklin Lakes, NJ, USA), and soluble ST2 (R&D systems, Minneapolis, MN, USA) were performed using commercially available ELISA kits, according to the manufacturer's instructions. Results are presented as amount of cytokine in cell-free supernatants. For soluble ST2 ELISA, the limit of detection for the assay was 31.3 pg/mL. In samples in which the concentration was below the lower limit of detection, it was not possible to determine whether the true value was 0, so, the concentration was arbitrarily assigned to be half the lower limit of detection.

Flow Cytometry

In this study, flow cytometry was used to evaluate (1) the expression of ST2 on ILCs and T-cells; and (2) the effect of IL-33 on RV-activated immune cells ($ST2^+$ ILCs, T cells, and NK cells) and the production of type 1 and type 2 cytokines in human PBMC. For cells undergoing intracellular cytokine staining, cells were incubated in the presence of Brefedlin A for 4 h prior to staining. Cell pellets were then harvested from 5-day cultures and washed twice with PBS and labeled with live/dead (L/D) aqua viability dye (Thermo Scientific, Waltham, MA, USA) for discrimination of dead cells. Cells were then stained with the following combinations of surface antibodies: (1) Lin-1-FITC (CD3/CD14/CD16/CD19/CD20/CD56), CD45-APC-H7, CD127-PEcy7, and ST2-PE; (2) CD3-APCcy7, CD4-FITC; or (3) CD56-AF488, for 30 min on ice and protected from light. Following surface staining, cells were then fixed and permeabilized using Fixation/Permeabilization solution kit (BD bioscience cat#554715). Cells were then intracellularly stained with IL-13-BV421 and IFNγ-PerCPcy5.5 for 30 min at room temperature, protected from light. Cells were fixed in 2% paraformaldehyde. Data were acquired using an LSRFortessa X-20 (BD Bioscience, San Jose, CA, USA) collecting 200,000 total events. Data were analyzed using FlowJo Tree star software (Version 7.6.1). Cell types have been defined as following: T cells ($CD3^+$ $CD4^+$), and NK cells ($CD56^+$). In some experiments, NK cells were further defined as $CD56^+$, CD3 negative. $ST2^+$ILCs were defined as $LIN1^-FcεR1^-CD45^+$ $CD127^+$ $ST2^+$ as previously described (16, 17). Information about the gating strategy and antibodies used in this work have been described in Figure S1 and Table S1 in Supplementary Material.

Statistical Analysis

Results were analyzed in Graphpad prism using ANOVA with Bonferroni correction, together with two-way comparisons used paired and unpaired *t*-tests where appropriate. Data are shown as mean ± SEM. Differences were regarded as statistically significant at $p \leq 0.05$.

RESULTS

Clinical Characteristics

Participants were between 18 and 53 years of age and comprised 18 people with mild/moderate asthma and 22 non-asthmatic volunteers. Based on skin-prick testing, people with asthma had a greater prevalence of allergic sensitization than non-asthmatic donors (66 versus 36%, respectively), though this difference was not statistically significant as determined by χ^2 analysis. Serum total IgE concentrations were significantly higher in the asthmatic group than in the non-asthmatic group (mean values = 361 and 194 IU/L, respectively; $p < 0.0125$).

Effects of IL-33 Pre-Exposure on RV-Induced Type 1 and 2 Cytokine Release

The effect of IL-33 on RV-induced type 1 and 2 cytokine release was evaluated 5 days post stimulation. In people with asthma, IL-33 pre-exposure led to significantly higher RV-induced (in red) IL-5 (**Figure 1A**) and IL-13 (**Figure 1B**) release (in red) compared to RV alone (in green) ($p = 0.02$ and $p = 0.003$ respectively). IL-33 alone (in blue) also induced IL-13 release in asthmatic individuals, which increased further in the presence of RV. Though IL-5 and IL-13 secretion tended to be higher in those with asthma than in healthy subjects, these differences were not statistically significant. In contrast, IL-33 pre-exposure had no significant effects on RV-induced IL-5 and IL-13 release by cells from healthy donors.

IFNγ release at day 5 post RV stimulation showed a very different response pattern (**Figure 1C**). RV-induced IFNγ release was lower in cells from asthmatics than in healthy donors ($p = 0.03$) and was not altered by IL-33 pre-exposure. In contrast, IL-33 pre-exposure led to significantly higher RV-induced IFNγ release by cells from healthy donors ($p = 0.001$).

Effects of IL-33 on Membrane-Bound ST2 (IL1-RL1) and Soluble ST2 (sST2) in Asthmatic and Healthy Individuals

The IL-33 membrane-bound receptor comprises two proteins, ST2 and its accessory receptor IL-1RAcP. However, ST2 can also exist as a soluble form named sST2. We, therefore, sought to determine whether IL-33 modulates ST2 and IL-1RAcP on RV-activated cells. Expression of *ST2* and *IL-1RAcP* were assessed using qRT-PCR (**Figure 2A**). Overall, cellular *ST2* expression was higher in asthmatic than healthy individuals (**Figure 2A**, left). RV-induced *ST2* expression was significantly higher in cells from asthmatic donors than in cells from healthy donors (in blue). Pre-exposure to IL-33 followed by RV (IL-33 + RV) (in red) further augmented the difference in *ST2* expression between asthmatic and healthy donors. In cells from asthmatics, IL-33 + RV stimulation lead to a ninefold increase in *ST2* expression, which was significantly higher than cells exposed to RV alone. In cells from healthy donors, RV and IL-33 (alone and in combination) had no significant effects on *ST2* expression. In contrast, *IL-1RAcP* expression was similar in cells from asthmatic and healthy individuals. Similarly, RV and IL-33 did not modify *IL-1RAcP* expression (**Figure 2A**, right).

To further investigate the increased *ST2* mRNA expression in asthmatic cells stimulated with IL-33 + RV, cell surface ST2 protein expression was assessed using flow cytometry, with a combination of surface antigens specific for innate lymphoid cells (ILC) (**Figure 2B**) and conventional T-cells (Figure S3 in

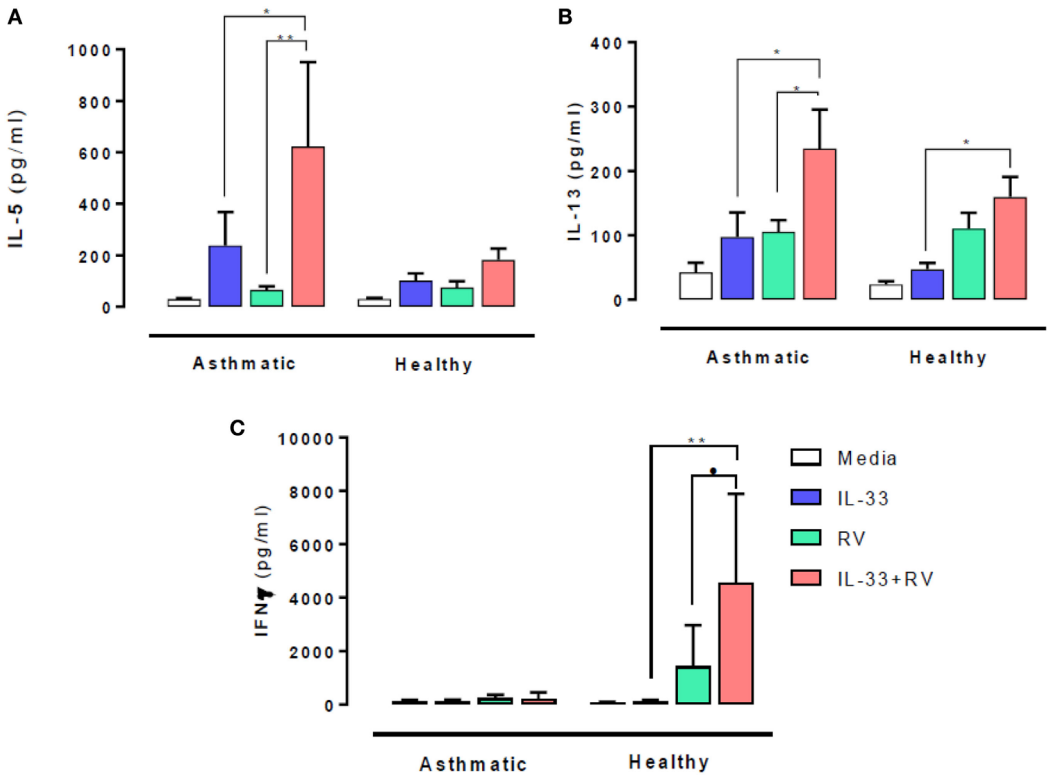


FIGURE 1 | Effects of IL-33 on rhinovirus (RV)-induced type 1 and 2 cytokine release. PBMCs from people with allergic asthma ($n = 14$) and non-allergic healthy controls ($n = 22$) were pre-exposed to media or IL-33 for 6 h and then cultured in the absence or presence of RV for 5 days. IL-5 **(A)**, IL-13 **(B)**, and IFNγ **(C)** production have been measured by ELISA. Data are expressed as mean (±SEM). Abbreviations: PBMC, peripheral blood mononuclear cells; IL-33, interleukin 33; RV, rhinovirus 16; ns, not significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Supplementary Material). Consistent with our qRT-PCR data, the frequency of ST2^{+}ILC was generally higher in asthmatic donors in comparison to healthy donors when stimulated under the same conditions (**Figure 2**, left). In asthmatic donors, IL-33 + RV caused a greater increase in the frequency of ST2^{+}ILC than RV and IL-33 individually (**Figure 2B**, right). In healthy donors, IL-33, RV, and IL-33 + RV had no effect on ST2^{+} ILC. Only a small proportion of unstimulated ILCs and T-cells were ST2^{+}, and there was no significant difference between cells from asthmatic and healthy donors (**Figure 2B**, Figure S3 in Supplementary Material). IL-33, RV, and IL-33 + RV had no appreciable effect on the proportion of ST2^{+} expressing T cells (Figure S3 in Supplementary Material).

Soluble ST2 (sST2) concentrations were measured from cell-free supernatants from 5-day PBMC cultures by ELISA (**Figure 2C**). In asthmatic individuals, the concentration of sST2 was significantly lower in IL-33 and IL-33 + RV stimulated cells than in unstimulated cells. There was a trend for lower sST2 concentrations with RV alone, but this was not statistically significant (**Figure 2C**). In contrast, both IL-33 and RV had minimal effects on sST2 concentrations in healthy individuals in comparison to unstimulated cells (**Figure 2C**). Interestingly, we found that IL-33 pre-exposure can further dampen RV-induced sST2 response, and this was observed in both asthmatic and healthy PBMC.

Which Cells Are Producing Type 1 and Type 2 Cytokines in Peripheral Blood?

We next sought to determine which leukocyte subsets respond to IL-33 and RV by producing type 1 and type 2 cytokines. Flow cytometry was used to determine the proportion of IL-13 or IFNγ producing ST2^{+}ILC, CD56^{+} cells, and T-cells (**Figures 3** and **4**). ST2^{+}ILC were the main IL-13 producers in asthmatic individuals (**Figure 3**). IL-33 alone significantly increased the frequency of IL-13 producing ST2^{+} ILC in asthmatic individuals in comparison to healthy. There was a trend toward a higher frequency of IL-13 producing ST2^{+}ILC in asthmatic donors in comparison to healthy donors when stimulated with RV alone and in combination with IL-33. However, this trend was not significant (**Figure 3**). In contrast, IL-33 pre-exposure had no significant effect on the frequency of IL-13 producing NK cells or T-cells in asthmatic and healthy individuals (**Figure 3**).

CD56^{+} cells were the predominant IFNγ-producing cells in both healthy and asthmatic donors (**Figure 4**), and this was most pronounced in unstimulated samples and those stimulated with IL-33 + RV (**Figure 4B**). Small numbers of IFNγ producing ST2^{+}ILC and T-cells were observed; however, there was little evidence that this was modified by IL-33 or RV (**Figure 4**). While CD56 can be expressed by both NK cells and conventional T-cells, it seems that the great majority of IFNγ producing CD56^{+} cells

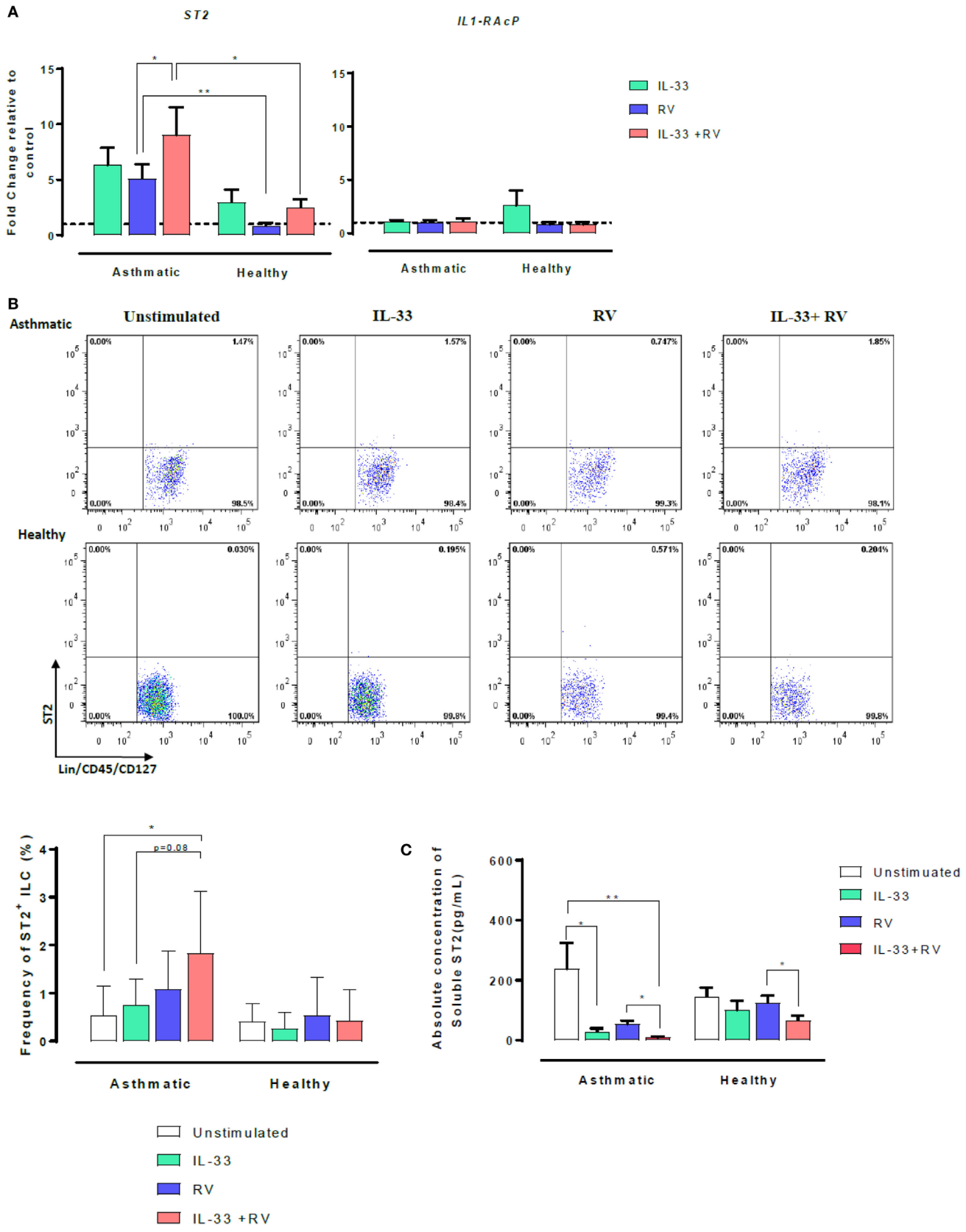


FIGURE 2 | Continued

FIGURE 2 | Effects of IL-33 on membrane-bound ST2 and soluble ST2 (sST2) in asthmatic and healthy individuals. PBMCs from patients with allergic asthma ($n = 12$) or non-allergic healthy controls ($n = 16$) were exposed to IL-33 or media for 6 h and then cultured in the absence or presence of RV for 5 days. **(A)** Relative expression of *ST2* and *IL-1RAcP* was assessed by qRT-PCR using primer pairs corresponding to each receptor and normalized to the house-keeping gene GAPDH. Basal expression is represented by a broken line. **(B)** The frequency of ST2L$^+$ ILC were then assessed by flow cytometry. ILCs were defined as Lin$^-$FcεR1$^-$CD45$^+$CD127$^+$ and then assessed for ST2. Raw dot plots representative of ST2$^+$ ILC from asthmatic and healthy donors. Data are presented as mean (±SEM) from 8 asthmatic and 6 healthy donors. **(C)** Soluble ST2 concentration was determined from cells free supernatants by ELISA. Data are expressed as mean (±SEM) from duplicate measurements. Abbreviations: PBMC, peripheral blood mononuclear cells; IL-33, interleukin 33; RV, rhinovirus 16; ST2$^+$ ILC2, ST2$^+$ innate lymphoid cells; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. $^*p < 0.05$, $^{**}p < 0.01$.

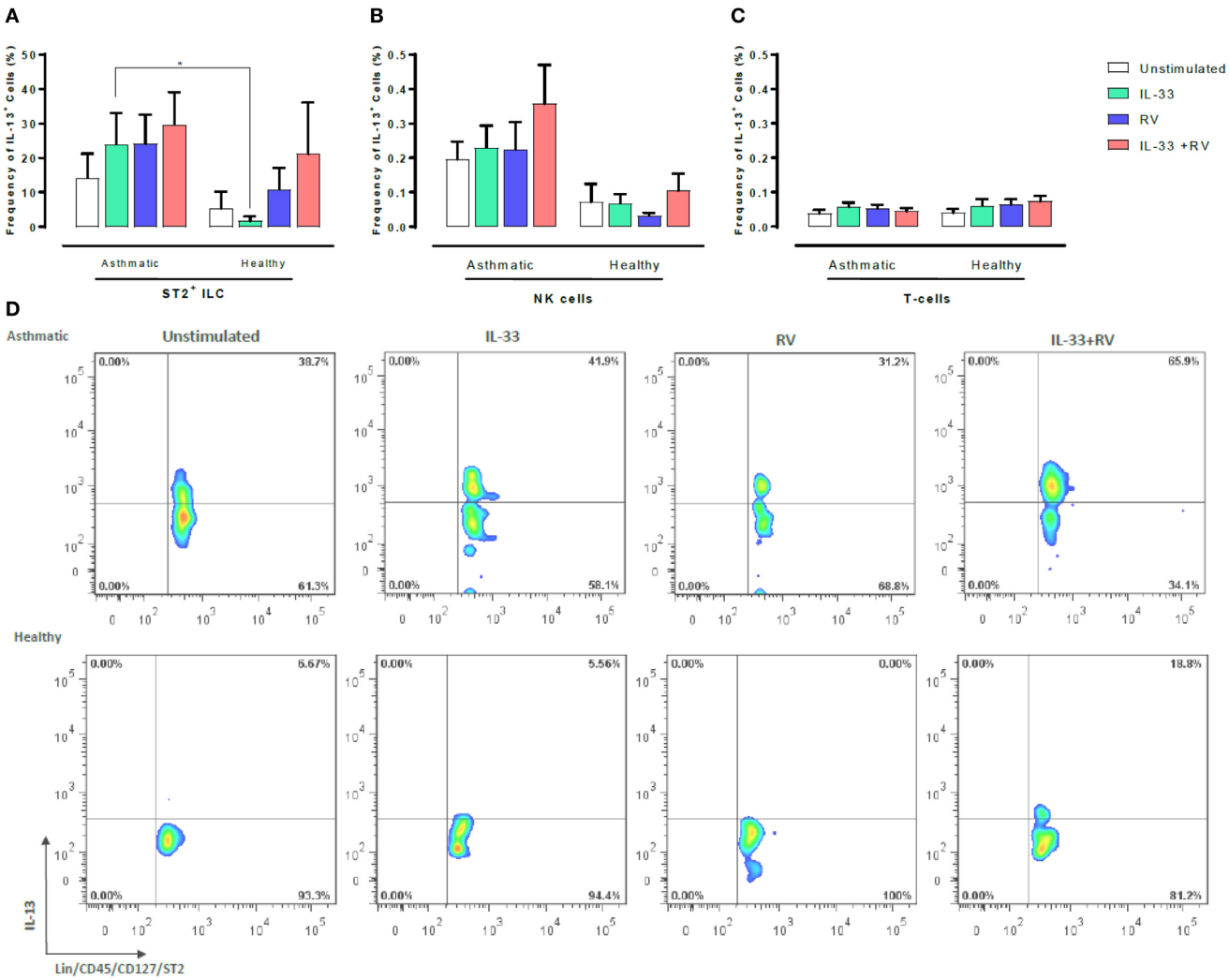


FIGURE 3 | Evaluation of IL-13-producing ST2$^+$ILC, CD56$^+$, and CD3$^+$ T cells in human PBMC. IL-13-producing cell subsets were evaluated using flow cytometry **(A–C)**. Raw dot plots representative of IL-13 + ST2 + ILC from 8 asthmatic and 6 healthy donors. PBMCs from people with allergic asthma and non-allergic healthy controls were pre-exposed to media or IL-33 for 6 h and then cultured in the absence or presence of RV for 5 days **(D)**. Abbreviations: PBMC, peripheral blood mononuclear cells; IL-33, interleukin 33; RV, rhinovirus 16, ST2$^+$ILCs, ST2$^+$ innate lymphoid cells. $^*p \leq 0.05$ and $^{**}p \leq 0.01$.

(**Figure 4**; Figure S4 in Supplementary Material) were NK cells rather than CD56$^+$ CD3$^+$ T-cells, as very few IFNγ-producing CD3$^+$ T-cells were identified. Moreover, additional experiments performed in a subset of participants identified that CD56$^+$ cells lacking CD3, CD14, and CD19 were the predominant source of IFNγ under the various stimuli used in our experiments (Figure S4 in Supplementary Material).

DISCUSSION

This study demonstrates that IL-33 has distinct effects on the *in vitro* cellular response to RV in asthmatic and healthy individuals. In asthmatics, IL-33 augments type 2 inflammatory responses to RV, but has little effect on type 1 immune responses. In healthy individuals, IL-33 enhances RV-induced type 1

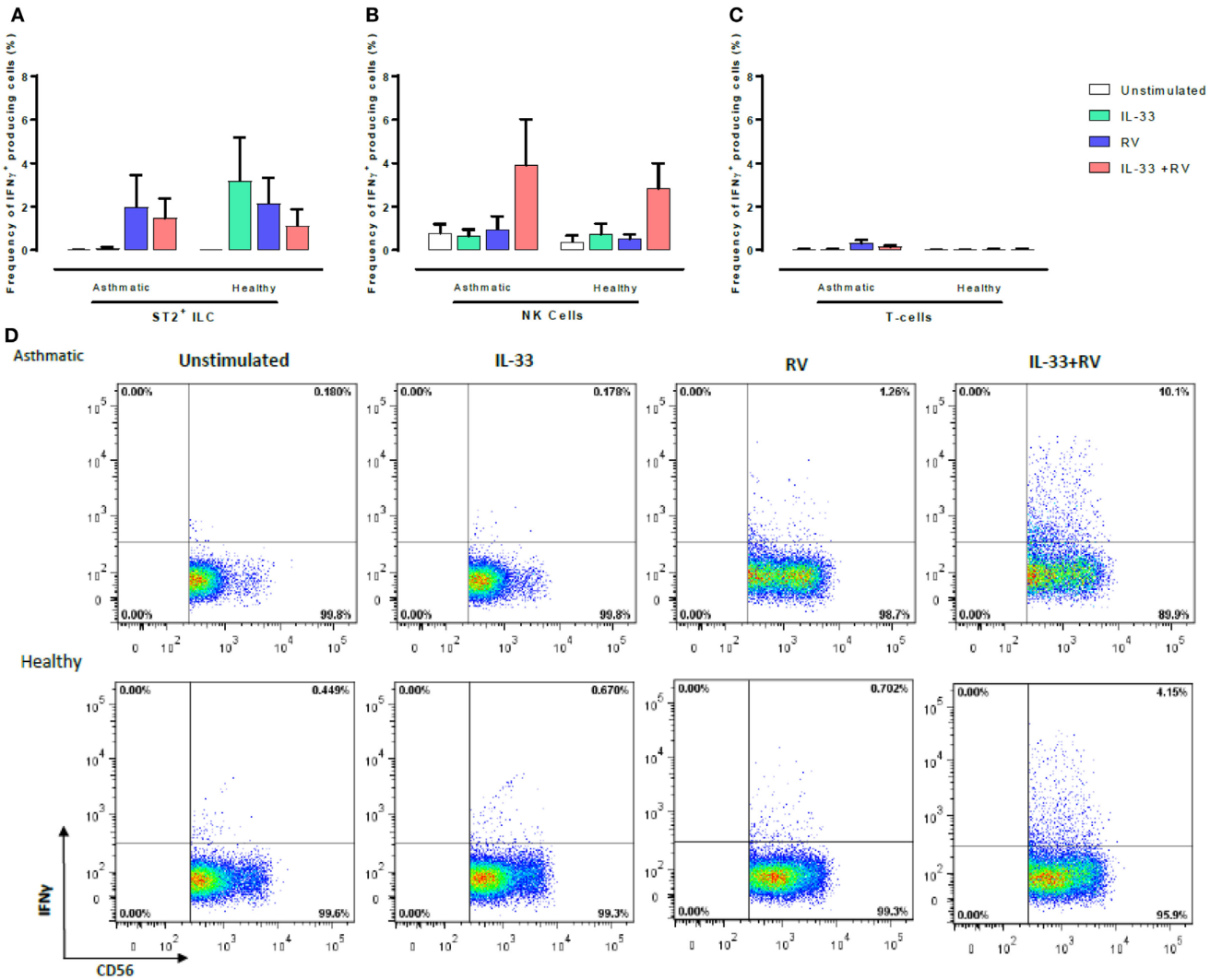


FIGURE 4 | Evaluation of IFNγ-producing ST2+ILC, CD56+, and CD3+ T cells in human PBMC. IFN-γ-producing cell subsets were evaluated using flow cytometry **(A–C)**. Raw dot plots representative of IFN-γ+CD56+ cells from 8 asthmatic and 6 healthy donors. PBMCs from people with allergic asthma and non-allergic healthy controls were pre-exposed to media or IL-33 for 6 h and then cultured in the absence or presence of RV for 5 days **(D)**. Abbreviations: PBMC, peripheral blood mononuclear cells; IL-33, interleukin 33; RV, rhinovirus 16; ST2+ILCs, ST2+ innate lymphoid cells. $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$.

immune response, but has no effect on type 2 responses. This is associated with differential regulation of the IL-33 receptor, with higher stimulated ST2 expression in cells from asthmatic than in cells from healthy individuals. In contrast, unstimulated ST2 expression and ST2 surface staining was similar in asthmatic and healthy participants. Our findings provide a possible mechanism by which IL-33 release amplifies eosinophilic inflammation in asthmatic individuals, yet has what appears to be a protective role in healthy individuals.

Given that ST2 was initially thought to be restricted to Th2 cells (18), it is surprising that circulating immune cells from both asthmatic and healthy individuals have a similar ability to respond to IL-33. More recent studies have identified that ST2 is more widely expressed on other immune cells (19). New evidence has emerged that Th1 effector cells can transiently express *ST2* during experimental viral infection and that Th1 effector cell differentiation and cytokine production is dependent on the IL-33/ST2 axis (20). This provides a plausible mechanism by which IL-33 was able to augment IFNγ release by RV-activated cells from healthy donors in the current study. Supporting this concept, our gene expression data show that cells from healthy donors express ST2 (**Figure 2A**).

Previous investigators have examined IL-33 receptor expression in asthma. Traister and colleagues demonstrated high airway epithelial ST2 expression in severe asthma, and importantly, linked ST2 expression to exacerbation risk and markers of type 2 inflammation (21). The IL-33 receptor is highly complex, as the *IL1RL1* gene is comprised of two splice variants that have opposing functional effects. The membrane bound form, ST2, which helps drive Th2 inflammation, and a

soluble form sST2, which acts as a decoy receptor and neutralizes the effect of IL-33 (12).

Our study for the first time simultaneously compared the relative balance of membrane bound ST2 and sST2 in asthmatic and healthy individuals, and how this is regulated *in vitro* by IL-33 and RV in the setting of asthma. In those with asthma, IL-33 pre-exposure and RV stimulation augmented *ST2* mRNA expression and elevated the frequency of ST2$^+$ILC, whereas sST2 production was low. In healthy subjects, IL-33 had little effect on RV-induced *ST2* mRNA and frequency of membrane bound ST2 on ILCs and T-cells, whereas sST2 was readily detected. This skewed expression of each splice variant may partially explain why IL-33 has the ability to amplify type 2 responses during viral infection in asthmatic individuals while having a protective role in healthy individuals. However, the underlying reason as to why one splice variant is translated over another remains unknown. Genome wide association studies have identified single nucleotide polymorphisms (SNPs) in the *IL1RL1* gene that are associated with asthma (21–23). Furthermore, SNPs located in the promoter region, have been linked to higher transcriptional activity of the *IL1RL1* gene (22), while non-synonymous SNPs in the coding region of this gene alters the ability of IL-33 to bind to its receptor (22). It is, therefore, possible that in those with asthma, one or more of these SNPs may be responsible for the translation of one splice variant over the other.

Jackson and colleagues recently demonstrated that RV-infected epithelial cells release IL-33 that induces peripheral blood naïve T-cells and ILC2 cells to produce type 2 cytokines under conditions of polyclonal activation (6). Our observations extend these findings to show that IL-33 can also enhance virus-specific type 2 cytokine release. We previously reported that RV-activated memory T-cells are responsible for much of the IFNγ and IL-13 production in healthy individuals (24). In contrast, the current study employed recently developed tools for accurately identifying human ILCs. We show herein that in those with asthma, ST2$^+$ILC are in fact the main IL-13 producers, rather than conventional T-cells. ILC2 are commonly identified with either ST2 or the prostaglandin D2 receptor, CRTH2 (17). Therefore, we used CRTH2 to further characterize this population. As per Smith and colleagues (17), we found that a large proportion of these cells also express this marker (data not shown) and believe this population to be ILC2. Consistent with this observation, ILC2 have been shown to be the primary producers of IL-13 in the context of IL-33 in the asthmatic airway (25).

Although the different experimental stimuli induced only subtle changes in the frequency of cells producing type 1 and 2 cytokines (**Figures 3** and **4**) between asthmatic and healthy individuals, it seems likely that greater difference would have occurred at earlier time points, especially as we observed that IL-33 + RV induced a marked increase in secreted type 1 and 2 cytokines in cultured supernatant between asthmatic and non-asthmatics, respectively (**Figure 1**). Functional studies in mice show that IL-13$^+$ ST2$^+$ ILC2 are most prominent between 6 and 10 days post infection (26, 27). However, this varies between models. In humans, it is unclear when the frequency of IL-13$^+$ILC2 peaks during RV Infection. In contrast, differences in IFNγ release in cultured supernatants between asthmatic and non-asthmatic donors can be detected as early as 48 h post infection (28). Therefore, greater differences in IFNγ$^+$ cells in healthy individuals may be observed at this time point. Future studies should assess how ILC2, NK cells, and T-cells interact over time to amplify type 1 or type 2 cytokines in the context of RV infection. Our preliminary experiments indicated that numbers of ST2$^+$ ILC and NK cells were similar in asthmatic and healthy participants (Figure S2 in Supplementary Material). However, it would be premature to read too much into these observations, which require confirmation in a much larger study.

Due to their substantial role in development of type 2 immune responses in allergic asthma, it is surprising that ST2$^+$ILC have the ability to produce IFNγ in response to IL-33 in healthy individuals. Although, it has been suggested that ILC2 have a protective role *in vivo* (29), no clear link has been established between this subset and type 1 cytokine production. However, new evidence in humans has emerged that ILC2 from non-allergic donors express higher levels of *NKG7*, *SOC1*, and *TBX21*. These genes are known to suppress type 2 associated transcriptional programs (30). Furthermore, low *SOC1* gene expression in the airways of severe asthmatics has been shown to be inversely associated with airway eosinophilia (31).

Another novel finding to emerge from this study is that RV and IL-33 act together to augment NK cell IFNγ production. Although T cells are also thought to produce IFNγ subsequent experiments showed little evidence of T cell involvement (Figure S5 in Supplementary Material). Interestingly, a recent study has shown that IL-33 exposed NK cells have the ability to negatively regulate ILC2s in mice *via* an IFNγ-dependent mechanism (32). Although our study supports that of Bi et al. (32), the possibility also exists that ILC2s are regulated by another pathway that then supports more favorable conditions for IFNγ production. In line with this concept, IFNα production from plasmacytoid dendritic cells in the context of IL-33 has also been shown to suppress proliferation of ILC2 and alleviate airway hyperreactivity (33). Future studies should also assess the extent to which IFNα and IFNγ influence ILC2 proliferation during experimental RV infection in the context of IL-33. This would provide a greater understanding into the mechanisms that regulate ILC2.

There are a number of limitations of the study that must be acknowledged. We studied a population of mild to moderate allergic asthmatics, and future studies will need to examine if IL-33 has similar effects across a broader range of asthma phenotypes and degrees of asthma severity. Second, although people with asthma had a greater prevalence of allergic sensitization than non-asthmatic donors, our study was not large enough to determine if our findings are confounded by atopy. In addition, we do not know if the effects of IL-33 on circulating cells also holds true for resident lymphocytes within the airway, and this needs to be examined in future studies. We do believe, however, that our *in vitro* experiments provide insight into the likely effects of airway IL-33 on lymphocyte populations that have recently migrated into the airway mucosa, with

responsiveness to IL-33 differing between lymphocytes from asthmatic and healthy individuals. Interestingly, findings from a large birth cohort study indicate that variations in RV-activated PBMC cytokine responses are associated with different clinical outcomes (3). Future studies should also directly examine interaction between lymphocyte populations and airway epithelial cells, and the extent to which this involves IL-33. Finally, there is evidence in asthma that T_{reg} cells are dysfunctional (34) and though we saw only minimal effects of IL-33 on T_{reg} (data not shown), our functional analysis was restricted to a small range of cytokines, and so this needs to be examined further.

In summary, these findings suggest that the effects of IL-33 on the cellular response to RV differ in asthmatic and healthy individuals, thus providing a potential mechanism by which RV infection in a high IL-33 tissue microenvironment can induce immunopathology in asthma but not in healthy people.

ETHICS STATEMENT

The Metro South Human Research Ethics Committee approved this study (Ethics approval number 2008000037).

AUTHOR CONTRIBUTIONS

JU, LJ, YX, and ML contributed to the conception of the study; JU, LJ, YX, ML, and MC contributed to the design of the study; LJ and YX performed majority of the experiments and the statistical analysis. LJ wrote the manuscript; JU, YX, ML, MC, and LM contributed to the revision of this manuscript and approved the submitted version.

ACKNOWLEDGMENTS

The study was supported by the National Health Medical Research Council of Australia (Project Grant and Centre for Research Excellence in Severe Asthma). We would also like to thank all the flow core staff of Translational Research Institute for the use of their facilities.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.01895/full#supplementary-material.

REFERENCES

1. Alper CM, Doyle WJ, Skoner DP, Buchman CA, Cohen S, Gwaltney JM. Prechallenge antibodies moderate disease expression in adults experimentally exposed to rhinovirus strain hanks. *Clin Infect Dis* (1998) 27:119–28. doi:10.1086/514634
2. Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy ClinImmunol* (2000) 105:692–8. doi:10.1067/mai.2000.104785
3. Custovic A, Belgrave D, Lin L, Bakhsoliani E, Telcian AG, Solari R, et al. Cytokine responses to rhinovirus and development of asthma, allergic sensitization, and respiratory infections during childhood. *Am J Respir Crit Care Med* (2018) 197:1265–74. doi:10.1164/rccm.201708-1762OC
4. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci U S A* (2008) 105:13562–7. doi:10.1073/pnas.0804181105
5. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. *Thorax* (2002) 57:328–32. doi:10.1136/thorax.57.4.328
6. Jackson DJ, Makrinioti H, Rana BMJ, Shamji BWH, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med* (2014) 190:1373–82. doi:10.1164/rccm.201406-1039OC
7. Zoltowska AM, Lei Y, Fuchs B, Rask C, Adner M, 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) 46:479–90. doi:10.1111/cea.12683
8. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, De Jongste JC, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy ClinImmunol* (2014) 134:170–7. doi:10.1016/j.jaci.2013.12.1080
9. Gordon ED, Simpson LJ, Rios CL, Ringel L, Lachowicz-Scroggins ME, Peters MC, et al. Alternative splicing of interleukin-33 and type 2 inflammation in asthma. *Proc Natl Acad Sci U S A* (2016) 113:8765–70. doi:10.1073/pnas.1601914113
10. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol* (2016) 16:676–89. doi:10.1038/nri.2016.95
11. Hentschke I, Graser A, Melichar VO, Kiefer A, Zimmermann T, Kroß B, et al. IL-33/ST2 immune responses to respiratory bacteria in pediatric asthma. *Sci Rep* (2017) 7:43426. doi:10.1038/srep43426
12. Palmer G, Lipsky BP, Smithgall MD, Meininger D, Siu S, Talabot-Ayer D, et al. The IL-1 receptor accessory protein (AcP) is required for IL-33 signaling and soluble AcP enhances the ability of soluble ST2 to inhibit IL-33. *Cytokine* (2008) 42:358–64. doi:10.1016/j.cyto.2008.03.008
13. Le Goffic R, Arshad MI, Rauch M, L'helgoualc'h A, Delmas B, Piquet-Pellorce C, et al. Infection with influenza virus induces IL-33 in murine lungs. *Am J Respir Cell Mol Biol* (2011) 45:1125–32. doi:10.1165/rcmb.2010-0516OC
14. Werder RB, Zhang V, Lynch JP, Snape N, Upham JW, Spann K, et al. 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. doi:10.1016/j.jaci.2017.07.051
15. Roponen M, Yerkovich ST, Hollams E, Sly PD, Holt PG, Upham JW. Toll-like receptor 7 function is reduced in adolescents with asthma. *Eur Respir J* (2010) 35:64. doi:10.1183/09031936.00172008
16. Christianson CA, Goplen NP, Zafar I, Irvin C, Good JT, Rollins DR, et al. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. *J Allergy ClinImmunol* (2015) 136:59–68.e14. doi:10.1016/j.jaci.2014.11.037
17. Smith SG, Chen R, Kjarsgaard M, Huang C, Oliveria JP, O'byrne PM, et al. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. *J Allergy ClinImmunol* (2016) 137:75–86.e8. doi:10.1016/j.jaci.2015.05.037
18. Vocca L, Di Sano C, Uasuf CG, Sala A, Riccobono L, Gangemi S, et al. IL-33/ST2 axis controls Th2/IL-31 and Th17 immune response in allergic airway diseases. *Immunobiology* (2015) 220:954–63. doi:10.1016/j.imbio.2015.02.005
19. Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol* (2016) 17:122–31. doi:10.1038/ni.3370
20. Baumann C, Bonilla WV, Fröhlich A, Helmstetter C, Peine M, Hegazy AN, et al. T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell responses. *Proc Natl Acad Sci U S A* (2015) 112:4056–61. doi:10.1073/pnas.1418549112
21. Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic and genotypic association of epithelial IL1RL1 to human TH2-like asthma. *J Allergy ClinImmunol* (2015) 135:92–9. doi:10.1016/j.jaci.2014.06.023

22. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. *J Allergy ClinImmunol* (2013) 131:856–65. doi:10.1016/j.jaci.2012.11.028
23. Ferreira MAR, Jansen R, Willemsen G, Penninx B, Bain LM, Vicente CT, et al. Gene-based analysis of regulatory variants identifies 4 putative novel asthma risk genes related to nucleotide synthesis and signaling. *J Allergy ClinImmunol* (2017) 139:1148–57. doi:10.1016/j.jaci.2016.07.017
24. Carroll ML, Yerkovich ST, Pritchard AL, Davies JM, Upham JW. Adaptive immunity to rhinoviruses: sex and age matter. *Respir Res* (2010) 11:184. doi:10.1186/1465-9921-11-184
25. Sugita K, Steer CA, Martinez-Gonzalez I, Altunbulakli C, Morita H, Castro-Giner F, et al. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. *J Allergy Clin Immunol* (2018) 141(1):300–10.e11. doi:10.1016/j.jaci.2017.02.038
26. Li BWS, Stadhouders R, De Bruijn MJW, Lukkes M, Beerens D, Brem MD, et al. Group 2 innate lymphoid cells exhibit a dynamic phenotype in allergic airway inflammation. *Front Immunol* (2017) 8:1684. doi:10.3389/fimmu.2017.01684
27. Stier MT, Goleniewska K, Cephus JY, Newcomb DC, Sherrill TP, Boyd KL, et al. STAT1 represses cytokine-producing group 2 and group 3 innate lymphoid cells during viral infection. *J Immunol* (2017) 199:510–9. doi:10.4049/jimmunol.1601984
28. Brooks GD, Buchta KA, Swenson CA, Gern JE, Busse WW. Rhinovirus-induced interferon-gamma and airway responsiveness in asthma. *Am J Respir Crit Care Med* (2003) 168:1091–4. doi:10.1164/rccm.200306-737OC
29. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* (2015) 519:242–6. doi:10.1038/nature14115
30. Lombardi V, Beuraud C, Neukirch C, Moussu H, Morizur L, Horiot S, et al. Circulating innate lymphoid cells are differentially regulated in allergic and nonallergic subjects. *J Allergy ClinImmunol* (2016) 138:305–8. doi:10.1016/j.jaci.2015.12.1325
31. Doran E, Choy DF, Shikotra A, Butler CA, O'rourke DM, Johnston JA, et al. Reduced epithelial suppressor of cytokine signalling 1 in severe eosinophilic asthma. *Eur Respir J* (2016) 48:715–25. doi:10.1183/13993003.00400-2015
32. Bi J, Cui L, Yu G, Yang X, Chen Y, Wan X. NK cells alleviate lung inflammation by negatively regulating group 2 innate lymphoid cells. *J Immunol* (2017) 198:3336–44. doi:10.4049/jimmunol.1601830
33. Maazi H, Banie H, Aleman Muench GR, Patel N, Wang B, Sankaranarayanan I, et al. Activated plasmacytoid dendritic cells regulate type 2 innate lymphoid cell-mediated airway hyperreactivity. *J Allergy Clin Immunol* (2018) 141(3):893–905.e6. doi:10.1016/j.jaci.2017.04.043
34. Chauhan A, Singh M, Agarwal A, Paul N. Correlation of TSLP, IL-33, and CD4 + CD25 + FOXP3 + T regulatory (Treg) in pediatric asthma. *J Asthma* (2015) 52:868–72. doi:10.3109/02770903.2015.1026441

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Jurak, Xi, Landgraf, Carroll, Murray and Upham. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

MINI REVIEW
published: 04 September 2018
doi: 10.3389/fimmu.2018.02004

Regulatory Mechanisms of IL-33-ST2-Mediated Allergic Inflammation

Hiroaki Takatori[1,2]*, *Sohei Makita*[1], *Takashi Ito*[1], *Ayako Matsuki*[1] and *Hiroshi Nakajima*[1]*

[1] Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan, [2] Department of Rheumatology, Hamamatsu Medical Center, Hamamatsu, Japan

Interleukin-33 (IL-33) plays multiple roles in tissue homeostasis, prevention of parasitic infection, and induction of allergic inflammation. Especially, IL-33-ST2 (IL-1RL1) axis has been regarded as the villain in allergic diseases such as asthma and atopic dermatitis and in autoimmune diseases such as rheumatoid arthritis. Indeed, a number of studies have indicated that IL-33 produced by endothelial cells and epithelial cells plays a critical role in the activation and expansion of group 2 innate lymphoid cells (ILC2s) which cause allergic inflammation by producing large amounts of IL-5 and IL-13. However, mechanisms that antagonize IL-33-ST2-mediated allergic responses remain largely unknown. Recently, several groups including our group have demonstrated cellular and molecular mechanisms that could suppress excessive activation of ILC2s by the IL-33-ST2 axis. In this review, we summarize recent progress in the regulatory mechanisms of IL-33-ST2-mediated allergic responses. Selective targeting of the IL-33-ST2 axis would be a promising strategy in the treatment of allergic diseases.

Keywords: IL-33, ST2, innate lymphoid cells (ILCs), regulatory T cells (Tregs), epithelial cells

OPEN ACCESS

Edited by:
Hui-Rong Jiang,
University of Strathclyde,
United Kingdom

Reviewed by:
Fons Van De Loo,
Radboud University Medical Center,
Netherlands
Paola Italiani,
Consiglio Nazionale Delle Ricerche
(CNR), Italy

***Correspondence:**
Hiroaki Takatori
takatorih@faculty.chiba-u.jp
Hiroshi Nakajima
nakajimh@faculty.chiba-u.jp

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *28 May 2018*
Accepted: *14 August 2018*
Published: *04 September 2018*

Citation:
Takatori H, Makita S, Ito T, Matsuki A and Nakajima H (2018) Regulatory Mechanisms of IL-33-ST2-Mediated Allergic Inflammation. Front. Immunol. 9:2004. doi: 10.3389/fimmu.2018.02004

INTRODUCTION

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family and mainly expressed in non-hematopoietic cells such as endothelial cells, epithelial cells, fibroblast-like cells, and myofibroblasts during homeostasis and in response to inflammation (1–5). IL-33 functions on target cells by binding to a heterodimeric receptor composed of suppression of tumorigenicity 2 (ST2, also known as IL-1RL1) and co-receptor, IL-1 receptor accessory protein (IL-1RAcP) (6, 7). Triggering of ST2/IL-1RAcP by IL-33 activates intracellular signaling pathways, which is initiated by homotypic protein-protein interactions with the adaptor molecule MyD88 and the further recruitment of IRAKs and TRAF6, leading to the expression of several inflammatory mediators through the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways (8, 9). IL-33 activates a variety of tissue-resident immune cells that express ST2, including group 2 innate lymphoid cells (ILC2s), mast cells, Th2 cells, regulatory T cells (Tregs), natural killer (NK) cells, eosinophils, basophils, dendritic cells, and alternatively activated macrophages (10–12).

Regarding *in vivo* relevance of IL-33-ST2 axis, it has been shown that bronchial epithelium is an important reservoir of IL-33 in the lung and that IL-33 expression is elevated in the airways of bronchial asthma along with disease severity (13). It has also been shown that increased IL-33 expression is associated with enhanced reticular basement membrane thickness in endobronchial tissues in children with severe therapy-resistant asthma (14). In addition, it has been reported that airway remodeling is absent in $ST2^{-/-}$ mice in house dust mite (HDM)-induced

neonatal asthma models (14). Moreover, IL-33 expression is not affected by steroid treatment in neonatal HDM-treated mice and in endobronchial tissues from children with severe therapy-resistant asthma (14). These findings suggest that IL-33-ST2 axis causes refractoriness in the allergic asthma.

Recently, numerous studies analyzing patient samples and murine models have revealed that ILC2s produce large amounts of IL-5 and IL-13 in response to IL-33 and IL-25 and that the activation of ILC2s by IL-33-ST2-mediated signaling contributes to anti-helminth responses and to the development of various allergic diseases such as asthma, atopic dermatitis, allergic rhinitis, and chronic rhinosinusitis (10–12, 15, 16). Furthermore, IL-33-ST2-mediated signaling seems to be involved in the development and exacerbation of non-allergic inflammatory conditions including arthritis, chronic obstructive pulmonary disease (COPD) with cigarette smoke, experimental autoimmune encephalomyelitis (EAE), periodontitis, and retina inflammation in murine models (12), although it remains unclear whether IL-33-ST2-ILC2s axis is involved in the pathogenesis of corresponding diseases in humans. Because IL-33-ST2-mediated signaling seems critical for the induction of excessive inflammatory responses in many conditions, it would be worthy of further investigation elucidating regulatory mechanisms against IL-33-ST2-mediated signaling to develop therapeutic strategies for these inflammatory diseases. In this review, we summarize recent progress in the regulatory mechanisms for IL-33-ST2-mediated allergic inflammation. Comprehensive reviews of the biology of IL-33 and its involvement in non-allergic conditions including autoimmune diseases and cancer have recently be described elsewhere (5, 9, 12).

REGULATORY MECHANISMS OF IL-33 EXPRESSION AND ITS INTERACTION WITH ST2

Bioactivity of IL-33-ST2 pathways is regulated by several distinct mechanisms (**Figure 1**). It has been shown that many of the single nucleotide polymorphisms (SNPs) in the human IL-33 gene associated with asthma are located in the promoter and intron 1, both of which are important for gene expression (17). Imai et al. have shown that keratinocyte-specific over-expression of IL-33 results in the spontaneous development of an atopic dermatitis (AD)-like skin disease, along with the activation of $ST2^+$ ILC2s (18). In addition, transgenic mice expressing IL-33 by a keratin 14 promoter spontaneously developed keratoconjunctivitis (19). These findings suggest that the expression level of IL-33 is critical for the induction of allergic inflammation and thus the suppression of IL-33 expression seems to be a good therapeutic strategy for the treatment of allergic diseases. In this regard, we have recently reported that IL-22, which belongs to the IL-10 family of cytokines, induces the expression of Reg3γ from lung epithelial cells in mice and that Reg3γ suppresses HDM-induced IL-33 expression and accumulation of ILC2s in the lung (20) (**Figure 1**). These results indicate that the IL-22-Reg3γ pathway is one of endogenous mechanisms that inhibit IL-33 expression. TGF-β is also shown to inhibit IL-33 expression in lung epithelial cells (21).

Recent studies indicate that microRNAs (miRNAs) also play pivotal roles in the regulation of the IL-33-ST2 axis. miRNAs are short noncoding RNAs that regulate the expression of various cytokines and pathogen recognition receptors at post-transcriptional levels by binding to the 3′-untranslated region (UTR) (22, 23). Regarding the regulation of IL-33 expression, Tang et al. have reported that miR-200b and miR-200c are significantly reduced in asthmatic patients and the induction of miR-200b and miR-200c decreases IL-33 expression in lung epithelial cells by binding 3′UTR of IL-33 mRNA (24). miR-487b has also been shown to suppress IL-33 expression in bone marrow-derived macrophages (25) and lung epithelial cells (26). On the other hand, RNA binding protein Mex-3B upregulates IL-33 expression by inhibiting miR-487b-3p-mediated repression of IL-33 (26). Additionally, it has been shown that miR-$155^{-/-}$ mice exhibit impaired IL-33 levels in lung in response to allergen challenge, suggesting the involvement of miR-155 in the induction of IL-33 expression (27). These findings indicate that multiple miRNAs are involved in the regulation of IL-33 expression during allergic responses.

The bioactivity of IL-33 is also regulated by posttranslational modification of IL-33 (5) (**Figure 2**). IL-33 is known to accumulate in the nucleus of producing cells by binding to histones and chromatin (1, 3, 28, 29). The N-terminal domain of IL-33 contains a chromatin-binding motif and a predicted nuclear localization sequence (28, 29). Importantly, the deletion of N-terminal domain containing a chromatin-binding motif has been shown to lead to early lethality in mice by constitutive secretion of IL-33 in serum, which causes severe multi-organ inflammation with infiltration of various immune cells including eosinophils, monocytes, neutrophils, and macrophages (30). These findings indicate that sequestration of IL-33 in the nucleus of IL-33-producing (or necrotic) cells is important to avoid the constitutive release of IL-33 and subsequent systemic inflammation. Interestingly, Osbourn et al. have recently shown that a product secreted by the parasite *Heligmosomoides polygyrus* (*H. polygyrus* Alarmin Release Inhibitor; HpARI) binds to IL-33 and also to nuclear DNA via its N-terminal complement control protein module pair (CCP1/2), tethering active IL-33 within necrotic cells, preventing its release, and inhibiting initiation of allergic responses (31). These findings suggest that *H. polygyrus* may escape from IL-33-mediated anti-parasitic responses via the production of HpARI.

It has also been reported that processing of IL-33 by proteases is crucial to regulate the bioactivity of IL-33 (**Figure 2**). IL-33 is cleaved by caspase-3 and caspase-7 during apoptosis, which generate two inactive IL-33 products (32–34). In addition, caspase-1 has been shown to cleave and inactivate IL-33 (32, 35, 36). Consistently, the administration of IL-33 greatly enhanced HDM-induced eosinophilic inflammation in caspase-1-deficient mice as compared with that in wild-type (WT) mice (36). Interestingly, cleaved chitin by acidic mammalian chitinase (AMCase) promotes the inactivation of IL-33 by inducing the activation of caspase-1 and subsequent activation of caspase-7, whereas uncleaved chitin promotes the release of IL-33 (35–37).

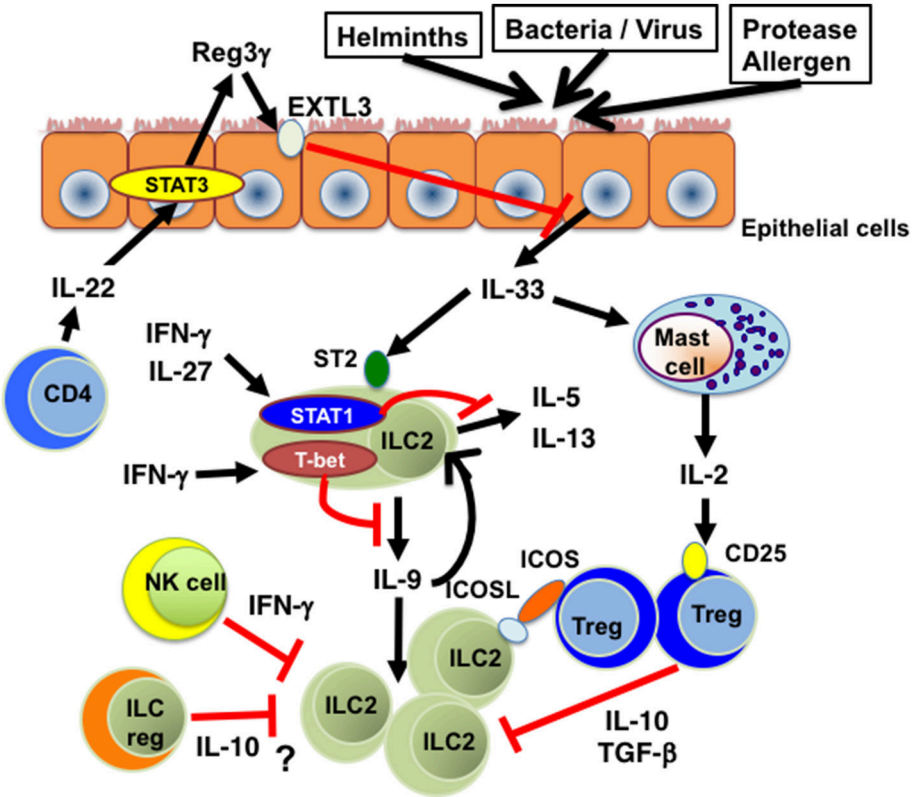


FIGURE 1 | The inhibitory effects of cytokines on IL-33-ST2-ILC2 axis**.** Th1 cell-related cytokines such as IFN-γ and IL-27 inhibit IL-33-induced cytokine production and proliferation of ILC2s in a STAT1-dependent manner. T-bet expressed in ILC2s suppresses IL-9 production and subsequent expansion of ILC2s. IL-22 produced by $CD4^+$ T cells suppresses IL-33 production from lung epithelial cells through Reg3γ induction. IL-2 produced by IL-33-stimulated mast cells promotes the expansion of Tregs, which suppress ILC2-mediated inflammation through the production of IL-10 and TGF-β. ICOS-ICOSL-mediated cell contact is also involved in Treg-mediated ILC2 suppression. In addition, IFN-γ produced by NK cells inhibits cytokine production and function of ILC2s. Moreover, IL-10-producing ILCregs could be a suppresser of IL-33-ST2-ILC2-mediated inflammation. EXTL3, exostatin-like 3; ICOS, inducible T-cell costimulator; ICOSL, inducible T-cell costimulator ligand; ILC2, group 2 innate lymphoid cells; ILCreg, regulatory innate lymphoid cell. NK cell, natural killer cell; Reg3γ, regenerating islet-derived protein 3 gamma; STAT1, signal transducer and activator or transcription 1; STAT3, signal transducer and activator or transcription 3; ST2, suppression of tumorigenicity 2; T-bet, T-box transcription factor expressed in T-cells; Treg, regulatory T cell.

Moreover, Cayrol et al. have recently shown that full-length IL-33 is cleaved by proteases derived from various environmental allergens such as fungi, HDM, cockroaches, and pollens and that the cleaved short mature forms of IL-33 are potent inducers of allergic airway inflammation (38). These findings suggest that the bioactivity of IL-33 is intricately regulated by endogenous and exogenous proteases.

The bioactivity of IL-33 is also controlled by the regulation of IL-33 binding to the receptor (**Figure 2**). IL-33 is shown to be rapidly inactivated in the extracellular lung environment by the oxidation of four critical cysteine residues and the formation of two disulfide bridges in the IL-1-like cytokine domain, resulting in an extensive conformational change that leads to the disruption of ST2 binding site (39). On the other hand, soluble spliced variant of ST2 (sST2), which directly binds to IL-33 and inhibits IL-33 bioactivity as a decoy receptor (40–42) is highly produced by mast cells and Th2 cells in asthmatic patients (40).

IL-33-ST2 signaling is also inhibited by SIGIRR (single immunoglobulin IL-1R-related molecule) which forms a complex with ST2 upon IL-33 stimulation (43). FBXL19, an orphan member of the Skp1-Cullin-F-box family of E3 ubiquitin ligases, selectively binds to ST2 to induce polyubiquitination and subsequent degradation of ST2 (44). Interestingly, IL-33 itself diminishes ST2 expression in mouse lung epithelial cells (MLE12 cells) in an FBXL19-dependent manner as one of negative feedback mechanisms (44).

REGULATORY MECHANISMS OF IL-33-ST2-ILC2 AXIS

Recent studies have shown that IL-33-ST2-ILC2s axis is inhibited by several distinct mechanisms (**Figure 1**). It has been reported that pro-inflammatory cytokines such as type 1 interferons and Th1 cell-inducing cytokines such as IFN-γ and IL-27 inhibit IL-33-ST2-ILC2s axis *in vivo* as well as *in vitro*. Type 1 interferons including IFN-β suppress the proliferation and cytokine production of IL-33-activated murine and human ILC2s

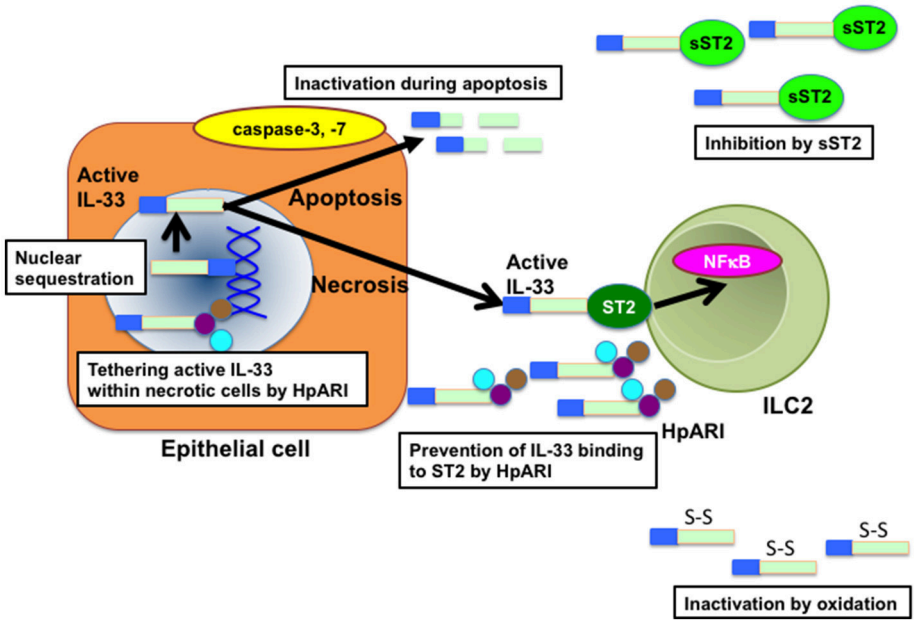


FIGURE 2 | Molecular mechanisms that affect IL-33 bioactivity. Nuclear localization of IL-33 by binding to histone and chromatin is essential for the suppression of IL-33 bioactivity. At cell necrosis, active IL-33 is released from the nuclei of producing cells. Released active IL-33 is abrogated by several mechanisms such as inhibition by decoy receptor sST2 and inactivation by the conformational change. In addition, active IL-33 is cleaved and inactivated by caspases such as caspase-3 and caspase-7 during apoptosis of producing cells. HpARI secreted by *H. polygylus* inhibits the release of IL-33 by binding directly to active IL-33 and nuclear DNA, tethering active IL-33 within necrotic cells. HpARI also prevents binding of active IL-33 to ST2. HpARI, *Heligmosomooides polygyrus* alarmin release inhibitor; NFκB, nuclear factor-kappa B. sST2, soluble suppression of tumorigenicity 2.

in a manner dependent on interferon-stimulated gene factor 3 (ISGF3) (45). Both IFN-γ and IL-27 have also been shown to inhibit cytokine production by ILC2s in IL-33-injected mice in a STAT1-dependent manner (45–48). In addition, STAT1-deficient mice have been shown to exhibit significantly increased IL-33 expression as compared with WT mice during respiratory syncytial virus (RSV) infection (49).

Both IFN-γ and IL-27 induce the expression of T-bet, a master regulator of Th1 cells (50), in $CD4^+$ T cells in a STAT1-dependent manner (51, 52). In this regard, we found that T-bet is expressed in lung ILC2s upon IFN-γ stimulation and that T-bet inhibits IL-33-induced accumulation of ILC2s in the lung and subsequent eosinophilic airway inflammation (16). Importantly, IL-9 production is significantly enhanced in T-$bet^{-/-}$ ILC2s and the neutralization of IL-9 markedly attenuates IL-33-induced eosinophilic airway inflammation in T-$bet^{-/-}$ mice (16). These results indicate that T-bet expressed in ILC2s plays an inhibitory role in IL-33-ST2-induced accumulation of lung ILC2s by inhibiting IL-9 production, consistent with a previous report showing that IL-9 promotes cell survival of ILC2s in an autocrine mechanism (53).

Regarding the other extracellular factors involved in the regulation of IL-33 bioactivity, it has been shown that prostaglandin D2 (PGD2) in combination with IL-33 stimulates IL-13 secretion and ST2 expression in human ILC2s through CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) (54, 55). On the other hand, lipoxin A4 (LXA4), which is a natural pro-resolving ligand for ALX/FPR2 receptors, decreases cytokine production by ILC2s in response to PGD2 and IL-33 (56). PGE2 and PGI2 have also been shown to exhibit a profound inhibitory effect on IL-33-mediated ILC2 expansion and cytokine production in mice and humans (57–59). Mechanistic studies revealed that PGE2- EP4 (E-prostanoid 4)-cyclic AMP signaling leads to the suppression of GATA3 and ST2 expression in ILC2s (59). These reports suggest that lipid mediators also seem to be critical factors for modulation of IL-33-ST2-ILC2 axis.

In addition, Laffont et al. have reported that androgen receptor-mediated signaling decreases the numbers of ILC2 progenitors in bone marrow and suppresses IL-33-induced ILC2-mediated airway inflammation (60). Moreover, upon IL-33 administration, the number of ILC2s is significantly increased in gonadectomized male mice as compared to sham-operated male mice (61). Consistently, testosterone attenuates alternaria extract-induced cytokine expression of ILC2s and eosinophils infiltration by decreasing the numbers of lung ILC2s (61). These results indicate that the imbalance in sexual hormones could be involved in the regulation of IL-33-mediated inflammatory diseases.

Recently, Moriyama et al. have reported that murine ILC2s express high levels of β2-adrenergic receptor (β2AR) (62) and the treatment with β2AR agonist (salmeterol) inhibits IL-33-induced IL-5 and IL-13 production of ILC2s, suggesting that the IL-33-ST2-ILC2 axis could be regulated by nervous systems including

β2AR signaling (63). Moreover, Thio et al. have shown that butyrate inhibits IL-33-induced GATA3 expression and cytokine production in ILC2s (64). On the other hand, Suzuki et al. have shown that IL-33 enhances the expression of Spred1 (sprouty-related Ena/VASP homology 1 domain-containing protein) in ILC2s, which negatively regulates ILC2 expansion and cytokine production by suppressing the Ras-ERK pathway (65).

IMMUNE CELL POPULATIONS INVOLVED IN THE REGULATION OF IL-33-ST2-ILC2 AXIS

Several cell types have been shown to regulate IL-33-ST2-ILC2 axis (**Figure 1**) in allergy. Roy et al. have shown that chymase derived from mast cells degrades IL-33 and that IL-33-induced TNF-α response is enhanced in chymase-deficient mice compared with WT mice (66). Moreover, it has been shown that IL-2 produced by IL-33-stimulated mast cells promotes the expansion of IL-10-producing $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs, thereby suppressing the development of IL-33-induced airway eosinophilia (67). These findings suggest that mast cells suppress IL-33-ST2-mediated inflammation through at least two distinct mechanisms. On the other hand, Rigas et al. have shown that iTregs attenuate IL-33-induced airway hyperreactivity by inhibiting IL-5 and IL-13 production from ILC2s and that the interaction of inducible T-cell costimulator ligand (ICOSL) on ILC2s with ICOS on Tregs is required for Treg-mediated suppression of ILC2 function (68). IFN-γ-producing NK cells also inhibit the cytokine expression and function of ILC2s (69).

More recently, Wang et al. have demonstrated that a regulatory subpopulation of ILCs (called ILCregs), which produce IL-10 and TGF-β, exists in murine and human intestine (70) and that ILCregs suppress intestinal inflammation driven by ILC1s and ILC3s via the secretion of IL-10 (70). Although it is reported that ILCregs isolated from the intestine do not suppress IL-33-induced IL-5 and IL-13 production from ILC2s (60), it remains unclear whether ILCregs are involved in the down-regulation of ILC2-mediated allergic responses *in vivo*.

POTENTIAL CLINICAL APPLICATIONS OF IL-33-ST2 AXIS INHIBITION FOR ALLERGIC DISEASES

Regarding the IL-33-ST2 axis in human allergic diseases, genome-wide association studies have clarified that a variety of SNPs in IL33 gene and IL1RL1 (ST2) gene are associated with asthma susceptibility (17). Additionally, Savenije et al. have reported that SNPs of IL1RAP and TRAF6, a downstream molecule of the IL-33-ST2 pathway, are associated with a phenotype of intermediate-onset wheeze, which is closely associated with sensitization (71), suggesting that polymorphisms of genes involved in IL-33-ST2 axis are associated with onset of asthma in childhood. Consistently, it has been shown that serum sST2 levels correlate well with the severity of asthma exacerbation (72). Moreover, a rare loss of function mutation in IL-33 gene has been shown to cause reduced numbers of eosinophils in blood and to protect against asthma (73). These finding suggest that the IL-33-ST2 axis seems to be a rational target in the treatment of allergic diseases.

Based on these findings, biologics including three anti-IL-33 monoclonal antibodies (ANB020; AnaptysBio, AMG282; Genentech, and REGN3500; Regeneron Pharmaceuticals) and an anti-ST2 monoclonal antibody (GSK3772847; GlaxoSmithKline) have already been under development and clinical trials have commenced (http://www.clinicaltrials.gov/). A phase 2 study of ANB020 for peanut allergy have been completed, and subjects are currently being recruited in phase 2 trials for severe eosinophilic asthma and atopic dermatitis. Phase 1 trials of AMG282 for mild atopic asthma and chronic rhinosinusitis with nasal polyps have also been completed. In addition, REGN3500 is being examined for asthma in a phase 1 trial. ST2 -targeting therapies with GSK3772847 for asthma are also currently in clinical trials. Because it is currently difficult to delete ILC2s *in vivo*, the neutralization of IL-33-ST2 pathway is a practical approach to regulate IL-33-ST2-ILC2 axis for the treatment of refractory allergic diseases.

CONCLUSION

In the past two decades, IL-33 has been regarded as an important initiator of immune responses. Additionally, recent studies have provided strong evidence that IL-33-ST2-ILC2s axis is one of central pathways in the pathogenesis of allergic inflammation. Accordingly, biologics targeting IL-33-ST2-ILC2 axis have already been under development. In addition, recent studies have shown that IL-33-ST2-ILC2s axis is negatively regulated by both cell intrinsic and extrinsic mechanisms. Given that IL-33-ST2-ILC2s axis seems to be deeply involved in the initiation phase of allergic diseases, these studies on the inhibitory mechanisms have yielded critical insights into the pathogenic mechanism underlying the onset of allergic diseases. Although further studies are needed to address how IL-33-ST2-ILC2s axis is negatively regulated in pathological situations in more detail, selective targeting of IL-33-ST2-ILC2s axis would be a promising strategy for the treatment of allergic diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported in part by Grants-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government, LGS Program (Leading Graduate School at Chiba University), MEXT, and Institute for Global Prominent Research, Chiba University, Japan.

REFERENCES

1. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, 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 USA.* (2007) 104:282–7. doi: 10.1073/pnas.0606854104
2. Küchler AM, Pollheimer J, Balogh J, Sponheim J, Manley L, Sorensen DR, et al. Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation. *Am J Pathol.* (2008) 173:1229–42. doi: 10.2353/ajpath.2008.080014
3. Moussion C, Ortega N, Girard JP. 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:e3331. doi: 10.1371/journal.pone.0003331
4. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, 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) 188:3488–95. doi: 10.4049/jimmunol.1101977
5. Cayrol C, Girard JP. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. *Immunol Rev.* (2018) 281:154–68. doi: 10.1111/imr.12619
6. Lingel A, Weiss TM, Niebuhr M, Pan B, Appleton BA, Wiesmann C, 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) 17:1398–410. doi: 10.1016/j.str.2009.08.009
7. Liu X, Hammel M, He Y, Tainer JA, Jeng US, Zhang L, et al. Structural insights into the interaction of IL-33 with its receptors. *Proc Natl Acad Sci USA.* (2013) 110:14918–23. doi: 10.1073/pnas.1308651110
8. Martin MU. Special aspects of interleukin-33 and the IL-33 receptor complex. *Semin Immunol.* (2013) 25:449–57. doi: 10.1016/j.smim.2013.10.006
9. Braun H, Afonina IS, Mueller C, Beyaert R. Dichotomous function of IL-33 in health and disease: from biology to clinical implications. *Biochem Pharmacol.* (2018) 148:238–52. doi: 10.1016/j.bcp.2018.01.010
10. Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* (2014) 31:31–7. doi: 10.1016/j.coi.2014.09.004
11. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity* (2015) 42:1005–19. doi: 10.1016/j.immuni.2015.06.006
12. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1016/j.ejphar.2015.04.047
13. Préfontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol.* (2010) 125:752–4. doi: 10.1016/j.jaci.2009.12.935
14. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol.* (2013) 132:676–85.e13. doi: 10.1016/j.jaci.2013.04.012
15. Kim BS, Wojno ED, Artis D. Innate lymphoid cells and allergic inflammation. *Curr Opin Immunol.* (2013) 25:738–44. doi: 10.1016/j.coi.2013.07.013
16. Matsuki A, Takatori H, Makita S, Yokota M, Tamachi T, Suto A, et al. T-bet inhibits innate lymphoid cell-mediated eosinophilic airway inflammation by suppressing IL-9 production. *J Allergy Clin Immunol.* (2017) 139:1355–67.e6. doi: 10.1016/j.jaci.2016.08.022
17. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. *J Allergy Clin Immunol.* (2013) 131:856–65. doi: 10.1016/j.jaci.2012.11.028
18. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, 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 USA.* (2013) 110:13921–6. doi: 10.1073/pnas.1307321110
19. Imai Y, Hosotani Y, Ishikawa H, Yasuda K, Nagai M, Jitsukawa O, et al. Expression of IL-33 in ocular surface epithelium induces atopic keratoconjunctivitis with activation of group 2 innate lymphoid cells in mice. *Sci Rep.* (2017) 7:10053. doi: 10.1038/s41598-017-10227-y
20. Ito T, Hirose K, Saku A, Kono K, Takatori H, Tamachi T, et al. IL-22 induces Reg3γ and inhibits allergic inflammation in house dust mite-induced asthma models. *J Exp Med.* (2017) 214:3037–50. doi: 10.1084/jem.20162108
21. Richards CD, Izakelian L, Dubey A, Zhang G, Wong S, Kwofie K, et al. Regulation of IL-33 by oncostatin M in mouse lung epithelial cells. *Mediators Inflamm.* (2016) 2016:9858374. doi: 10.1155/2016/9858374
22. Foster PS, Plank M, Collison A, Tay HL, Kaiko GE, Li J, et al. The emerging role of microRNAs in regulating immune and inflammatory responses in the lung. *Immunol Rev.* (2013) 253:198–215. doi: 10.1111/imr.12058
23. Fleshner M, Crane CR. Exosomes, DAMPs and miRNA: Features of stress physiology and immune homeostasis. *Trends Immunol.* (2017) 38:768–76. doi: 10.1016/j.it.2017.08.002
24. Tang X, Wu F, Fan J, Jin Y, Wang J, Yang G. Posttranscriptional regulation of Interleukin-33 expression by microRNA-200 in bronchial asthma. *Mol Ther.* (2018) 26:1808–17. doi: 10.1016/j.ymthe.2018.04.016
25. Xiang Y, Eyers F, Herbert C, Tay HL, Foster PS, Yang M. MicroRNA-487b is a negative regulator of macrophage activation by targeting IL-33 production. *J Immunol.* (2016) 196:3421–8. doi: 10.4049/jimmunol.1502081
26. Yamazumi Y, Sasaki O, Imamura M, Oda T, Ohno Y, Shiozaki-Sato Y, et al. The RNA binding protein Mex-3B is required for IL-33 induction in the development of allergic airway inflammation. *Cell Rep.* (2016) 16:2456–71. doi: 10.1016/j.celrep.2016.07.062
27. Johansson K, Malmhäll C, Ramos-Ramírez P, Rådinger M. MicroRNA-155 is a critical regulator of type 2 innate lymphoid cells and IL-33 signaling in experimental models of allergic airway inflammation. *J Allergy Clin Immunol.* (2017) 139:1007–16.e9. doi: 10.1016/j.jaci.2016.06.035
28. Baekkevold ES, Roussigné M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol.* (2003) 163:69–79. doi: 10.1016/S0002-9440(10)63631-0
29. Roussel L, Erard M, Cayrol C, Girard JP. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. *EMBO Rep.* (2008) 9:1006–12. doi: 10.1038/embor.2008.145
30. Bessa J, Meyer CA, de Vera Mudry MC, Schlicht S, Smith SH, Iglesias A, et al. Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. *J Autoimmun.* (2014) 55:33–41. doi: 10.1016/j.jaut.2014.02.012
31. Osbourn M, Soares DC, Vacca F, Cohen ES, Scott IC, Gregory WF, et al. HpARI protein secreted by a helminth parasite suppresses Interleukin-33. *Immunity* (2017) 47:739–51.e5. doi: 10.1016/j.immuni.2017.09.015
32. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA.* (2009) 106:9021–6. doi: 10.1073/pnas.0812690106
33. Lüthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* (2009) 31:84–98. doi: 10.1016/j.immuni.2009.05.007
34. Ali S, Nguyen DQ, Falk W, Martin MU. Caspase 3 inactivates biologically active full length interleukin-33 as a classical cytokine but does not prohibit nuclear translocation. *Biochem Biophys Res Commun.* (2010) 391:1512–6. doi: 10.1016/j.bbrc.2009.12.107
35. Kim LK, Morita R, Kobayashi Y, Eisenbarth SC, Lee CG, Elias J, et al. AMCase is a crucial regulator of type 2 immune responses to inhaled house dust mites. *Proc Natl Acad Sci USA.* (2015) 112:E2891–9. doi: 10.1073/pnas.1507393112
36. Madouri F, Guillou N, Fauconnier L, Marchiol T, Rouxel N, Chenuet P, et al. Caspase-1 activation by NLRP3 inflammasome dampens IL-33-dependent house dust mite-induced allergic lung inflammation. *J Mol Cell Biol.* (2015) 7:351–65. doi: 10.1093/jmcb/mjv012
37. Lamkanfi M, Kanneganti TD, Van Damme P, Vanden Berghe T, Vanoverberghe I, Vandekerckhove J, et al. Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. *Mol Cell Proteomics* (2008) 7:2350–63. doi: 10.1074/mcp.M800132-MCP200
38. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat Immunol.* (2018) 19:375–85. doi: 10.1038/s41590-018-0067-5
39. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun.* (2015) 6:8327. doi: 10.1038/ncomms9327
40. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem.* (2007) 282:26369–80. doi: 10.1074/jbc.M704916200

41. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. *Eur J Immunol.* (2015) 45:3034–44. doi: 10.1002/eji.201545501
42. Hayakawa H, Hayakawa M, Tominaga SI. Soluble ST2 suppresses the effect of interleukin-33 on lung type 2 innate lymphoid cells. *Biochem Biophys Rep.* (2016) 5:401–7. doi: 10.1016/j.bbrep.2016.02.002
43. Bulek K, Swaidani S, Qin J, Lu Y, Gulen MF, Herjan T, et al. The essential role of single Ig IL-1 receptor-related molecule/Toll IL-1R8 in regulation of Th2 immune response. *J Immunol.* (2009) 182:2601–9. doi: 10.4049/jimmunol.0802729
44. Zhao J, Wei J, Mialki RK, Mallampalli DF, Chen BB, Coon T, et al. F-box protein FBXL19-mediated ubiquitination and degradation of the receptor for IL-33 limits pulmonary inflammation. *Nat Immunol.* (2012) 13:651–8. doi: 10.1038/ni.2341
45. Duerr CU, McCarthy CD, Mindt BC, Rubio M, Meli AP, Pothlichet J, et al. Type I interferon restricts type 2 immunopathology through the regulation of group 2 innate lymphoid cells. *Nat Immunol.* (2016) 17:65–75. doi: 10.1038/ni.3308
46. Kudo F, Ikutani M, Seki Y, Otsubo T, Kawamura YI, Dohi T, et al. Interferon-γ constrains cytokine production of group 2 innate lymphoid cells. *Immunology* (2016) 147:21–9. doi: 10.1111/imm.12537
47. Mchedlidze T, Kindermann M, Neves AT, Voehringer D, Neurath MF, Wirtz S. IL-27 suppresses type 2 immune responses *in vivo* via direct effects on group 2 innate lymphoid cells. *Mucosal Immunol.* (2016) 9:1384–94. doi: 10.1038/mi.2016.20
48. Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, et al. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. *Nat Immunol.* (2016) 17:76–86. doi: 10.1038/ni.3309
49. Stier MT, Goleniewska K, Cephus JY, Newcomb DC, Sherrill TP, Boyd KL, et al. STAT1 represses cytokine-producing group 2 and group 3 innate lymphoid cells during viral infection. *J Immunol.* (2017) 199:510–9. doi: 10.4049/jimmunol.1601984
50. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* (2000) 100:655–69. doi: 10.1016/S0092-8674(00)80702-3
51. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, et al. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. *Proc Natl Acad Sci USA.* (2001) 98:15137–42. doi: 10.1073/pnas.261570598
52. Takeda A, Hamano S, Yamanaka A, Hanada T, Ishibashi T, Mak TW, et al. Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. *J Immunol.* (2003) 170:4886–90. doi: 10.4049/jimmunol.170.10.4886
53. Turner JE, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, Renauld JC, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med.* (2013) 210:2951–65. doi: 10.1084/jem.20130071
54. Chang JE, Doherty TA, Baum R, Broide D. Prostaglandin D2 regulates human type 2 innate lymphoid cell chemotaxis. *J Allergy Clin Immunol.* (2014) 133:899–901.e3. doi: 10.1016/j.jaci.2013.09.020
55. Xue L, Salimi M, Panse I, Mjösberg JM, McKenzie AN, Spits H, et al. Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. *J Allergy Clin Immunol.* (2014) 133:1184–94. doi: 10.1016/j.jaci.2013.10.056
56. Barnig C, Cernadas M, Dutile S, Liu X, Perrella MA, Kazani S, et al. Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med.* (2013) 5:174ra26. doi: 10.1126/scitranslmed.3004812
57. Zhou W, Toki S, Zhang J, Goleniewksa K, Newcomb DC, Cephus JY, et al. Prostaglandin I2 signaling and inhibition of group 2 innate lymphoid cell responses. *Am J Respir Crit Care Med.* (2016) 193:31–42. doi: 10.1164/rccm.201410-1793OC
58. Maric J, Ravindran A, Mazzurana L, Björklund Å, Van Acker A, Rao A, et al. Prostaglandin E_2 suppresses human group 2 innate lymphoid cell function. *J Allergy Clin Immunol.* (2018) 141:1761–73.e6. doi: 10.1016/j.jaci.2017.09.050
59. Zhou Y, Wang W, Zhao C, Wang Y, Wu H, Sun X, et al. Prostaglandin E_2 inhibits group 2 innate lymphoid cell activation and allergic airway inflammation through E-prostanoid 4-cyclic adenosine monophosphate signaling. *Front Immunol.* (2018) 9:501. doi: 10.3389/fimmu.2018.00501
60. Laffont S, Blanquart E, Savignac M, Cénac C, Laverny G, Metzger D, et al. Androgen signaling negatively controls group 2 innate lymphoid cells. *J Exp Med.* (2017) 214:1581–92. doi: 10.1084/jem.20161807
61. Cephus JY, Stier MT, Fuseini H, Yung JA, Toki S, Bloodworth MH, et al. Testosterone attenuates group 2 innate lymphoid cell-mediated airway inflammation. *Cell Rep.* (2017) 21:2487–99. doi: 10.1016/j.celrep.2017.10.110
62. Moriyama S, Brestoff JR, Flamar AL, Moeller JB, Klose CSN, Rankin LC, et al. β_2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science* (2018) 359:1056–61. doi: 10.1126/science.aan4829
63. Veiga-Fernandes H, Artis D. Neuronal-immune system cross-talk in homeostasis. *Science* (2018) 359:1465–6. doi: 10.1126/science.aap9598
64. Thio CL, Chi PY, Lai AC, Chang YJ. Regulation of type 2 innate lymphoid cell-dependent airway hyperreactivity by butyrate. *J Allergy Clin Immunol.* (2018). doi: 10.1016/j.jaci.2018.02.032. [Epub ahead of print].
65. Suzuki M, Morita R, Hirata Y, Shichita T, Yoshimura A. Spred1, a suppressor of the Ras-ERK pathway, negatively regulates expansion and function of group 2 innate lymphoid cells. *J Immunol.* (2015) 195:1273–81. doi: 10.4049/jimmunol.1500531
66. Roy A, Ganesh G, Sippola H, Bolin S, Sawesi O, Dagälv A, et al. Mast cell chymase degrades the alarmins heat shock protein 70, biglycan, HMGB1, and interleukin-33 (IL-33) and limits danger-induced inflammation. *J Biol Chem.* (2014) 289:237–50. doi: 10.1074/jbc.M112.435156
67. Morita H, Arae K, Unno H, Miyauchi K, Toyama S, Nambu A, et al. An Interleukin-33-mast cell-Interleukin-2 axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. *Immunity* (2015) 43:175–86. doi: 10.1016/j.immuni.2015.06.021
68. Rigas D, Lewis G, Aron JL, Wang B, Banie H, Sankaranarayanan I, et al. Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator-inducible T-cell costimulator ligand interaction. *J Allergy Clin Immunol.* (2017) 139:1468–77.e2. doi: 10.1016/j.jaci.2016.08.034
69. Bi J, Cui L, Yu G, Yang X, Chen Y, Wan X. NK cells alleviate lung inflammation by negatively regulating group 2 innate lymphoid cells. *J Immunol.* (2017) 198:3336–44. doi: 10.4049/jimmunol.1601830
70. Wang S, Xia P, Chen Y, Qu Y, Xiong Z, Ye B, et al. Regulatory innate lymphoid cells control innate intestinal inflammation. *Cell* (2017) 171:201–16.e18. doi: 10.1016/j.cell.2017.07.027
71. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy Clin Immunol.* (2014) 134:170–7. doi: 10.1016/j.jaci.2013.12.1080
72. Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S, et al. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med.* (2001) 164:277–81. doi: 10.1164/ajrccm.164.2.2008120
73. Smith D, Helgason H, Sulem P, Bjornsdottir US, Lim AC, Sveinbjornsson G, et al. A rare IL33 loss-of-function mutation reduces blood eosinophil counts and protects from asthma. *PLoS Genet* .(2017) 13:e1006659. doi: 10.1371/journal.pgen.1006659

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Takatori, Makita, Ito, Matsuki and Nakajima. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 24 October 2018
doi: 10.3389/fimmu.2018.02432

IL-33/ST2 Axis in Organ Fibrosis

Ourania S. Kotsiou[1,2]*, *Konstantinos I. Gourgoulianis*[1] *and Sotirios G. Zarogiannis*[1,2]

[1] *Department of Respiratory Medicine, Faculty of Medicine, School of Health Sciences, University of Thessaly, BIOPOLIS, Larissa, Greece,* [2] *Department of Physiology, Faculty of Medicine, School of Health Sciences, University of Thessaly, BIOPOLIS, Larissa, Greece*

Interleukin 33 (IL-33) is highly expressed in barrier sites, acting via the suppression of tumorigenicity 2 receptor (ST2). IL-33/ST2 axis has long been known to play a pivotal role in immunity and cell homeostasis by promoting wound healing and tissue repair. However, it is also involved in the loss of balance between extensive inflammation and tissue regeneration lead to remodeling, the hallmark of fibrosis. The aim of the current review is to critically evaluate the available evidence regarding the role of the IL-33/ST2 axis in organ fibrosis. The role of the axis in tissue remodeling is better understood considering its crucial role reported in organ development and regeneration. Generally, the IL-33/ST2 signaling pathway has mainly anti-inflammatory/anti-proliferative effects; however, chronic tissue injury is responsible for pro-fibrogenetic responses. Regarding pulmonary fibrosis mature IL-33 enhances pro-fibrogenic type 2 cytokine production in an ST2- and macrophage-dependent manner, while full-length IL-33 is also implicated in the pulmonary fibrotic process in an ST2-independent, Th2-independent fashion. In liver fibrosis, evidence indicate that when acute and massive liver damage occurs, the release of IL-33 might act as an activator of tissue-protective mechanisms, while in cases of chronic injury IL-33 plays the role of a hepatic fibrotic factor. IL-33 signaling has also been involved in the pathogenesis of acute and chronic pancreatitis. Moreover, IL-33 could be used as an early marker for ulcer-associated activated fibroblasts and myofibroblast trans-differentiation; thus one cannot rule out its potential role in inflammatory bowel disease-associated fibrosis. Similarly, the upregulation of the IL-33/ST2 axismay contribute to tubular cell injury and fibrosis via epithelial to mesenchymal transition (EMT) of various cell types in the kidneys. Of note, IL-33 exerts a cardioprotective role via ST2 signaling, while soluble ST2 has been demonstrated as a marker of myocardial fibrosis. Finally, IL-33 is a crucial cytokine in skin pathology responsible for abnormal fibroblast proliferation, leukocyte infiltration and morphologic differentiation of human endothelial cells. Overall, emerging data support a novel contribution of the IL-33/ST2 pathway in tissue fibrosis and highlight the significant role of the Th2 pattern of immune response in the pathophysiology of organ fibrosis.

Keywords: epithelial cells, inflammation, Interleukin-33, myofibroblasts, organ fibrosis, ST2, tissue remodeling

OPEN ACCESS

Edited by:
Fang-Ping Huang,
University of Hong Kong, Hong Kong

Reviewed by:
Remo Castro Russo,
Universidade Federal de Minas Gerais, Brazil
Marcela A. Hermoso,
Universidad de Chile, Chile

***Correspondence:**
Ourania S. Kotsiou
raniakotsiou@gmail.com

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *04 June 2018*
Accepted: *02 October 2018*
Published: *24 October 2018*

Citation:
Kotsiou OS, Gourgoulianis KI and Zarogiannis SG (2018) IL-33/ST2 Axis in Organ Fibrosis. Front. Immunol. 9:2432. doi: 10.3389/fimmu.2018.02432

INTRODUCTION

Interleukin (IL) 33 was first described in 1999 as a protein (DV27) overexpressed in vasospastic cerebral arteries in a canine subarachnoid hemorrhage model (1). Later, in 2003, it was characterized at the molecular level as a nuclear factor abundantly expressed in human high endothelial venule cells (2). The mechanisms mediating its effects were elucidated by Schmitz et al., as well as Dinarello, that identified IL-33 as a cytokine of the IL-1 superfamily (3, 4). Its name was given due to its adherence to the style of the IL-1 superfamily nomenclature, indicating that IL-33 was not merely a functional copy of IL-1α and IL-1β proteins (3, 4). The role of IL-33 as an alarmin was established participating in tissue homeostasis, signaling via the IL-1 receptor-related suppression of tumorigenicity 2 receptor (ST2), and inducing T helper type 2 (Th2) immune responses (3, 4).

Normally human IL-33 is mainly expressed and stored in the nucleus of endothelial and epithelial cells (5). IL-33 is a dual-function cytokine: the full-length IL-33 protein (flIL-33) serves as an intranuclear gene regulator, and the mature IL-33 (mIL-33) serves as an extracellular cytokine upon release from damaged or necrotic cells (6). IL-33 is passively and rapidly released from damaged cells, as a tissue-barrier component in response to stimuli or cell injury. However, it can also be actively secreted by immune cells. The abundant basal IL-33 expression in tissues can be further increased during inflammation (7). Notably, it has been documented that the fraction of IL-33 produced by tissues rather than that provided by immune cells, is necessary for Th2-induced airway inflammation (8). An inflammatory microenvironment may exacerbate disease-associated functions of IL-33 through the generation of highly active mature forms (9). Neutrophil serine proteases such as cathepsin G and elastase which secreted during inflammation have been shown to regulate IL-33 activity, by processing flIL-33 and generate biologically highly active mature forms of IL-33, *in vivo* (10). Furthermore, serine proteases secreted by activated mast cells (chymase and tryptase) generate mIL-33 with potent activity on lymphoid cell type 2 ILC2s (11). On the contrary, it is still unknown whether and which endogenous proteases have a similar capacity (7). It has only been reported an endogenous calcium-dependent caspase which is called calpain that mediates pro-IL-33 cleavage and mIL-33 production. Calpain is secreted when the cells are severely damaged by external stimulation such as inflammatory stimuli; and subsequently, the level of intracellular calcium ion is raised by an influx of extracellular ion or a release from an intracellular store (7, 12). Although both flIL-33 and mIL-33 can bind to and signal through ST2, mIL-33 exhibit 10-fold higher affinity and bioactivity than flIL-33 (6, 10).

Conversely, the ST2 receptor is predominantly expressed by immune cells involved in innate immunity, including mast cells, ILC2s, macrophages, dendritic cells (DCs), eosinophils, basophils, natural killer cells (NK cells). Furthermore, ST2 is expressed by cells participating in adaptive immunity such as CD4 +, CD8 + T cells, and T-regulatory cells (Tregs) (13, 14).

In humans, there are three ST2 isoforms. IL-33 signals via the ST2L receptor which has a membrane-bound domain, an extracellular segment composed of three linked immunoglobulin-like motifs, and a cytosolic Toll/interleukin-1 receptor domain. The soluble ST2 (sST2) isoform lacks the transmembrane and cytoplasmic domains and includes a unique nine amino-acid C-terminal sequence, constitutes a decoy receptor that does not signal. The ST2V isoform which is characterized by the absence of an immunoglobulin-like motif and alternative splicing of the C-terminal portion of ST2 is thought to be a form which is primarily found in gastrointestinal tissues (15, 16).

The IL-33/ST2 axis has been widely studied in respiratory, digestive, urogenital, heart and liver pathologies and the abundance of literature suggests a pivotal role of this pathway in the pathogenesis of an increasing number of diseases (**Table 1**). Emerging data have shown that IL-33/ST2 axis is involved in a variety of biological processes such as the development and regulation of immune responses, restoration of normal tissue homeostasis by promoting wound healing and repair. However, the IL-33/ST2 signaling pathway is involved in the loss of balance between extensive inflammation and tissue regeneration lead to remodeling that constitutes the hallmark of fibrosis (14, 72).

Despite the burden of human organ fibrosis, there are still many things unknown regarding the underlying mechanisms. On this note, it has been supported that the IL-33/ST2 axis exerts anti-inflammatory and anti-proliferative effects in many diseases; however, it has also been shown that results in fibrotic effects in others. Although several lines of evidence demonstrate that there

Abbreviations: AP, acute pancreatitis; ASCF, American College of Cardiology Foundation; AHA, American Heart Association; Acute Kidney Injury; ALI, Acute Lung Injury; AP-1, Activator Protein 1; a-SMA, α-Smooth Muscle Actin; BAL, Bronchoalveolar Lavage; BLM, Bleomycin; BM, Bone Marrow; CKD, Chronic Kidney Disease; CXCL, Chemokine (C-X-C motif) Ligand; DAMPs, Damage Associated Molecular Patterns; ECM, Extracellular Matrix; EGFR, Epidermal Growth Factor Receptor; EMT, Epithelial to Mesenchymal Transition; ERK, Extracellular Signal-Regulated Kinase, eSOD, Erythrocyte Superoxide Dismutase; ESRD, End-Stage Renal Disease; Fbln1, fibulin-1; Fli1, Friend Leukemia Virus Integration; flIL-33, full-length Interleukin 33; FoxO3a, Forkhead box O3a; Gal-3, Galectin-3; GFLs, glial cell-line derived neurotrophic factor family ligands; HF, Heart Failure; HK, Human Kidney; HSCs, Hepatic Stellate Cells; HSP, Heat Shock Protein; IBD, Inflammatory Bowel Disease; IL, Interleukin; IL-13Ra1, IL-13 Receptor subunit alpha 1; ILC2s, Innate Lymphoid Cell type 2; IFN-γ, Interferon-γ; IPF, Idiopathic Pulmonary Fibrosis; IRI, Ischemia-Reperfusion Injury; JNK, Jun N-terminal kinase; LPS, Lipopolysaccharide; LVAD, Left Ventricular Assist Device; MAPK, Mitogen-Activated Protein Kinase; MCP-1, Monocyte Chemoattractant Protein-1; MEK, Mitogen-Activated Protein Kinase\ERK kinase; mIL-33, mature Interleukin 33; MMP, Matrix Metalloproteinase; MyD88, Myeloid Differentiation primary response 88; NAFLD, Non-alcoholic Fatty Liver Disease; NK cells, Natural Killers cells; NASH, Non-alcoholic Steatohepatitis; NF, Nuclear Factor; PDGF, Platelet-Derived Growth Factor; PDGFR, Platelet-Derived Growth Factor Receptor; pDCs, plasmacytoid Dendritic Cells; PGE2, Prostaglandin E2; PRIDE, Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department; PSCs, Pancreatic Stellate Cells; RAG, Recombination-Activating Gene; SEMFs, Subepithelial Myofibroblasts; SLE, Systemic Lupus Erythematosus; SSc, Systemic Sclerosis; STAT6, Signal Transducer and Activator of Transcription 6; ST2, Suppression of Tumorigenicity 2; sST2, soluble ST2; ST2L, transmembrane ST2; TGF-β, Transforming Growth Factor β; Th1, T helper type 1; Th2, T helper type 2; TIMP1, Tissue Inhibitor of Metalloproteinases 1; TNF-a, Tumor Necrosis Factor a; Tregs, T-regulatory cells; rIL-33, recombinant Interlrukin-33; UC, Ulcerative Colitis.

TABLE 1 | The main roles of IL-33 in organ fibrosis.

Fibrotic disease	IL-33 sources	Role of IL-33	IL-33/ST2 Axis	References
PULMONARY FIBROSIS				
IPF	Airway epithelial cells	Pro-fibrogenetic role	*mIL-33:* ST2-, Th2-, and macrophage-dependent fashion	(17, 18)
SSc-related fibrosis	Endothelial cells			(19)
	Fibroblasts			
BLM-induced fibrosis	Myofibroblasts			(6, 20–23)
	Innate immune cells			
			fllL-33: ST2-independent, Th2-independent fashion	
LIVER FIBROSIS				
Cirrhosis	Hepatocytes	Pro-fibrogenetic role	ST2-, Th2-, ILC2s-dependent fashion	(20, 24–28)
	Hepatic stellate cells			
Viral hepatitis				(29)
Primary biliary cirrhosis				(30, 31)
NASH				(32, 33)
PANCREATIC FIBROSIS				
Acute and chronic pancreatitis	Pancreatic stellate cells	Pro-inflammatory role	ST2- dependent fashion	(18, 34–37)
Pancreatic cancer	Pancreatic myofibroblasts	Pro-fibrogenetic role	Proliferation and migration of pancreatic myofibroblasts	
	Pancreatic acinar cells			
Murine autoimmune pancreatitis	Plasmacytoid dendritic cells	Pro-inflammatory role	ST2- dependent fashion	(38)
Human IgG4-related AIP		Pro-fibrogenetic role		
INTESTINE FIBROSIS				
IBD	Colonic subepithelial	Pro-inflammatory role	ST2-, Th2- dependent fashion	(39–47)
(UC	myofibroblasts	Pro-fibrogenetic role		
Pediatric Crohn's ileitis)	Stromal cells			
	Epithelial cells			
	Pericryptal fibroblasts			
RENAL FIBROSIS				
Transplanted kidney interstitial fibrosis	Peritubular capillary endothelial cells	Pro-inflammatory role	ST2-, Th2-, ILC2s- dependent fashion	(48–51)
		Pro-fibrogenetic role		
	Human kidney cells	Renal tubular Epithelial to Mesenchymal Transition		
IRI-induced renal fibrosis				(52–54)
Diabetic kidney disease				(19, 55, 56)
SLE, SSc associated Kidney fibrosis				
HEART FIBROSIS				
Heart failure	Cardiac fibroblasts	Cardioprotective effects	ST2-, Th2- dependent fashion	(57–64)
Myocardial infarction	Cardiomyocytes	Anti-hypertrophic effects		
Post-implantation		Anti-fibrotic effects		
SKIN FIBROSIS				
Cutaneous fibrosis	Dermal fibroblasts	Pro-fibrogenetic role	Eosinophil-mediated Th2 immune responses	(3, 65–67)
Irradiation-induced fibrosis		Pro-inflammatory role	ST2-, Th2- Tregs-dependent fashion	
SSc-associated fibrosis		Morphologic differentiation of human endothelial cells		(19, 68–70)
BLM-induced skin fibrosis				(71)

BLM, Bleomycin; EMT, Epithelial to Mesenchymal Transition; fllL-33, full-length Interleukin 33; IBD, Inflammatory Bowel Disease; IL-33, Interleukin 33; ILC2s, Innate Lymphoid Cell type 2; IPF, Idiopathic Pulmonary Fibrosis; IRI, Ischemia-Reperfusion Injury; mIL-33, mature Interleukin 33; NASH, Non-alcoholic Steatohepatitis; SLE, Systemic Lupus Erythematosus; SSc, Systemic Sclerosis; ST2, Suppression of Tumorigenicity 2; Th2, T helper type 2; UC, Ulcerative Colitis.

is a potential role of IL-33/ST2 in remodeling and differentiation processes in, there is still room for better understanding. The aim of this review is to critically evaluate the available evidence regarding the role of the IL-33/ST2 axis in organ fibrosis.

IL33/ST2 AXIS PARTICIPATES IN THE TH2-MEDIATED INFLAMMATORY RESPONSE AND EXACERBATES TISSUE REMODELING

Acute wounds initiate an early inflammatory response, directing the subsequent phases of healing (73). However, the triggers of inflammation and wound repair upon injury are still unclear (73). IL-33 has been found to participate in the early inflammatory process as other members of the IL-1 family do (73). More specifically, increased mRNA and protein expression after scratching *in vivo* have been demonstrated; suggesting a role for IL-33 in wound healing (74). Furthermore, ST2 receptor signaling improves wound closure by promoting the transition of macrophages from an inflammatory to a non-inflammatory state during healing, supporting epidermal closure, angiogenesis, and reduced scarring (73). During wound healing extracellular mIL-33 interacts with the ST2L receptor and the complex of IL-33/ST2L activates myeloid differentiation primary response 88 (MyD88) intracellular cascades that drive production of type 2 cytokines (such as IL-13) from polarized Th2 cells (73). Besides, it has been suggested that proteolytically uncleaved, flIL-33, which remains predominantly intracellular and intranuclear, promotes inflammation in an ST2-independent fashion through regulation of gene expression (75).

The key role of the IL-33/ST2 axis in tissue remodeling is better understood considering the crucial role of immune responses during tissue regeneration. Of note, increased production of epithelial IL-33 could lead to accumulation of innate type 2 cells during the alveolar period of lung development; that is when the lung is maximally remodeled (76). IL-33 promotes ILC2s function that enhance tissue healing, remodeling, and homeostasis in the post-partum period. More convincingly, studies in adult murine models showed that IL-33 activates lung ILC2s and renders them resistant to interferon-γ (IFN-γ)-mediated suppression of IL-5 and IL-13 production (22). Th2 cytokines such as IL-13 are crucial mediators of inflammation and remodeling (22). In addition, IL-33 activates alternatively activated M2 macrophages that control tissue remodeling during lung postnatal branching morphogenesis (77).

In other words, type 2 immunity influences lung development and/or remodeling while the spontaneous activation of type 2 cells in the embryonic period have been found to require extracellular IL-33 and ST2 signaling (76, 78). Meanwhile, IL-33 contributes to the development of type 2 immune environment in lungs at a young age as it lowers the threshold for innate immune responses to allergens (76).

IL-33/ST2 AXIS IN PULMONARY FIBROSIS

Pulmonary fibrosis is a non-neoplastic pulmonary disease that is primarily caused by an uncontrolled wound-healing response. Idiopathic pulmonary fibrosis (IPF) is a highly lethal pathological entity of unknown etiology that is characterized by inflammation, fibroblast accumulation, and excessive collagen deposition. Importantly, IL-33 mRNA and protein levels have been found significantly increased in the bronchoalveolar lavage (BAL) fluids of patients with IPF (17) and systemic sclerosis (SSc)-related fibrosis as compared to healthy controls (19). Furthermore, the expression of IL-33 mRNA was also enhanced in IPF lung tissue (23).

IL-33 is also elevated in the bleomycin (BLM)-induced murine model of lung injury and fibrosis (20, 21, 23). The lungs of BLM-treated mice showed a substantial accumulation of IL-33-positive cells (20, 21, 23). Specifically, it has been documented that IL-33 and BLM result in synergistic effects on pulmonary fibrosis *in vivo* (6). In detail, mIL-33 production is induced in macrophages by BLM (6, 20, 21, 23). Subsequently, IL-33 enhances the polarization of macrophages toward an M2 phenotype (6, 79, 80). It is well established that the pro-fibrogenic activity of IL-33 is mainly attributed to its involvement in M2 macrophage polarization, as macrophages with alternative activation, rather than classical activation, serve to accelerate pulmonary fibrosis (6, 79, 80). A clear link between biologic splice variants of IL-33 (mIL-33) and a Th2 innate immune response has been demonstrated (80). Mature forms of IL-33 have been reported to drive production of extremely high levels of Th2 cytokines such as IL-13 (80). Furthermore, M2 macrophages are polarized by IL-13, and they further promote a Th2 reaction through the IL-13 and the transforming growth factor β (TGF-β) production, vice versa (20–23).

Several studies have demonstrated the importance of Th2 cells in fibrosis since IL-4, IL-5, and IL-13 have been causally linked to fibrosis (80). In detail, IPF fibroblasts are hyper-responsive to cytokines such as IL-13, at the same time, fibroblasts and innate immune cells are important sources of IL-33 (80). Enhanced production of TGF-β and IL-13 are essential for the development of pulmonary fibrosis by inducing myofibroblast differentiation and stimulating the production of extracellular matrix components, such as collagen (79, 80). Therefore, these data support that precise control of alveolar TGF-β activation and IL-13 are essential for alveolar homeostasis (79).

Previous work has shown that deficiency of the Akt2 isoform resulted in M2 macrophages polarization producing IL-13 and TGF-β and in the expansion of IL-13 recruiting ILC2s (6). Thus, Akt2 regulates pulmonary fibrosis by up-regulating the pro-fibrotic TGF-β and IL-13 production by macrophages (81). It is likely that the induction of IL-13 precedes and is essential for the subsequent enhanced M2 macrophage polarization by IL-33 (6). Moreover, it has been shown that in response to IL-33 treatment, $Akt2^{-/-}$macrophages displayed decreased production of IL-13 and TGF-β1 and attenuated phosphorylation of transcription factor Forkhead box O3a (FoxO3a) to stop acting as a trigger for

apoptosis (81). Inhibition of Akt2 marked as a potential strategy for treating IPF (81).

Interestingly, BLM can also induce fIL-33 secretion from airway epithelial cells and alveolar macrophages in an ST2-independent, Th2-independent fashion, likely through cytokine regulation of several non-Th2 cytokines, such as TGF-β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) and possibly by engaging heat shock protein (HSP) 70 (6). flIL-33 can then be processed into various mature forms of IL-33 by neutrophil proteases. mIL-33 subsequently stimulate macrophages and ILC2s to produce IL-13 (75). On the other hand, this response may be mediated only by nuclear-located flIL-33 affecting gene expression. Collectively, these data suggest that flIL-33 is also potentially implicated in the pulmonary fibrotic process (6, 75).

ST2 is mainly expressed in endothelial and type II alveolar epithelial cells as well as innate immune cells such as macrophages and ILC2s in the lungs. sST2 levels were also found increased in serum and BAL of acute exacerbation of IPF and BLM-induced lung fibrosis, respectively (82). ST2 mRNA expression has been reported to be increased in the BLM-induced lung fibrosis model *in vivo*, as well as in a human lung fibroblast and a human type II alveolar epithelial cell line, possibly reflecting the development of a type 2 pattern of inflammatory process in the fibrotic lung tissue (82, 83).

Li et al. reported that ST2 deficiency or administration of an anti-IL-33 antibody were able to attenuate bone marrow (BM)-induced pulmonary fibrosis (6). Similarly, intranasal administration of a lentivirus for epithelial over-expression of sST2 has been reported to attenuate pulmonary fibrotic change by inhibiting the expression of pro-inflammatory and pro-fibrotic mediators, such as IL-13, IL-33, and TGF-β1 along with improved survival rates in BLM-treated mice (20).

Increased numbers of lung ILC2s have also been implicated in BLM-induced fibrosis in the mouse (6). Especially, BM-derived ST2-expressive ILC2s have been recently reported to be recruited to the fibrotic lung through the IL-33/ST2 pathway and contributed to fibroblast activation perhaps via transforming TGF-β (84). Furthermore, adoptive ILC2 transfer into recipient mice enhances lung fibrosis, whereas blocking IL-33 or ST2 deficiency diminish fibrosis. The increase in IL-33 responsive ILC2s is not unique to animal models of pulmonary fibrosis since they are also increased in SSc, correlating with the extent of fibrosis. ILC2s are also present in the IPF lung tissue and BAL, wherein they are associated with upregulated expression of lung IL-33.

Even in cases of asthma, experimental results showed that direct murine airway exposure to IL-33 could induce local fibrotic changes. IL-33/ST2 axis is thought to at least partially mediate the fibroblast function and local expression of matrix metalloproteinases (MMPs) and their inhibitors as well as other fibrosis-related proteins (85). Additionally, IL-33 is probably related to prostaglandin E2 (PGE2) production, stimulating mast cells to produce a large quantity of PGE2 demonstrating potent anti-fibrotic activity in the IPF lung (17, 86). IL-33 can regulate deposition of extracellular matrix (ECM) and promote the process of pulmonary fibrosis by inducing the imbalance between MMP9 and tissue inhibitor of MMP9 (TIMP-1) (17, 86).

Finally, fibulin-1 (Fbln1), an important ECM component involved in a matrix organization and wound repair, has been found to predict disease progression in IPF patients (87). Genetically inhibited Fbln1 has been associated with reduced levels of pro-inflammatory cytokines such as IL-33 and pulmonary inflammatory cells in a murine COPD model (87).

Hence, IL-33 is thought to be a novel cytokine that promotes the initiation and progression of pulmonary fibrosis by recruiting and directing inflammatory cell function and enhancing pro-fibrogenic cytokine production in an ST2- and macrophage-dependent manner (6, 79, 80). However, fIL-33 secretion from airway epithelial cells and alveolar macrophages acts in an ST2-independent, Th2-independent fashion in the fibrotic process. The over-expression of sST2 decoy receptor or any other exogenous inhibition of IL-33/ST2L signaling result in markedly lower levels of IL-33 and other pro-inflammatory and pro-fibrotic mediators thus attenuate the fibrotic process.

IL-33/ST2 AXIS IN LIVER FIBROSIS

Liver fibrosis is a reversible wound healing response to acute or chronic hepatocellular injury from various etiologies, including viral infection, cholestasis, metabolic diseases, and alcohol abuse. It has been suggested that damage associated molecular patterns (DAMPs) act as molecular links between hepatocyte death and liver fibrogenesis (27). Evidence indicates that when acute and massive liver damage occurs, the release of IL-33 by injured hepatocytes might act as an activator of tissue-protective mechanisms, while in cases of chronic injury IL-33 plays the role of a hepatic fibrotic factor (27). For instance, melatonin which exerts cytoprotective effects via inhibition of oxidative stress and apoptosis in liver ischemia-reperfusion injury (IRI) has been proposed to inhibit liver fibrosis through suppressing necroptotic DAMPs signaling cascades, such as the IL-33 signaling pathway (88).

IL-33/ST2 axis has been implicated in several hepatic diseases, such as cirrhosis, virus infection, fatty liver disease and toxic liver damage leading further to liver fibrosis (24, 27–29). Indeed, IL-33 has shown potential liver fibrosis promoting effect (24, 32). It has been found that in murine and human fibrotic livers, IL-33 levels as well as the mRNA expression of both IL-33 and ST2, are higher as compared to healthy liver (24, 28). Their expression is significantly increased along with the severity of fibrosis, especially in cirrhotic livers (33). Likewise in patients with primary biliary cirrhosis, an autoimmune liver disease that could result in liver failure, and hepatoma carcinoma, the serum IL-33 levels were positively correlated with disease severity (30, 31).

The role of ST2 has also been highlighted in liver fibrosis. It has been documented that liver injury, inflammatory cell infiltration, and fibrosis are reduced in the absence of the receptor ST2L (24). Furthermore, it has been found that ST2L deficient mice did not increase collagen production when challenged with carbon tetrachloride, an organic compound

with pro-fibrotic effects (24, 28). Similarly, the absence of ST2L prevented liver inflammation both in the acute and chronic phases, with attenuated activation of mitogen-activated protein kinase (MEK)\extracellular signal-regulated kinase(ERK)/p38-mitogen-activated protein kinase (MAPK) signaling cascade(25, 32).

Besides, sST2 has been regarded as a circulating biomarker to reflect IL-33 activation and fibrosis in patients with liver diseases. In fact, sST2 serum levels differ between hepatitis B virus-infected patients were dependent on the severity of hepatic fibrosis (89). Especially, the plasma levels of sST2 were found to be associated with mortality in patients with HBV-related acute-on-chronic liver failure (89). Notably, in a murine liver fibrosis model, the antibody blockade of sST2 enhances the severity of fibrosis (89).

It has been reported that the major source of IL-33 in fibrotic livers is the hepatic stellate cells (HSCs) that have also been suggested to be the leading producers of ECM proteins (25, 27, 32). Injury-associated immunological processes supporting trans-differentiation of quiescent HSC to fibrogenic myofibroblasts in the course of liver injury are particularly important in fibrosis (90). Moreover, an ST2 expression has been observed on the membrane of HSCs (32). However, other data derived from murine and human studies demonstrated that hepatocytes are the primary sources of IL-33 both in the fibrotic liver and in healthy liver (27, 32). More specifically, IL-33 release as a DAMP upon hepatocyte damage may have a direct effect on HSCs that increases secretion of cytokines and production of collagen (27, 32). Besides, another study showed that activation of HSCs was decreased in ST2-deficient liver fibrosis mice (24). IL-33-mediated Th2 immune response promotes HSCs proliferation, TGF-β synthesis, and fibrogenesis (27). As in lung tissue, Th2 pro-fibrotic cytokines production such as IL-4, IL-5, and IL-13 are known to play a critical role in liver fibrosis (27). On the other hand, Th1 cytokines lead to a rapid and intense inflammatory response while causing little fibrosis.

Interestingly, vector-encoded overexpression of IL-33 was sufficient to induce fibrosis in the liver without administration of chemicals, demonstrating the pro-fibrotic role of IL-33, predominantly exerted through the IL-13 induction (27). In particular, IL-13 could initiate activation and differentiation of HSCs by enhancing TGF-β signaling through IL-4Rα and signal transducer and activator of transcription 6 (STAT6) in HSCs, promoting liver fibrosis (28). Some other data suggest that IL-13, rather than TGF-β, primarily activates HSCs in liver fibrosis (32). Furthermore, the IL-33/ST2/IL-13 pathway is thought to be Galectin-3 (Gal-3) dependent (91–93). Gal-3 has been found to attenuate steatosis while promoting liver injury, inflammation and fibrosis in an obesogenic mouse model of non-alcoholic steatohepatitis (NASH) (91–93). Therefore, Gal-3 inhibitors have been suggested to protect against fibrotic disorders (91, 92).

More convincingly, the stimulation of *in vitro* activated HSCs with recombinant IL-33 (rIL-33) induced the MAPK pathways that were found to be mediated by ERK, Jun N-terminal kinase (JNK) and p38 protein kinases (32). Moreover, HSCs activated by rIL-33 *in vitro*, released IL-6, TGF-β, and resulted in the stimulation of α-smooth muscle actin (a-SMA) and collagen expression (32). These data suggest a direct fibrogenic role of IL-33 in HSCs, which is potentially synergistic with its effects on ILC2s (26, 28). Hence, another mechanism proposed to be involved in liver fibrosis is through the activation of ILC2s via the ST2 signaling pathway, resulting again in a release of several Th2 cytokines (30, 33, 94). IL-33 induces the activation and expansion of ILC2s to express IL-13 and IL-5, which subsequently causes M2 macrophage and eosinophil accumulation and regulates $ST2^+$ Tregs homeostasis in liver adipose tissue through attenuating adipose tissue inflammation (95–97). In fact, the upregulation of IL-33 was positively correlated with an increase of ILC2s (32, 98). Activated ILC2-derived IL-13 initiated activation and differentiation of HSCs via the IL-4Rα-STAT6 transcription factor-dependent pathway, as previously described (28).

Non-alcoholic Fatty Liver Disease (NAFLD) which comprises simple steatosis, NASH, cirrhosis and possibly liver carcinoma, is potentially related to a severe form of the fibrotic liver disease; however, how fat deposition renders hepatocytes susceptibility to inflammatory, lipid and oxidative stress mediators is still unidentified. In obesity, immune cells infiltrating the visceral adipose tissue mediate chronic low-grade inflammation that plays a critical role in the pathogenesis of NAFLD (99). It has been demonstrated that administration of rIL-33 aggravates liver fibrosis in an ST2-dependent manner during experimental NAFLD, which is further shown by a substantial reduction of experimentally-induced liver fibrosis in mice lacking IL-33 (28, 33).

In NASH, through secreted cytokines, intrahepatic innate and adaptive immune cells sustain chronic inflammation and induce trans-differentiation of HSCs into myofibroblasts, which are critical cells for liver fibrosis (90). Remarkably, IL-33 treatment has been proposed to attenuate diet-induced hepatic steatosis on the one hand, but aggravate hepatic fibrosis in an ST2-dependent manner on the other hand (33). These findings provide evidence for a dual role of the IL-33/ST2 axis in diet-induced NASH in mice. Similarly, additional results were recently obtained showing that injury-induced endogenous IL-33 release is sufficient to cause inflammation and fibrosis in the bile duct ligated mouse model, which is not further enhanced by rIL-33 (32). More contradictory data reported that IL-33 deficiency in mice does not lessen liver fibrosis during diet-induced steatohepatitis (100).

Hence, in liver fibrosis, evidence indicate that when acute and massive liver damage occurs, the release of IL-33 might act as an activator of tissue-protective mechanisms, while in cases of chronic injury IL-33 shows a significant liver fibrosis promoting effect in an ST2-, Th2- dependent fashion across the entire spectrum of liver pathology.

IL-33/ST2 AXIS IN ANCREATIC FIBROSIS

Pancreatic fibrosis is one of the characteristic histopathological findings in cases of chronic pancreatitis. The fibrosis develops as a result of abnormal activation of stromal cells and deposition of ECM proteins. Identification of essential regulators of

pancreatic fibrosis, mainly the pancreatic stellate cells (PSCs), has contributed significantly to the understanding of the cellular and molecular basis of these pathogenic processes (101). There is accumulating evidence that PSCs play a key role in the development of pancreatic fibrosis in chronic pancreatitis and pancreatic cancer (34, 102). Additionally, IL-33 is a novel factor involved in the pathogenesis of chronic pancreatitis and possibly pancreatic cancer. In addition, IL-33 has been found to exacerbate acute pancreatic (AP) inflammation in mice (35).

IL-33 is expressed in the nucleus of activated PSCs. Baseline IL-33 expression was reported to be low in quiescent rat PSCs but increased upon cellular activation with mediators such as IL-1b, tumor necrosis factor a (TNF-a), lipopolysaccharide (LPS), platelet-derived growth factor (PDGF)-BB (36). In detail, IL-1b induces IL-33 expression via activation of the nuclear factor (NF)-kβ and ERK pathways and partially through p38 MAPK, whereas PDGF-BB induces IL-33 expression primarily via activating the ERK signaling pathway (18).

It has been recently proposed that IL-33 induction is associated with the transformation to an α-SMA positive PSCs myofibroblastic phenotype. However, treatment of PSCs with rIL-33 did not stimulate any specific phenotype, while a reduction of IL-33 expression resulted in decreased proliferation of PSCs in response to PDGF-BB. Pancreatic myofibroblasts responded to IL-33 by the expression of pro-inflammatory mediators, and increased proliferation and migration, thus playing a crucial role in the progression of pancreatic fibrosis (103). Vice versa, pancreatic myofibroblasts express and secrete modest levels of IL-33 mRNA and protein, respectively. Expression of the ST2 was detected in PSCs and pancreatic myofibroblasts (36).

Moreover, Watanabe et al. found that IL-33 secretion by pancreatic acinar cells under the influence of type I IFN plays a significant role in the development of pancreatic fibrosis occurring in a model of conventional pancreatitis (38). Furthermore, substance P released by pancreatic acinar cells was shown to synergize IL-33 and augment mast cell activation that subsequently regulates the release of several inflammatory mediators in the initiation and progression of AP (35). Remarkably, a triangular link between the cytokine IL-33, pancreatic acinar cells, and mast cells in the development and progression of AP exists (35, 37). Recently Leema G et al. investigated the protective effects of scopoletin, a coumarin compound with anti-inflammatory activities on AP and associated lung injury in mice and found an anti-inflammatory effect by down-regulating substance P signaling via Nf-κB pathway (104).

Besides, activation of plasmacytoid dendritic cells (pDCs) producing IFN-α and IL-33 plays a pivotal role in the chronic fibro-inflammatory responses underlying murine autoimmune pancreatitis (AIP) and human IgG4-related AIP (38).

Watanabe et al. also suggested the possibility that microbe-associated molecular patterns act as pDC activators in AIP, indicating that this form of pancreatic inflammation is initiated and/or driven by gut bacterial components (38). However, further studies defining the gut microbiome in AIP, as well as the demonstration that gut bacteria are translocated into the circulation and can thus contact pancreatic cells, will be required to fully establish this concept (38).

Therefore, IL-33 is considered to be a novel factor implicated in the pathogenesis of acute and chronic pancreatitis and potentially in tissue fibrosis. Specifically, the expression of the ST2 in PSCs, pancreatic myofibroblasts, and pDCs implying a role for IL-33 signaling within the pancreas in an autocrine, and/or paracrine fashion.

IL-33/ST2 AXIS IN INTESTINAL FIBROSIS

IL-33/ST2 axis seems to represent an important mediator in intestinal fibrosis. Normal epithelium and stroma of the intestine express large amounts of IL-33 and ST2 during the homeostatic turnover of the intestinal mucosa (39). It has been demonstrated that intestinal baseline IL-33 expression was present in pericryptal fibroblasts and was increased during infection (105). A role for IL-33/ST2 signaling in the differentiation of stem cells in organoid culture was also elucidated (105).

IL-33 has been associated with areas of compromised barrier function and plays a critical role in maintaining normal gut homeostasis (44). Uncontrolled IL-33 expansion potentially leads to barrier dysfunction of epithelium, chronic relapsing inflammation, and fibrotic lesions (41–43). Additionally, IL-33 induces enteric glia to secrete glial cell-line derived neurotrophic factor family ligands (GFLs) that play an essential role in intestinal epithelial barrier homeostasis by maintaining tight junctions and negatively regulating local inflammatory response (106, 107). Moreover, IL-33 influences the enteric nervous system to induce intestine hypermotility t to expel invading parasites from the intestine, while is an important regulator of the gut microbiome (108, 109).

Within the gut mucosa, colonic subepithelial myofibroblasts SEMFs are primary sources of IL33, particularly in ulcerated lesions from patients with ulcerative colitis (UC) and induce potent Th2 immune responses (45, 46). The localization of IL-33-producing SEMFs in mucosal ulcerations suggests a significant role of the cytokine in wound healing. Actually, it could be used as an early marker for ulcer-associated activated fibroblasts and myofibroblasts trans-differentiation. Overall, one cannot rule out the potential role of IL-33 in gut-associated fibrosis, particularly in the setting of turnover of chronic tissue damage and repair, characteristics of inflammatory bowel disease (IBD) (45, 46).

The IL-33 expression is enhanced specifically in inflamed mucosa in UC, while exogenous IL-33 treatment in mice, modulated higher colonic mucin release (45, 46). Moreover, IL-33 mRNA levels have been associated with UC disease activity (18, 44, 47). Sponheim et al. found that a feature of IBD-associated mRNA IL-33 expression is the accumulation of both fibroblasts and myofibroblasts in UC lesions. In ulcerations, the fibroblast marker HSP47, platelet-derived growth factor receptor (PDGFR) β, and in part the SEMFs marker α-SMA were expressed (46). Epidermal growth factor has also been demonstrated to contribute to increased IL-33 production and ST2 expression (110).

Epithelial-IL-33 was also increased in pediatric Crohn's ileitis and strongly associated with clinical and histopathological findings, ileal eosinophilia, and complicated fibrostenotic disease (40). Furthermore, neutralization of IL-33 interferes with the massive influx of eosinophils into the gut mucosa and potently decreases fibrogenic gene expression and fibrosis (111).

sST2 constitutes a marker of IBD severity, found to be significantly increased in both the gut mucosa and the serum in both patients and experimental models of IBD. However, in IBD patients, ST2L mRNA expression remained similar to that of healthy controls (14, 44).

IL-33 promotes ILC2s in the gut to produce the growth factor amphiregulin (AREG) that binds to the epidermal growth factor receptor (EGFR) which are responsible for tissue repair during inflammation and restoration of mucosal integrity (42, 112). Disruption of the AREG/EGFR signaling pathway is involved in human patients and murine models of IBD (113, 114). On the other hand, IL-33 stimulation of ILC2s could be beneficial in intestinal inflammation outside the setting of infection for instance in cases of helminth intestinal infection (115). ATP released by parasite-infected cells stimulates local mast cells to produce IL-33, which then activates IL-13-producing ILC2s necessary for helminth expulsion (115).

The role of the IL-33 is not limited to Th2 responses but could also amplify Th1-mediated inflammation (41). IL-33 is an activator of Tregs, activation of which appears to be a compensatory mechanism for intestinal inflammation (112, 116). Colonic Tregs preferentially express ST2. Signaling through ST2/IL-33 promotes both Treg accumulation and maintenance in the gut, enhancing their protective function (117).

The role of IL-33 in colitis as well as in colon cancer is controversial, being very dependent on the timing of alarmin activation or expression relative to the damaging insult (41, 118). Regarding the colon cancer, on the one hand, it has been supported that IL-33 promotes an IFN-γ-mediated immune protective mechanism that helps guard against the development of sporadic colon cancer that links to inflammation (39). IL-33 inhibits colon cancer growth by suppressing cellular proliferation and promoting apoptosis (119). Interestingly, IL-33 has been found to potently induce a neutralizing ant-IL-13 receptor that plays an important protective role in cases of mucosal damage (120–122). Conversely, Zhang et al., found that tumor-derived IL-33 following the activation of tumor cells by pro-inflammatory cytokines such as IL-13, modulates the tumor microenvironment to potently promote colon carcinogenesis and liver metastasis in murine models (123). Furthermore, IL-33 stimulation of human colonic SEMFs has been found to induce the expression of growth factors associated with intestinal tumor progression and extracellular matrix components (124). Similarly, experimental studies in a murine model showed that overexpression of IL-33 promoted the expansion of ST2+Tregs, increased Th2 cytokine milieu, and induced M2 macrophages in the gut, thereby increasing tumor development (125). Furthermore, lower expression of ST2L has been reported in human colon tumors. ST2L expression was negatively associated with the higher tumor grade (126). Inhibition of the IL-33/ST2 pathway may limit mucositis and thus improve the effectiveness of chemotherapy (127).

It is ambiguous if IL-33 is a consequence of intestinal inflammation or if IL-33 is a critical instigator in promoting an inflammatory response. Taken together, there is clear evidence that IL-33/ST2 axis participates in maintaining normal gut homeostasis, particularly in promoting mucosal wound healing and repair. When deregulated, this important ligand-binding pair can also play a critical role in the progression of chronic inflammation and fibrosis, leading to gastrointestinal-related disorders such as IBD as well as colon cancer.

IL-33/ST2 AXIS IN RENAL FIBROSIS

Renal fibrosis is characterized by progressive connective tissue expansion through kidney parenchyma, leading to detrimental renal function deterioration (128, 129). Almost all the cell types in the kidneys participate in the pathogenesis of renal fibrosis, illustrating the complexity of this process (48, 51, 52). Specifically, a study by Manetti et al. found that nuclear IL-33 expression in the fibrotic kidneys of patients with SSc was absent in the endothelial cells of peritubular capillaries while ST2 has been expressed abundantly in kidney glomeruli, tubules, and peritubular capillaries (19). Conversely, in control human kidney (HK) specimens, IL-33 was found to be constitutively expressed in peritubular capillary endothelial cells (49). The levels of IL-33 and sST2 were relevant to the progressive deterioration of kidney function while a significant correlation between the serum level of sST2 and disease severity has been shown (50).

Epithelial to Mesenchymal Transition (EMT) of podocytes, tubular epithelial cells, and circulating fibrocytes which are transformed to mesenchymal fibroblasts migrating to adjacent interstitial parenchyma constitutes the principal mechanism of renal fibrosis (129, 130). Other key events in tubulointerstitial fibrosis that also take place in the glomeruli after injury are glomerular infiltration of inflammatory cells and myofibroblastic activation of the mesangial cells (129, 131–133).

Emerging evidence supported that the renal tubular EMT is a remarkable process in the pathogenesis of renal interstitial fibrosis, mediated by IL-33 (50). IL-33 has been found to participate in transplanted kidney interstitial fibrosis promoting EMT of HK-2 cells in a dose- and time-dependent manner, via the activation of the p38 MAPK signaling pathway (129). These data suggest that therapies are targeting a reduction in IL-33 levels or on the downregulation of the p38 MAPK signaling axis may be an effective strategy in the prevention of kidney interstitial fibrosis (129).

IL-33 is also a marker of IRI that contributes to innate immune cell recruitment and development of renal graft damage associated with renal transplantation in humans (53). Furthermore, it plays a significant role in the pathogenesis of IRI-induced renal fibrosis through regulating myeloid fibroblast accumulation, inflammatory cell infiltration, and cytokine and chemokine expression (52, 53). Th2- cytokines, including IL-4, IL-5, and IL-13, all induced by IL-33, play an essential role in the renal fibrotic disease (52, 53).

Experimental data revealed that IL-33-treated IRI mice had increased levels of IL-4 and IL-13 in serum and renal tissue as well as more ILC2s, Tregs, and anti-inflammatory M2 macrophages, as compared to control-treated IRI mice (54). Furthermore, it has been reported that depletion of ILC2s substantially abolished the protective effect of IL-33 on renal IRI (54). Conversely, adoptive transfer of *ex vivo*-expanded ILC2 prevented renal injury in mice subjected to IRI. Besides, treatment of mice with IL-33 or ILC2 after IRI had a protective effect associated with induction of M2 macrophages in kidney and required the ILC2 production of amphiregulin (54). Interestingly, mice treated with sST2 exhibited less severe renal dysfunction and fibrosis in response to IRI compared with vehicle-treated mice (54). Furthermore, inhibition of IL-33 suppressed BM-derived fibroblast accumulation and myofibroblast formation in the kidneys after IRI stress, which was associated with less expression of ECM proteins (54). Hence, IL-33 signaling in ILC2s plays a critical role in the pathogenesis of IRI–induced renal fibrosis and treatment with IL-33 inhibitor reduced pro-inflammatory cytokine and chemokine levels in the kidneys of mice following IRI insult (54).

Furthermore, it is increasingly recognized that episodes of acute kidney injury (AKI) increase the susceptibility of chronic kidney disease (CKD) and end-stage renal disease (ESRD) that are characterized by organ fibrogenesis (134, 135). It has been found that the administration of rIL-33 exacerbated cisplatin-induced AKI by acting as a pro-inflammatory cytokine (49). In a cisplatin-induced mouse AKI model, IL-33 was reported to promote AKI through CD4+ T cell-mediated production of chemokine (C-X-C motif) ligand (CXCL) 1, which could exacerbate the renal damage. In addition, high expression levels of IL-33 have been observed in LPS-induced acute glomerular injury (49, 136).

Additionally, IL-33 released from necrotic cells has been implicated in autophagy, which can balance increased apoptosis secondary to contrast-induced nephropathy in diabetic kidney disease (55). Another study also reported that IL-33 contributes to kidney fibrosis associated with systemic lupus erythematosus (SLE).

Thus, emerging data indicate that the upregulation of the IL-33/ST2 signaling pathway may promote tubular cell injury and fibrosis predominantly via EMT in the kidneys (56).

IL-33/ST2 AXIS IN HEART FIBROSIS

Heart failure (HF) and cardiac fibrosis are associated with IL-33 mainly in cases of a mechanical strain of cardiac fibroblasts (57, 64). IL-33 demonstrates anti-hypertrophic and anti-fibrotic effects on cardiomyocytes, transduced by ST2L. In 2007, Sanada et al. first documented that IL-33 prevents cardiomyocyte apoptosis, reduces infarct size, fibrosis, and apoptosis through induction of anti-apoptotic proteins after ischemia-reperfusion in rats and improves cardiac function and survival after myocardial infarction (57). IL-33 correlated with the expression kinetics of the anti-apoptotic gene B-cell lymphoma 2 (Bcl-2), which is in agreement with its anti-apoptotic role (58). Thereafter, multiple experimental studies have also illustrated that IL-33 attenuates cardiac fibrosis induced by the increased cardiovascular load, showing that IL-33 directly inhibits pro-fibrotic activities of cardiac fibroblasts (58, 59, 137). Treatment of rat cardiac fibroblasts with IL-33 was also found to impair the migratory activity of fibroblasts or their precursors into the stressed myocardium (57, 138). IL-33 levels were found to be significantly elevated upon a cyclic stretch of cardiac fibroblasts *in vitro*, and the administration of IL-33 was shown to inhibit myocyte amino acid incorporation and growth thus protecting against cardiac hypertrophy (57). IL-33 protected cardiomyocytes from hypoxia-induced apoptosis *in vitro*, and this effect was partially inhibited by sST2, highlighting the critical role of IL-33 in regulating cardiac myocyte function and its protective role in cardiac fibrotic diseases (58).

Others have reported that ablation of IL-33 gene caused exaggerated cardiac remodeling in both ischemic and non-ischemic HF, It leads to cardiomyocyte hypertrophy and cardiac fibrosis upon mechanical stress, impaired cardiac function, and survival (60). Furthermore, it has been recently shown that IL-33 acts by reducing a form of erythrocyte superoxide dismutase (eSOD) production, thus eSOD is found decreased in chronic HF (61). eSOD is a protective enzyme against oxidative stress in chronic HF (61). Alternatively, the *in vitro* administration of IL-33 significantly decreased cardiac interstitial fibrosis in wild-type mice underwent transaortic constriction surgery to increase cardiovascular load (57).

Of note, the aforementioned IL-33 benefits were absent in mice with deletion of the ST2 gene, so these data indicate that IL-33 exerts its cardioprotective role only through the ST2 receptor signaling. Moreover, the microRNA-587b has been proposed to ameliorate cell apoptosis, inflammatory reaction of myocarditis, and fibrosis through inhibition of the IL-33/ST2 pathway by suppressing IL-33 (139).

In contrast, sST2 disrupts the cardioprotective effects of IL-33 by sequestering its availability for binding with the transmembrane receptor ST2L. sST2 has been demonstrated as a marker of myocardial fibrosis and HF progression. Both cardiac fibroblasts and cardiomyocytes express IL-33 and sST2, and expression levels are increased as a response to myocardial stress (15). This issue is supported from a clinical perspective, given that sST2 concentrations have repeatedly been found high in patients with acute myocardial infarction and acute HF and correlate with parameters of infarct magnitude, cardiac dysfunction, hemodynamic impairment, and neurohormonal derangement (15). Based on the above sST2 is thought to be a biomarker for poor outcome in patients with cardiovascular disease (140–142). Moreover, cardiogenic shock and increased C-reactive protein levels are associated with higher sST2 levels. The PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study highlighted the potential applications of sST2 in acute HF (143). Thereafter, several studies that followed emphasized on its diagnostic and prognostic utility (144–146). sST2 may also identify patients who benefit most from cardiac resynchronization therapy defibrillators (147), titration of beta blockers (62) and angiotensin-converting enzyme inhibitors (148). Weir et al. showed that sST2 could predict functional

recovery and left ventricle remodeling during the post-infarction period (149). The sST2 levels were positively correlated with the degree of cardiac fibrosis (150). Along these lines, it has been recently observed that left ventricular assist device (LVAD) resulted in a significant drop in sST2 levels with normalization within 3 months post-implantation, thus lessened heart fibrosis and inflammation (151).

According to the above-mentioned studies, recently, a highly sensitive ELISA for sST2 (Presage ST2) as well as a rapid quantitative lateral flow immunoassay for measurement of sST2 in human plasma has been developed, allowing for point-of-care testing (142). The first one was approved by regulatory agencies both in Europe and the United States for prognostication in HF. The second one (Aspect-PLUS ST2 test, Critical Diagnostics, San Diego, CA, USA) has received regulatory approval in Europe, but it has yet to be approved by the Food and Drug Administration in the United States (142). The American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guidelines of 2013 have incorporated sST2 as a relevant marker of fibrosis. They recommend it for additive risk stratification in patients with acutely decompensated HF (level of evidence A) or chronic HF (level of evidence B) (63).

Additionally, the metabolic activity of epicardial adipose tissue has been recently associated with a decrease in the IL-33 levels, thus was closely related to the development of cardiac fibrosis at 1-year post-myocardial infraction (150).

Moreover, Gal-3 has been associated with left ventricular remodeling along with an increased risk of incident HF and mortality (152). It has been reported that Gal-3 promotes myocardial fibrosis, whereas myocardial fibrosis and hypertrophy are prevented through interaction between IL-33 and sST2 (57).

Hence, IL-33 demonstrates cardioprotective, anti-hypertrophic and anti-fibrotic effects on cardiomyocytes, transduced by ST2L, and disturbing by sST2.

IL-33/ST2 AXIS IN SKIN FIBROSIS

IL-33 is released by dermal fibroblasts (3). It has been documented that the IL-33/ST2 signaling is associated with abnormal fibroblast proliferation, leukocyte infiltration and morphologic differentiation of human endothelial cells, resulting in increased endothelial permeability, consistently with increased angiogenesis and ECM deposition *in vivo* (65).

IL-33 has been reported to be a crucial signaling cytokine in skin pathology by inducing IL-13-dependent cutaneous fibrosis mechanism, required both eosinophils and recombination-activating gene (RAG)-dependent lymphocytes. Eosinophils contribute to tissue remodeling and fibrosis (65). It is known that in skin diseases, eosinophil expresses a broad spectrum of Th2 cytokines such as IL-4, IL-5, IL-13, and C-C motif chemokine-11 (CCL11/eotaxin) (66). The different cytokine expression patterns suggest distinct functional roles of eosinophils in various diseases that might be related to host defense, immunomodulation, fibrosis, and/or tumor development (67).

More convincingly, in cutaneous fibrosis, the injection of rIL-33 induces collagen production via ST2-dependent recruitment of BM-derived eosinophils that further secrete IL-13 in response to IL-33 stimulation (22, 65). IL-33 has also been proposed as a critical molecule operating in eosinophil-mediated fibrosis in the high-dose-per fraction irradiated skin (66). In detail, vascular endothelial cells damaged by high-dose radiation secrete IL-33, which may stimulate fibrotic responses via eosinophil recruitment and eosinophil-mediated Th2 immune responses.

Rankin et al. demonstrated that IL-33 induces cutaneous fibrosis and intense inflammation that are associated with large numbers of CD3+ cells and F4/80+ myeloid cells, except infiltrating eosinophils (22). Additionally, IL-33 was also shown to induce several others cytokines such as IL-4, IL-5, tissue inhibitor of metalloproteinases 1 (TIMP1), MMP12, and MMP13 gene expression; however, it did not induce the expression of TGF-β1 which participated with varying degrees in fibrogenesis (22).

As far as SSc is concerned the most severe variant is characterized by aggressive skin fibrosis (68). These patients experience a low health-related quality of life that is directly related to the extent of the dermal fibrosis (68). Serum levels of IL-33 are elevated in SSc, even more pronounced in diffuse cutaneous SSc than in limited cutaneous SSc; thus these levels are positively correlated with the total fibrotic skin score. In other words, serum IL-33 levels are likely to reflect the degree of endothelial damage in patients with SSc (68).

IL-33 might mediate very early pathogenic events of SSc through recruitment and stimulation of ST2-expressing cells (immune cells and fibroblast/myofibroblast) (68). Other studies reported that increased circulating levels of IL-33 in SSc correlate with early disease stage and microvascular involvement being a serum marker for vascular abnormalities in SSc (69). IL-33 induces migration of Th2 lymphocytes and enhances Th2 cytokine production. Remarkably, owing to the Th2-type signature, there are elevated levels of both IL-4 and IL-13 in SSc patient's sera (67), while SSc patients exhibit substantial Th2 cytokine production in cultures of CD4+ T lymphocytes isolated from their affected skin (153). It is likely that the signaling mechanism in the dermal fibroblasts mediated by IL-13 is STAT6 (67, 154, 155).

A recent study demonstrated that the rs7044343 polymorphism of the IL-33 gene was associated with susceptibility to SSc in a Turkish population. No similar studies were found in the literature (156).

Conversely, sST2 constitutes a potential marker for disease progression in limited cutaneous SSc with disease duration over 9 years. On the contrary, sST2 was not elevated in healthy controls or SSc patients with early skin involvement or disease duration shorter than 9 years (157). Furthermore, sST2 serum levels were lowered by iloprost (prostacyclin) treatment (157). The question remains why sST2 is elevated in limited cutaneous SSc and not in diffuse cutaneous SSc patients. Diffuse cutaneous SSc and limited cutaneous SSc may be two pathophysiologically different diseases rather than two subtypes of one disease showing significant differences of organ involvement, disease progression and significantly different chemokine levels between two entities

(158, 159). Accordingly, sST2 could be a marker for pathological alterations and higher sST2 in limited cutaneous SSc could be partially beneficial by blocking the high inflammatory capacity of IL-33 by neutralizing its bioactivity.

In addition, evidence is presented to support the high tissue-localized expression of IL-33 in patients with SSc, as well as IL-33-dependant skin-localized Tregs trans-differentiation into Th2-like cells, combined with expression of the ST2 receptor on Tregs. In other words, IL-33 might be an important stimulator of tissue-localized loss of normal Tregs function (70). Moreover, friend leukemia virus integration 1 (Fli1) -a predisposing factor of SSc- haploinsufficiency increases Th2- and Th17-like Tregs proportions in BLM-induced pro-fibrotic skin condition, in which IL-33-producing dermal fibroblasts contribute to Th2-like Tregs trans-differentiation (71).

Consequently, IL-33 is a crucial cytokine in skin pathology responsible for abnormal fibroblast proliferation, leukocyte infiltration and morphologic differentiation of human endothelial cells, leading to fibrotic skin conditions.

CONCLUSION

In this review, we spotlight the distinctive contribution of IL-33/ST2 signaling in organ fibrosis as well as the significant role of the Th2 pattern of immune response in the pathophysiology of organ fibrosis. The IL-33/ST2 axis widely participates in the fibrotic process of many vital organs, demonstrating clear direct effects on wound healing and remodeling. Generally, the IL-33/ST2 signaling pathway has mainly anti-inflammatory/anti-proliferative effects. However, chronic tissue injury is responsible for pro-inflammatory/pro-fibrogenetic responses. At the basal level, both flIL-33 and mIL-33 forms have been reported to contribute to fibrogenesis. The axis influences the capacity of various cells to trans-differentiate into extracellular matrix-secreting activated myofibroblasts which constitute the main cell population of fibrosis, in an organ-specific underlying mechanism. Furthermore, the IL-33/ST2 axis is involved in angiogenesis, production of matrix components, ECM deposition. Importantly, elevated levels of IL-33 and/or sST2 constitute markers of dysfunction and severity in many fibrotic diseases. IL-33/ST2 axis seems to be a promising therapeutic target in fibrosis constitutes, therefore, a critical area for further investigation.

AUTHOR CONTRIBUTIONS

OK took part in decision on structure and content of the review, performing literature, search, and writing the review. KG revised the draft critically; gave final approval of the submitted version. SZ took part in decision on structure and content of the review, contributed to writing the review and gave thorough feedback throughout the process, and accepting the final version.

REFERENCES

1. Onda H, Kasuya H, Takakura K, Hori T, Imaizumi T, Takeuchi T, et al. Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. *J Cerebr Blood Flow Met.* (1999) 19:1279–88. doi: 10.1097/00004647-199911000-00013
2. Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol.* (2003) 163:69–79. doi: 10.1016/S0002-9440(10)63631-0
3. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
4. Dinarello CA. An IL-1 family member requires caspase-1 processing and signals through the ST2 receptor. *Immunity* (2005) 23:461–2. doi: 10.1016/j.immuni.2005.10.004
5. Moussion C, Ortega N, Girard JP. 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:e3331. doi: 10.1371/journal.pone.0003331
6. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, et al. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J Allergy Clin Immunol.* (2014) 134:1422.e11–32. doi: 10.1016/j.jaci.2014.05.011
7. Drake LY, Kita H. L-33: biological properties, functions, and roles in airway disease. *Immunol Rev.* (2017) 278:173–84. doi: 10.1111/imr.12552
8. Nakanishi W, Yamaguchi S, Matsuda A, Suzukawa M, Shibui A, Nambu A, 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:e78099. doi: 10.1371/journal.pone.0078099
9. Lefrançais E, Cayrol C. Mechanisms of IL-33 processing and secretion: differences and similarities between IL-1 family members. *Eur Cytokine Netw.* (2012) 23:120–7. doi: 10.1684/ecn.2012.0320
10. Lefrancais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, Girard JP, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc Natl Acad Sci USA.* (2012) 109:1673–8. doi: 10.1073/pnas.1115884109
11. Lefrançais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc Natl Acad Sci USA.* (2014) 111:15502–7. doi: 10.1073/pnas.1410700111
12. Hayakawa M, Hayakawa H, Matsuyama Y, Tamemoto H, Okazaki H, Tominaga S. Mature interleukin-33 is produced by calpain-mediated cleavage *in vivo*. *Biochem Biophys Res Commun.* (2009) 387:218–22. doi: 10.1016/j.bbrc.2009.07.018
13. Gajardo Carrasco T, Morales RA, Pérez F, Terraza C, Yáñez L, Campos-Mora M, et al. Alarmin' immunologists: IL-33 as a putative target for modulating T cell-dependent responses. *Front Immunol.* (2015) 6:232. doi: 10.3389/fimmu.2015.00232
14. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol.* (2017) 8:475. doi: 10.3389/fimmu.2017.00475
15. Pascual-Figal DA, Januzzi JL. The biology of ST2: the international ST2 consensus panel. *Am J Cardiol.* (2015) 115:3B—7. doi: 10.1016/j.amjcard.2015.01.034
16. Mueller T, Jaffe AS. Soluble ST2–analytical considerations. *Am J Cardiol.* (2015) 115:8B—21. doi: 10.1016/j.amjcard.2015.01.035
17. Lee JU, Chang HS, Lee HJ, Jung CA, Bae DJ, SongHJ, et al. Upregulation of interleukin-33 and thymic stromal lymphopoietin levels in the lungs of idiopathic pulmonary fibrosis. *BMC Pulm Med.* (2017) 17:39. doi: 10.1186/s12890-017-0380-z
18. Gao Q, Li Y, Li M. The potential role of IL-33/ST2 signaling in fibrotic diseases. *J Leukoc Biol.* (2015) 98:15–22. doi: 10.1189/jlb.3RU0115-012R
19. Manetti M, Ibba-Manneschi L, Liakouli V, Guiducci S, Milia AF, Benelli G, 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) 69:598–605. doi: 10.1136/ard.2009.119321

20. Gao Q, Li Y, Pan X, Yuan X, Peng X, Li M. Lentivirus expressing soluble ST2 alleviates bleomycin-induced pulmonary fibrosis in mice. *Int Immunopharmacol.* (2016) 30:188–93. doi: 10.1016/j.intimp.2015.11.015
21. Xu J, Zheng J, Song P, Zhou Y, Guan S. IL33/ST2 pathway in a bleomycin induced pulmonary fibrosis model. *Mol Med Rep.* (2016) 14:1704–8. doi: 10.3892/mmr.2016.5446
22. Rankin AL, Mumm JB, Murphy E, Turner S, Yu N, McClanahan TK, et al. IL-33 induces IL-13-dependent cutaneous fibrosis. *J Immunol.* (2010) 184:1526–35. doi: 10.4049/jimmunol.0903306
23. Luzina IG, Kopach P, Lockatell V, Kang PH, Nagarsekar A, Burke AP. Interleukin-33 potentiates bleomycin-induced lung injury. Interleukin-33 potentiates bleomycin-induced lung injury. *Am. J. Respir. Cell. Mol. Biol.* (2013) 49:999–1008. doi: 10.1165/rcmb.2013-0093OC
24. Sun Z, Chang B, Gao M, Zhang J, Zou Z. IL-33-ST2 axis in liver disease: progression and challenge. *Mediators Inflamm.* (2017) 2017:5314213. doi: 10.1155/2017/5314213.
25. Weiskirchen R, Tacke F. Interleukin-33 in the pathogenesis of liver fibrosis: alarming ILC2 and hepatic stellate cells. *Cell Mol Immunol.* (2017) 14:143–5. doi: 10.1038/cmi.2016.62
26. Hammerich L, Tacke F. Interleukins in chronic liver disease: lessons learned from experimental mouse models. *Clin Exp Gastroenterol.* (2014) 7:297–306. doi: 10.2147/CEG.S43737. eCollection 2014
27. Marvie P, Lisbonne M, L'Helgoualc'h A, Rauch M, Turlin B., Preisser L, et al. Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. *J Cell Mol Med.* (2010) 14:1726–39. doi: 10.1111/j.1582-4934.2009.00801.x
28. McHedlidze T, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, et al. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity* (2013) 39:357–71. doi: 10.1016/j.immuni.2013.07.018
29. Wang J, Cai Y, Ji H, Feng J, Ayana DA, Niu J, et al. Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis B. *J Interferon Cytokine Res.* (2012) 32:248–53. doi: 10.1089/jir.2011.0109
30. Sun Y, Zhang JY, Lv S, Wang H, Gong M, Du N, et al. Interleukin-33 promotes disease progression in patients with primary biliary cirrhosis. *Tohoku J Exp Med.* (2014) 234:255–61. doi: 10.1620/tjem.234.255
31. Li J, Razumilava N, Gores GJ, Walters S, Mizuochi T, Mourya R, et al. Biliary repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation. *J Clin Invest.* (2014) 124:3241–51. doi: 10.1172/JCI73742
32. Tan Z, Liu Q, Jiang R, Lv L, Shoto SS, Maillet I, et al. Interleukin-33 drives hepatic fibrosis through activation of hepatic stellate cells. *Cell. Mol. Immunol.* (2017) 15:388–98. doi: 10.1038/cmi.2016.63
33. Gao Y, Liu Y, Yang M, Guo X, Zhang M, Li H, et al. IL-33 treatment attenuated diet-induced hepatic steatosis but aggravated hepatic fibrosis. *Oncotarget* (2016) 7:33649–61. doi: 10.18632/oncotarget.9259
34. Pang TCY, Wilson JS, Apte MV. Pancreatic stellate cells: what's new? *Curr Opin Gastroenterol.* (2017) 33:366–73. doi: 10.1097/MOG.0000000000000378
35. Kempuraj D, Twait EC, Williard DE, Yuan Z, Meyerholz DK, Samuel I. The novel cytokine interleukin-33 activates acinar cell proinflammatory pathways and induces acute pancreatic inflammation in mice. *PLoS ONE* (2013) 8:e56866. doi: 10.1371/journal.pone.0056866
36. Masamune A, Watanabe T, Kikuta K, Satoh K, Kanno A, Shimosegawa T. Nuclear expression of interleukin-33 in pancreatic stellate cells. *Am J Physiol Gastrointest Liver Physiol.* (2010) 299:G821–32. doi: 10.1152/ajpgi.00178.2010
37. Dib M, Zhao X, Wang X, Andersson R. Role of mast cells in the development of pancreatitis-induced multiple organ dysfunction. *Br J Surg.* (2002) 89:172–8. doi: 10.1046/j.0007-1323.2001.01991.x
38. Watanabe T, Yamashita K, Arai Y, Minaga K, Kamata K, Nagai T, et al. Chronic fibro-inflammatory responses in autoimmune pancreatitis depend on IFN-α and IL-33 produced by plasmacytoid dendritic cells. *J Immunol.* (2017) 198:3886–96. doi: 10.4049/jimmunol.1700060
39. Eissmann MF, Dijkstra C, Wouters MA, Baloyan D, Mouradov D, Nguyen PM, et al. Interleukin 33 signaling restrains sporadic colon cancer in an interferon-γ-dependent manner. *Cancer Immunol. Res.* (2018) 6:409–21. doi: 10.1158/2326-6066.CIR-17-0218
40. Masterson JC, Capocelli KE, Hosford L, Biette K, McNamee EN, de Zoeten EF, et al. Eosinophils and IL-33 perpetuate chronic inflammation and fibrosis in a pediatric population with Stricturing Crohn's Ileitis. *Inflamm Bowel Dis* (2015) 21:2429–40. doi: 10.1097/MIB.0000000000000512
41. Lopetuso LR, Scaldaferri F, Pizarro TT. Opposing functions of classic and novel IL-1 family members in gut health and disease. *Front Immunol.* (2013) 4:181. doi: 10.3389/fimmu.2013.00181
42. Monticelli A, Osborne LC, Noti M, Tran SV, Zaiss DMW, Artis D. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin–EGFR interactions. *Proc Natl Acad Sci USA.* (2015) 112:10762–67. doi: 10.1073/pnas.1509070112
43. Pastorelli L, De Salvo C, Cominelli MA, Vecchi M, Pizarro TT. Novel cytokine signaling pathways in inflammatory bowel disease: insight into the dichotomous functions of IL-33 during chronic intestinal inflammation. *Therap Adv Gastroenterol.* (2011) 4:311–23. doi: 10.1177/1756283X11410770
44. Beltran CJ, Nunez LE, Diaz-Jimenez D, Farfan N, Candia E, Heine C, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1097–107. doi: 10.1002/ibd.21175
45. Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. *J Gastroenterol.* (2010) 45:999–1007. doi: 10.1007/s00535-010-0245-1
46. Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarström C, Loos T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am J Pathol.* (2010) 177:2804–15. doi: 10.2353/ajpath.2010.100378
47. Seidelin JB, Bjerrum JT, Coskun M, Widjaya B, Vainer B, Nielsen OH. IL-33 is upregulated in colonocytes of ulcerative colitis. *Immunol. Lett.* (2010) 128:80–5. doi: 10.1016/j.imlet.2009.11.001
48. Humphreys BD. Mechanisms of renal fibrosis. *Annu Rev Physiol.* (2018) 80:309–26. doi: 10.1146/annurev-physiol-022516-034227
49. Akcay A, Nguyen Q, He Z, Turkmen K, Won D, Hernando AA, et al. IL-33 exacerbates acute kidney injury. *J Am Soc Nephrol.* (2011) 22:2057–67. doi: 10.1681/ASN.2010091011
50. Chen WY, Chang YJ, Su CH, Tsai TH, Chen SD, Hsing CH, et al. Upregulation of Interleukin-33 in obstructive renal injury. *Biochem Biophys Res Commun.* (2016) 473:1026–32. doi: 10.1016/j.bbrc.2016.04.010
51. Liu Y. Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol.* (2011) 7:684–96. doi: 10.1038/nrneph.2011.149
52. Liang H, Xu F, Wen XJ, Liu HZ, Wang HB, Zhong JY, et al. Interleukin-33 signaling contributes to renal fibrosis following ischemia reperfusion. *Eur J Pharmacol.* (2017) 812:18–27. doi: 10.1016/j.ejphar.2017.06.031
53. Ferhat M, Robin A, Giraud S, Sena S, Goujon JM, Touchard G, et al. Endogenous IL-33 contributes to kidney ischemia-reperfusion injury as an alarmin. *J Am Soc Nephrol.* (2018) 29:1272–88. doi: 10.1681/ASN.2017060650.
54. Cao Q, Wang Y, Niu Z, Wang C, Wang R, Zhang Z, et al. Potentiating tissue-resident type 2 innate lymphoid cells by IL-33 to prevent renal ischemia-reperfusion injury. *J Am Soc Nephrol.* (2018) 29:961–76. doi: 10.1681/ASN.2017070774
55. Demirtas L, Turkmen K, Kandemir FM, Ozkaraca M, Kucukler S, Gürbüzel M, et al. The possible role of interleukin-33 as a new player in the pathogenesis of contrast-induced nephropathy in diabetic rats. *Ren Fail.* (2016) 38:952–60. doi: 10.3109/0886022X.2016.1165034
56. Chen WY, Li LC, Yang YL. Emerging roles of IL-33/ST2 axis in renal diseases. *Int J Mol Sci.* (2017) 18:783. doi: 10.3390/ijms18040783
57. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest.* (2007) 117:1538–49. doi: 10.1172/JCI30634
58. Seki K, Sanada S, Kudinova AY, Steinhauser ML, Handa V, Gannon J, et al. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. *Circ Heart Fail.* (2009) 2:684–91. doi: 10.1161/CIRCHEARTFAILURE.109.873240
59. Sánchez-Más J, Lax A, Asensio-López Mdel C, Fernandez-Del Palacio MJ, Caballero L, Santarelli G, et al. Modulation of IL-33/ST2 system in postinfarction heart failure: correlation with cardiac remodelling markers. *Eur J Clin Invest.* (2014) 44:643–51. doi: 10.1111/eci.12282

60. Veeraveedu PT, Sanada S, Okuda K, Fu HY, Matsuzaki T, Araki R, et al. Ablation of IL-33 gene exacerbate myocardial remodeling in mice with heart failure induced by mechanical stress. *Biochem Pharmacol*. (2017) 138:73–80. doi: 10.1016/j.bcp.2017.04.022
61. Zhang HF, Xie SL, Chen YX, Mai JT, Wang JF, Zhu WL, et al. Altered serum levels of IL-33 in patients with advanced systolic chronic heart failure: correlation with oxidative stress. *J Transl Med*. (2012) 10:120. doi: 10.1186/1479-5876-10-120
62. Gaggin HK, Motiwala S, Bhardwaj A, Parks KA, Januzzi JL Jr. Soluble concentrations of the interleukin receptor family member ST2 and beta-blocker therapy in chronic heart failure. *Circ Heart Fail*. (2013) 6:1206–13. doi: 10.1161/CIRCHEARTFAILURE.113.000457
63. Yancy CW, Jessup M, Chair V, Bozkurt B, Butler J, Casey DE, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. (2013) 128:e240–327. doi: 10.1016/j.jacc.2013.05.019
64. Kakkar R, Hei H, Dobner S, Lee RT. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J Biol Chem*. (2012) 287:6941–8. doi: 10.1074/jbc.M111.298703
65. Artlett CM. The IL-1 family of cytokines. Do they have a role in scleroderma fibrosis? *Immunol Lett*. (2018) 195:30–7. doi: 10.1016/j.imlet.2017.11.012
66. Lee EJ, Kim JW, Yoo H, Kwak W, Choi WH, Cho S, et al. Single high-dose irradiation aggravates eosinophil-mediated fibrosis through IL-33 secreted from impaired vessels in the skin compared to fractionated irradiation. *Biochem Biophys Res Commun*. (2015) 464:20–6. doi: 10.1016/j.bbrc.2015.05.081
67. O'Reilly S. Role of interleukin-13 in fibrosis, particularly systemic sclerosis. *Biofactors* (2013) 39:593–6. doi: 10.1002/biof.1117
68. Yanaba K, Yoshizaki A, Asano Y, Kadono T, Sato S. Serum IL-33 levels are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *Clin Rheumatol*. (2011) 30:825–30. doi: 10.1007/s10067-011-1686-5
69. Duan L, Chen J, Gong F, Shi G. The role of IL-33 in rheumatic diseases. *Clin Dev Immunol*. (2013) 2013:924363. doi: 10.1155/2013/924363
70. MacDonald KG, Dawson NA, Huang Q, Dunne JV, Levings MK, Broady R. Regulatory T cells produce profibrotic cytokines in the skin of patients with systemic sclerosis. *J Allergy Clin Immunol*. (2015) 135:946.e9. doi: 10.1016/j.jaci.2014.12.1932
71. Saigusa R, Asano Y, Taniguchi T, Hirabayashi M, Nakamura K, Miura S, et al. Fli1-haploinsufficient dermal fibroblasts promote skin-localized transdifferentiation of Th2-like regulatory T cells. *Arthritis Res Ther*. (2018) 20:23. doi: 10.1186/s13075-018-1521-3
72. Ueha S, Shand FHW, Matsushima K. Cellular and molecular mechanisms of chronic inflammation-associated organ fibrosis. *Front Immunol*. (2012) 3:71. doi: 10.3389/fimmu.2012.00071
73. Lee JS, Seppanen E, Patel J, Rodero MP, Khosrotehrani K. ST2 receptor invalidation maintains wound inflammation, delays healing and increases fibrosis. *Exp Dermatol*. (2016) 25:71–4. doi: 10.1111/exd.12833
74. Yin H, Li X, Hu S, Liu T, Yuan B, Gu H, et al. IL-33 accelerates cutaneous wound healing involved in upregulation of alternatively activated macrophages. *Mol. Immunol*. (2013) 56:347–53. doi: 10.1016/j.molimm.2013.05.225
75. Luzina IG, Pickering EM, Kopach P, Kang PH, Lockatell V, Todd NW, et al. Full-length IL-33 promotes inflammation but not Th2 response *in vivo* in an ST2-independent fashion. *J Immunol*. (2012) 189:403–10. doi: 10.4049/jimmunol.1200259
76. de Kleer IM, Kool M, de Bruijn MJ, Willart M, van Moorleghem J, Schuijs MJ, et al. *Immunity* (2016) 45:1285–98. doi: 10.1016/j.immuni.2016.10.031.
77. Kudo F, Ikutani M, Seki Y, Otsubo T, Kawamura YI, Dohi T, et al. Interferon-γ constrains cytokine production of group 2 innate lymphoid cells. *Immunology* (2016) 147:21–9. doi: 10.1111/imm.12537
78. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol*. (2009) 183:6469–77. doi: 10.4049/jimmunol.0901575
79. John AE, Wilson MR, Habgood A, Porte J, Tatler AL, Stavrou A, et al. Loss of epithelial Gq and G11 signaling inhibits TGFβ production but promotes IL-33-mediated macrophage polarization and emphysema. *Sci Signal*. (2016) 9:ra104. doi: 10.1126/scisignal.aad5568
80. Cayrol C, Girard JP. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. *Immunol Rev*. (2018) 281:154–68. doi: 10.1111/imr.12619
81. Arranz AC, Doxaki E, Vergadi Y, Martinez de la Torre K, Vaporidi ED, Lagoudaki E, et al. Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. *Proc. Natl. Acad. Sci. USA*. (2012) 109:9517–22. doi: 10.1073/pnas.1119038109
82. Tajima S, Oshikawa K, Tominaga S, Sugiyama Y. The increase in serum soluble ST2 protein upon acute exacerbation of idiopathic pulmonary fibrosis. *Chest* (2003) 124: 1206–14. doi: 10.1378/chest.124.4.1206
83. Tajima S, Bando M, Ohno S, Sugiyama Y, Oshikawa K, Tominaga S, et al. ST2 gene induced by type 2 helper T cell (Th2) and proinflammatory cytokine stimuli may modulate lung injury and fibrosis. *Exp Lung Res*. (2007) 33:81–97. doi: 10.1080/01902140701198583
84. Zhao Y, Gonzalez De Los Santos F, Wu Z, Liu T, Phan SH. An ST2-dependent role of bone marrow derived group 2 innate lymphoid cells in pulmonary fibrosis. *J. Pathol*. (2018) 245:399–409. doi: 10.1002/path.5092
85. An G, Zhang X, Wang W, Huang Q, Li Y, Shan S, et al. The effects of interleukin-33 on airways collagen deposition and matrix metalloproteinase expression in a murine surrogate of asthma. *Proc Natl Acad Sci USA*. (2018) 109:9517–22. doi: 10.1111/imm.12911
86. Park GY, Christman JW. Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. *Am J Physiol Lung Cell Mol Physiol*. (2006) 290:L797–805. doi: 10.1152/ajplung.00513.2005
87. Liu G, Cooley MA, Jarnicki AG, Hsu AC, Nair PM, Haw TJ, et al. Fibulin-1 regulates the pathogenesis of tissue remodeling in respiratory diseases. *JCI Insight* (2016) 1:e86380. doi: 10.1172/jci.insight.86380
88. Kim SH, Lee SM. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. *J Pineal Res*. (2008) 44:165–71. doi: 10.1111/j.1600-079X.2007.00504.x
89. Oztas E, Kuzu UB, Zengin NI, Kalkan IH, Onder FO, Yildiz H, et al. Can serum ST2 levels be used as a marker of fibrosis in chronic hepatitis B infection? *Medicine* (2015) 94:e1889. doi: 10.1097/MD.0000000000001889
90. Friedman SL. Evolving challenges in hepatic fibrosis. *Nat Rev Gastroenterol Hepatol*. (2010) 7:425–36. doi: 10.1038/nrgastro.2010.97
91. de Oliveira SA, de Freitas Souza BS, SáBarreto EP, Kaneto CM, Neto HA, et al. Reduction of galectin-3 expression and liver fibrosis after cell therapy in a mouse model of cirrhosis. *Cytotherapy* (2012) 14:339–49. doi: 10.3109/14653249.2011.637668
92. Traber PG, Chou H, Zomer E, Hong F, Klyosov A, Fiel MI, et al. Regression of fibrosis and reversal of cirrhosis in rats by galectin inhibitors in thioacetamide-induced liver disease. *PLoS ONE* (2013) 8:e75361. doi: 10.1371/journal.pone.0075361
93. Jeftic I, Jovicic N, Pantic J, Arsenijevic N, Lukic ML, Pejnovic N. Galectin-3 ablation enhances liver steatosis, but attenuates inflammation and IL-33-dependent fibrosis in obesogenic mouse model of nonalcoholic steatohepatitis. *Mol Med*. (2015) 21:453–65. doi:10.2119/molmed.2014.00178
94. Forkel M, Berglin L, Kekäläinen E, Carlsson A, Svedin E, Michaëlsson J, et al. Composition and functionality of the intrahepatic innate lymphoid cell-compartment in human nonfibrotic and fibrotic livers. *Eur J Immunol*. (2017) 47:1280–94. doi: 10.1002/eji.201646890
95. Molofsky AB, Nussbaum JC, Liang HE, Van Dyken SJ, Cheng LE, Mohapatra A, et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med*. (2013) 210:535–49. doi: 10.1084/jem.20121964
96. Han JM, Wu D, Denroche HC, Yao Y, Verchere CB, Levings M. IL-33 reverses an obesity-induced deficit in visceral adipose tissue ST2+ T regulatory cells and ameliorates adipose tissue inflammation and insulin resistance. *J Immunol*. (2015) 194:4777–83. doi: 10.4049/jimmunol.1500020
97. Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, Kawamoto S, et al. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development

and maintenance of adipose tissue-resident regulatory T cells. *Nat Immunol.* (2015) 16:276–85. doi: 10.1038/ni.3085

98. Jeffery HC, McDowell P, Lutz P, Wawman RE, Roberts S, Bagnall C, et al. Human intrahepatic ILC2 are IL-13 positive amphiregulin positive and their frequency correlates with model of end stage liver disease score. *PLoS ONE* (2017) 12:e0188649. doi: 10.1371/journal.pone.0188649
99. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest*. (2011) 121:2111–7. doi: 10.1172/JCI57132
100. Vasseur P, Dion S, Filliol A, Genet V, Lucas-Clerc C, Jean-Philippe G, et al. Endogenous IL-33 has no effect on the progression of fibrosis during experimental steatohepatitis. *Oncotarget* (2017) 25:848563–74. doi: 10.18632/oncotarget.18335
101. Ferdek PE, Jakubowska MA. Biology of pancreatic stellate cells—more than just pancreatic cancer. *Pflugers Arch*. (2017) 469:1039–50. doi:10.1007/s00424-017-1968-0
102. Schmieder A, Multhoff G, Radons J. Interleukin-33 acts as a pro-inflammatory cytokine and modulates its receptor gene expression in highly metastatic human pancreatic carcinoma cells. *Cytokine* (2012) 60:514–21. doi: 10.1016/j.cyto.2012.06.286
103. Nishida A, Andoh A, Imaeda H, Inatomi O, Shiomi H, Fujiyam AY. Expression of interleukin 1-like cytokine interleukin 33 and its receptor complex (ST2L and IL1RAcP) in human pancreatic myofibroblasts. *Gut* (2010) 59:531–41. doi: 10.1136/gut.2009.193599
104. Leema G, Tamizhselvi R. Protective effect of scopoletin against cerulein-induced acute pancreatitis and associated lung injury in mice. *Pancreas* (2018) 47:577–85. doi: 10.1097/MPA.0000000000001034
105. Mahapatro M, Foersch S, Hefele M, He GW, Giner-Ventura E, McHedlidze T, et al. Programming of intestinal epithelial differentiation by IL-33 derived from pericryptal fibroblasts in response to systemic infection. *Cell Rep*. (2016) 15:1743–56. doi: 10.1016/j.celrep.2016.04.049
106. Zhang DK, He FQ, Li TK, Pang XH, Cui DJ, Xie Q, et al. Glial-derived neurotrophic factor regulates intestinal epithelial barrier function and inflammation and is therapeutic for murine colitis. *J Pathol*. (2010) 222:213–22. doi: 10.1002/path.2749
107. Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut-brain communications. *Nat. Rev. Gastroenterol. Hepatol.* (2017) 14:143–59. doi: 10.1038/nrgastro.2016.191
108. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol*. (2016) 16:676–89. doi: 10.1038/nri.2016.95
109. Malik A, Sharma D, Zhu Q, Karki R, Guy CS, Vogel P, et al. IL-33 regulates the IgA-microbiota axis to restrain IL-1α-dependent colitis and tumorigenesis. *J Clin Invest*. (2016) 126:4469–81. doi: 10.1172/JCI88625
110. Islam MS, Horiguchi K, Iino S, Kaji N, Mikawa S, Hori M, et al. Epidermal growth factor is a critical regulator of the cytokine IL-33 in intestinal epithelial cells. *Br J Pharmacol*. (2016) 173:2532–42. doi: 10.1111/bph.13535
111. De Salvo C, Wang XM, Pastorelli L, Mattioli B, Omenetti S, Buela KA, et al. IL-33 drives eosinophil infiltration and pathogenic type 2 helper T-cell immune responses leading to chronic experimental ileitis. *Am J Pathol*. (2016) 186:885–98. doi: 10.1016/j.ajpath.2015.11.028
112. Hodzic Z, Schill EM, Bolock AM, Good M. IL-33 and the intestine: the good, the bad, and the inflammatory. *Cytokine* (2017) 100:1–10. doi: 10.1016/j.cyto.2017.06.017
113. Sipos F, Muzes G, Valcz G, Galamb O, Toth K, Leiszter K, et al. Regeneration associated growth factor receptor and epithelial marker expression in lymphoid aggregates of ulcerative colitis. *Scand J Gastroenterol*. (2010) 45:440–8. doi: 10.3109/00365521003624144
114. Yan F, Cao H, Cover TL, Washington MK, Shi Y, Liu L, et al. Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. *J Clin Invest.* (2011) 121:2242–53. doi: 10.1172/JCI44031
115. Shimokawa C, Kanaya T, Hachisuka M, Ishiwata K, Hisaeda H, Kurashima Y, et al. Mast cells are crucial for induction of Group 2 innate lymphoid cells and clearance of helminth infections. *Immunity* (2017) 46:863.e4–74. doi: 10.1016/j.immuni.2017.04.017
116. Fernandes C, Wanderley CWS, Silva CMS, Muniz HA, Teixeira MA, Souza NRP, et al. Role of regulatory T cells in irinotecan-induced intestinal mucositis. *Eur J Pharm Sci*. (2018) 115:158–66. doi: 10.1016/j.ejps.2018.01.006
117. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* (2014) 513:564–8. doi: 10.1038/nature13577
118. Judd LM, Chalinor HV, Walduck A, Pavlic DI, Däbritz J, Dubeykovskaya Z, et al. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. *Am J Physiol Gastrointest Liver Physiol.* (2015) 308:G12–24. doi: 10.1152/ajpgi.00172.2014
119. Chen X, Lu K, Timko NJ, Weir DM, Zhu Z, Qin C, et al. IL-33 notably inhibits the growth of colon cancer cells. *Oncol Lett.* (2018) 16:769–74. doi: 10.3892/ol.2018.8728
120. Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* (2002) 17:629–38. doi: 10.1016/S1074-7613(02)00453-3
121. Kawashima R, Kawamura YI, Kato R, Mizutani N, Toyama-Sorimachi N, Dohi T. IL-13 receptor alpha2 promotes epithelial cell regeneration from radiation-induced small intestinal injury in mice. *Gastroenterology* (2006) 131:130–41. doi: 10.1053/j.gastro.2006.04.022
122. Sezaki T, Hirata Y, Hagiwara T, Kawamura YI, Okamura T, Takanashi R, et al. Disruption of the TWEAK/Fn14 pathway prevents 5-fluorouracil-induced diarrhea in mice. *World J Gastroenterol.* (2017) 23:2294–307. doi: 10.3748/wjg.v23.i13.2294
123. Zhang Y, Davis C, Shah S, Hughes D, Ryan JC, Altomare D, et al. L-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis. *Mol Carcinog* (2017) 56:272–87. doi: 10.1002/mc.22491
124. Maywald RL, Doerner SK, Pastorelli L, De Salvo C, Benton SM, Dawson EP, et al. IL-33 activates tumor stroma to promote intestinal polyposis. *Proc Natl Acad Sci USA*. (2015) 112:E2487–96. doi: 10.1073/pnas.1422445112
125. He Z, Chen L, Souto FO, Canasto-Chibuque C, Bongers G, Deshpande M, et al. Epithelial-derived IL-33 promotes intestinal tumorigenesis in Apc Min/+ mice. *Sci Rep.* (2017) 7:5520. doi: 10.1038/s41598-017-05716-z
126. O'Donnell C, Mahmoud A, Keane J, Murphy C, White D, Carey S, et al. An antitumorigenic role for the IL-33 receptor, ST2L, in colon cancer. *Oncol Lett*. (2018) 16:769–774. doi: 10.1038/bjc.2015.433
127. Guabiraba R, Besnard AG, Menezes GB, Secher T, Jabir MS, Amaral SS, et al. IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice. *Mucosal Immunol.* (2014) 7:1079–93. doi: 10.1038/mi.2013.124
128. Rabb H, Griffin MD, McKay DB, Swaminathan S, Pickkers P, Rosner MH, et al. Inflammation in AKI: current understanding, key questions, and knowledge gaps. *J Am Soc Nephrol*. (2016) 27:371–9. doi: 10.1681/ASN.2015030261
129. Xu Z, Zhao C, Wang Z, Tao J, Han Z, Zhang W, et al. Interleukin-33 levels are elevated in chronic allograft dysfunction of kidney transplant recipients and promotes epithelial to mesenchymal transition of human kidney (HK-2) cells. *Gene* (2017) 644:113–21. doi: 10.1016/j.gene.2017.11.010
130. Stone RC, Pastar I, Ojeh N, Chen V, Liu S, Garzon KI, et al. Epithelial-mesenchymal transition in tissue repair and fibrosis. *Cell Tissue Res.* (2016) 365:495–506. doi: 10.1007/s00441-016-2464-0.
131. Li Y, Kang YS, Dai C, Kiss LP, Wen X, Liu Y. Epithelial-to-mesenchymal transition is a potential pathway leading to podocyte dysfunction and proteinuria. *Am J Pathol.* (2008) 172:299–308. doi: 10.2353/ajpath.2008.070057
132. Yamaguchi Y, Iwano M, Suzuki D, Nakatani K, Kimura K, Harada K, et al. Epithelial-mesenchymal transition as an explanation for podocyte depletion in diabetic nephropathy. *Am J Kidney Dis*. (2009) 54:653–64. doi: 10.1053/j.ajkd.2009.05.009
133. Kang YS, Li Y, Dai C, Kiss LP, Wu C, Liu Y. Inhibition of integrin-linked kinase blocks podocyte epithelial-mesenchymal transition and ameliorates proteinuria. *Kidney Int*. (2010) 78:363–73. doi: 10.1038/ki.2010.137
134. Mutsaers HA, Stribos EG, Glorieux G, Vanholder R, Olinga P. Chronic kidney disease and fibrosis: the role of uremic retention solutes. *Front Med.* (2015) 2:60. doi: 10.3389/fmed.2015.00060

135. Ó hAinmhire E, Humphreys BD. Fibrotic changes mediating acute kidney injury to chronic kidney disease transition. *Nephron* (2018) 137:264–7. doi: 10.1159/000474960
136. Lee SJ, Borsting E, Declèves AE, Singh P, Cunard R. Podocytes express IL-6 and lipocalin 2/neutrophil gelatinase-associated lipocalin in lipopolysaccharide-induced acute glomerular injury. *Nephron Exp Nephrol*. (2012) 121:e86–96. doi: 10.1159/000345151
137. Millar NL, O'Donnell C, McInnes IB, Brint E. Wounds that heal and wounds that don't - The role of the IL-33/ST2 pathway in tissue repair and tumorigenesis. *Semin Cell Dev Biol*. (2017) 61:41–50. doi: 10.1016/j.semcdb.2016.08.007
138. Zhu J, Carver W. Effects of interleukin-33 on cardiac fibroblast gene expression and activity. *Cytokine* (2012) 58:368–79. doi: 10.1016/j.cyto.2012.02.008
139. Wang EW, Jia XS, Ruan CW, Ge ZR. miR-487b mitigates chronic heart failure through inhibition of the IL-33/ST2 signaling pathway. *Oncotarget* (2017) 8:51688–702. doi: 10.18632/oncotarget.18393
140. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* (2003) 107:721–6.
141. Sabatine MS, Morrow DA, Higgins LJ, MacGillivray C, Guo W, Bode C, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. *Circulation* (2008) 117:1936–44. doi: 10.1161/CIRCULATIONAHA.107.728022
142. McCarthy CP, Januzzi JL Jr. Soluble ST2 in heart failure. *Heart Fail Clin* (2018) 14:41–8. doi: 10.1016/j.hfc.2017.08.005
143. Januzzi JL Jr, Peacock WF, Maisel AS, Chae CU, Jesse RL, Baggish AL, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J Am Coll Cardiol*. (2007) 50:607–13. doi: 10.1016/j.jacc.2007.05.014
144. Ky B, French B, McCloskey K, Rame JE, McIntosh E, Shahi P, et al. High-sensitivity ST2 for prediction of adverse outcomes in chronic heart failure. *Circ Heart Fail*. (2011) 4:180–7. doi: 10.1161/CIRCHEARTFAILURE.110.958223
145. Dieplinger B, Mueller T. Soluble ST2 in heart failure. *Clin Chim Acta*. (2015) 443:57–70. doi: 10.1016/j.cca.2014.09.021
146. Aimo A, Vergaro G, Ripoli A, Bayes-Genis A, Pascual Figal DA, de Boer RA, et al. Meta-analysis of soluble suppression of tumorigenicity-2 and prognosis in acute heart failure. *JACC Heart Fail*. (2017) 5:287–96. doi: 10.1016/j.jchf.2016.12.016
147. Skali H, Gerwien R, Meyer TE, Snider JV, Solomon SD, Stolen CM. Soluble ST2 and risk of arrhythmias, heart failure, or death in patients with mildly symptomatic heart failure: results from MADIT-CRT. *J Cardiovasc Transl Res*. (2016) 9:421–8. doi: 10.1007/s12265-016-9713-1
148. Anand IS, Rector TS, Kuskowski M, Snider J, Cohn JN. Prognostic value of soluble ST2 in the valsartan heart failure trial. *Circ Heart Fail*. (2014) 7:418–26. doi: 10.1161/CIRCHEARTFAILURE.113.001036
149. Weir RA, Miller AM, Murphy GE, Clements S, Steedman T, Connell JM, et al. Serum soluble ST2: a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction. *J Am Coll Cardiol*. (2010) 55:243–50. doi: 10.1016/j.jacc.2009.08.047
150. Gruzdeva O, Uchasova E, Dyleva Y, Borodkina D, Akbasheva O, Belik E, et al. Relationships between epicardial adipose tissue thickness and adipofibrokine indicator profiles post-myocardial infarction. *Cardiovasc Diabetol*. (2018) 17:40. doi: 10.1186/s12933-018-0679-y
151. Tseng CCS, Huibers MMH, Gaykema LH, Siera-de Koning E, Ramjankhan FZ, Maisel AS, et al. Soluble ST2 in end-stage heart failure, before and after support with a left ventricular assist device. *Eur J Clin Invest*. (2018) 48:e12886. doi: 10.1111/eci.12886
152. Shah RV, Januzzi JL Jr. Soluble ST2 and galectin-3 in heart failure. *Clin Lab Med*. (2014) 34, 87–97. doi: 10.1016/j.cll.2013.11.009
153. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol*. (2007) 37:2779–86. doi: 10.1002/eji.200737547
154. Aoudjehane L, Pissaia AJr, Scatton O, Podevin P, Massault PP, Chouzenoux S, et al. Interleukin-4 induces the activation and collagen production of cultured human intrahepatic fibroblasts via the STAT-6 pathway. *Lab Invest*. (2008) 88:973–85. doi: 10.1038/labinvest.2008.61
155. Fuschiotti P, Larregina AT, Ho J, Feghali-Bostwick C, Medsger TA Jr. Interleukin-13-producing CD81 T cells mediate dermal fibrosis in patients with systemic sclerosis. *Arthritis Rheum*. (2013) 65:236–46. doi: 10.1002/art.37706
156. Koca SS, Pehlivan Y, Kara M, Alibaz-Oner F, Oztuzcu S, Yilmaz N, et al. The IL-33 gene is related to increased susceptibility to systemic sclerosis. *Rheumatol Int*. (2016) 36:579–84. doi: 10.1007/s00296-015-3417-8
157. Wagner A, Köhm M, Nordin A, Svenungsson E, Pfeilschifter JM, Radeke HH. Increased serum levels of the IL-33 neutralizing sST2 in limited cutaneous systemic sclerosis. *Scand J Immunol*. (2015) 82:269–74. doi: 10.1111/sji.12317
158. Hachulla E, Launay D. Diagnosis and classification of systemic sclerosis. *Clinic Rev Allerg Immunol*. (2011) 40:78–83. doi: 10.1007/s12016-010-8198-y
159. Mathes AL, Christmann RB, Stifano G, Affandi AJ, Radstake TR, Farina GA, et al. Global chemokine expression in systemic sclerosis (SSc): CCL19 expression correlates with vascular inflammation in SSc skin. *Ann Rheum Dis*. (2014) 73:1864–72. doi: 10.1136/annrheumdis-2012-202814

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Kotsiou, Gourgoulianis and Zarogiannis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

MINI REVIEW
published: 26 October 2018
doi: 10.3389/fimmu.2018.02506

The Pro-tumorigenic IL-33 Involved in Antitumor Immunity: A Yin and Yang Cytokine

*Jean-Jacques Fournié and Mary Poupot**

INSERM UMR 1037 Centre de Recherche en Cancérologie de Toulouse (CRCT), ERL 5294 CNRS, Université Toulouse III Paul Sabatier, Laboratoire d'excellence Toucan, Toulouse, France

Interleukin-33 (IL-33), considered as an alarmin released upon tissue stress or damage, is a member of the IL-1 family and binds the ST2 receptor. First described as a potent initiator of type 2 immune responses through the activation of T helper 2 (T_H2) cells and mast cells, IL-33 is now also known as an effective stimulator of T_H1 immune cells, natural killer (NK) cells, iNKT cells, and CD8 T lymphocytes. Moreover, IL-33 was shown to play an important role in several cancers due to its pro and anti-tumorigenic functions. Currently, IL-33 is a possible inducer and prognostic marker of cancer development with a direct effect on tumor cells promoting tumorigenesis, proliferation, survival, and metastasis. IL-33 also promotes tumor growth and metastasis by remodeling the tumor microenvironment (TME) and inducing angiogenesis. IL-33 favors tumor progression through the immune system by inducing M2 macrophage polarization and tumor infiltration, and upon activation of immunosuppressive cells such as myeloid-derived suppressor cells (MDSC) or regulatory T cells. The anti-tumor functions of IL-33 also depend on infiltrated immune cells displaying T_H1 responses. This review therefore summarizes the dual role of this cytokine in cancer and suggests that new proposals for IL-33-based cancer immunotherapies should be considered with caution.

Keywords: interleukin-33, immunity, cancer, immunosuppression, microenvironment

OPEN ACCESS

Edited by:
Hui-Rong Jiang,
University of Strathclyde,
United Kingdom

Reviewed by:
Paola Italiani,
Consiglio Nazionale Delle Ricerche (CNR), Italy
Kesley Attridge,
Aston University, United Kingdom

****Correspondence:***
Mary Poupot
mary.poupot@inserm.fr

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *28 May 2018*
Accepted: *10 October 2018*
Published: *26 October 2018*

Citation:
Fournié J-J and Poupot M (2018) The Pro-tumorigenic IL-33 Involved in Antitumor Immunity: A Yin and Yang Cytokine. Front. Immunol. 9:2506. doi: 10.3389/fimmu.2018.02506

INTRODUCTION

Cancer development depends on hallmarks such as self-sufficient proliferation, escape to anti-apoptotic signals, resistance to apoptosis, immune evasion, infinite replication, nurture of vascularization, and ability for invasion and metastasis (1). However, these hallmarks do not only concern the cancer cell but also the tumor microenvironment (TME) which is essential for tumorigenesis. The TME consists of fibroblasts, endothelial cells, immune cells, pericytes, and smooth muscle cells which are recruited by cancer cells as non-malignant cells but then modified to take part in tumor development (2–4). Besides cellular components, acellular components such as matrix, chemokines, and cytokines are also essential for tumor development (4). Cytokines as central mediators, favor the interaction between cells in the inflammatory tumor microenvironment (5). Amongst these cytokines, Interleukin-33 (IL-33), a member of the IL-1 superfamily of cytokines (6), is well-known now to have an important role in innate and adaptive immunity through its contribution to tissue homeostasis and responses to stress such as tumor

development. IL-33 is constitutively expressed at high levels in the nucleus of human and mouse tissue lining and in various cell types including vascular endothelium (7), endothelial cells of endothelial venules (HEVs) (8, 9) and epithelial cells in barrier tissues that are exposed to the environment such as bronchial epithelial cells (10), keratinocytes, epithelial cells of the stomach, and salivary glands (7). Fibroblastic reticular cells (FRCs) in lymphoid tissues and cells of the central nervous system represent a major source of IL-33 (7, 11). IL-33 was first described in HEV as an intracellular nuclear factor with transcriptional regulatory properties (8). It was then shown that IL-33 binds a heterodimer formed by the specific ST2 receptor and a co-receptor, the IL-1 receptor accessory protein (6, 12). To exert its cytokine activity and alert the immune system, IL-33 is not secreted extracellularly like a conventional cytokine but after cell injury following cell stress or damage (11, 13–16). Full-length IL-33 is thus considered as an alarmin produced as a result of an injury to the central nervous system (15), a mechanical stress (17, 18), necroptosis (19) but also in pathological wound repair and fibrosis (20–22). The IL-33/ST2 axis is also associated with many inflammatory diseases such as asthma (23–25), rheumatoid arthritis, psoriatic arthritis or osteoarthritis (26), pulmonary fibrosis (27) or dermatitis and allergic contact dermatitis (28, 29). Many publications have summarized the important role of IL-33 in these diverse inflammatory diseases (30–34). IL-33/ST2 signaling is transduced by MyD88 and the kinase-4 associated to the ST2 receptor, which is a downstream adaptor protein, shared with other IL-1 family members and Toll-like receptors (35). Moreover, the soluble form of ST2 (sST2) produced from 3′-UTR promoter or splice variants mRNA, can be a decoy receptor for IL-33 (36–38). IL-33 was first described as a potent initiator of type 2 immune responses through the activation of many cell types, including the T_H2 subset of helper cells, type 2 innate lymphoid cells (ILC2s), mast cells, basophils, eosinophils, and myeloid cells such as myeloid-derived antigen-presenting cells including macrophages and dendritic cells (DCs) (6, 35, 39–45). Furthermore, IL-33-exposed DCs or mast cells also selectively support $FOXP3^+$ regulatory T cell (T_{reg} cells) expansion through IL-33-induced secretion of IL-2 (40, 41) and favor T_H17 cell differentiation through IL-1β and IL-6 secretion (46). IL-33 was detected in the serum of patients with T_H1/T_H17 mediated diseases (47, 48). However, besides this pro-inflammatory function of IL-33, its protective role in atherosclerosis, obesity, type2 diabetes and cardiac remodeling also holds an important place (16, 49–51). Moreover, IL-33 can also activate type 1 immune responses via TNF-α and IFN-γ expression by CD8 T lymphocytes, natural killer (NK) cells or iNKT cells. The latter can be stimulated by IL-33 upon its ligation to their cell surface ST2 receptors (13, 52–55). Finally, several studies have shown an important involvement of IL-33 in several types of cancer with pro or anti-tumorigenic functions depending on the immune status of the tumor. The goal of this review is to summarize the hallmarks of IL-33 in cancer, both in terms of its pro-tumorigenic function targeting resident T_H2 immune cells of the TME, and as a tumor suppressor molecule activating the competent T_H1 immune cells.

These properties therefore position IL-33 as a possible inducer and prognostic marker of cancer development, as reviewed here.

IL-33 AS A MARKER FOR GOOD OR POOR PROGNOSIS

IL-33 has been shown to be a promising biomarker in several types of cancer for tumor detection and as a predictor of prognosis and therapeutic response. Recently, IL-33 was shown to be correlated with a bad prognosis in several types of cancer, although in some cases IL-33 behaves as a tumor suppressor by inducing an immune response. In terms of bad prognosis, high levels of IL-33 were detected in the serum and tumors of patients with glioma (56), gastric cancer (57), hepatocellular carcinoma (58), uterine leiomyoma (59), lung cancer (60), colorectal cancer (61), head and neck squamous cell carcinoma (62), and breast cancer (63), when compared to corresponding healthy tissues. The Cancer Genome Atlas Pan-Cancer analysis project showed and declared that the level of IL-33 expression is altered in only 3% of ∼580 tumors and that the most common genetic alteration is the deletion of the IL-33 gene (64).

Lu and collaborators detected "significantly higher IL-33 expression in glioma tissues than in normal brain tissues through immune-histochemical (IHC) analysis" (56). High IL-33 expression in glioma was correlated with shorter overall survival (OS) and progression-free survival (PFS) (56). In women, IL-33 highly promotes epithelial cell proliferation and tumorigenesis in breast cancer, since IL-33 increases Cancer Osaka Thyroid (COT) phosphorylation via ST2-COT interaction in normal epithelial and breast cancer cells. This induces the activation of MEK-ERK, JNK-cJun, and STAT3 signaling pathways, both leading to cell proliferation (65). The expression levels of IL-33 and ST2 proteins were also positively correlated with the expression of Ki-67 in epithelial ovarian cancer tumors and at the metastatic site, and negatively correlated with the patient survival time (66). High expression of IL-33, assessed by IHC staining, was associated with advanced stage clear-cell renal carcinoma and abnormally high amounts of serum IL-33 was detected in patients with hepatocellular carcinoma or gastric cancer. Hence, IL-33 is correlated with a bad prognosis in these types of cancer (57, 58, 67). sST2 was also described as a negative prognostic marker when its serum concentration was associated with OS of patients with hepatocellular carcinoma (68). Nevertheless, this soluble IL-33 receptor can also be associated with a good prognosis in colorectal cancer, as the trapping of soluble IL-33 in the TME inhibits cancer growth and metastases (69). Moreover, the level of IL-33 protein has been inversely correlated with tumor grade and size in patients with pulmonary adenocarcinoma, showing an association of low IL-33 expression level with a poor prognosis (70–72). Likewise, a genome-wide association study unveiled a correlation between high IL-33 expression and a good prognosis in patients with osteosarcoma (73). However, if IL-33 can have a pro-tumor effect by directly targeting cancer cells, the tumor suppressor functions displayed by IL-33 are indirectly promulgated by immune surveillance as we will show hereafter.

IL-33 AS A PRO-TUMORIGENIC CYTOKINE THROUGH ACTIONS ON CANCER CELLS AND TME

As described above, IL-33 is considered as a prognostic biomarker when expressed in tumors. This intratumoral IL-33 is expressed by cancer cells as well as by other cell components of the TME. For instance, in patients with head and neck squamous cell carcinoma and oral squamous cell carcinoma, intratumoral IL-33 has been shown to be expressed in cancer-associated fibroblasts (CAF) (62, 74). This infiltrating IL-33 has a direct pro-tumorigenic effect on cancer cells and indirect effects on cellular components of the TME.

In the first case, Wang and collaborators showed that the IL-33/ST2 pathway up-regulated membrane glucose transporter 1 in non-small-cell lung cancer cells, enhancing their glucose uptake and glycolysis, thus favoring *in vitro* outgrowth of human lung cancer and its metastasis in a mouse model (60). By *in vitro* and *in vivo* experiments, IL-33 was also shown to be able to promote growth, invasion and migration of gastric cancer and colorectal cancer cells due to the autocrine secretion of several metalloproteases (MMP3, MMP9, MMP2), IL-6 and CXCR4 via the ST2-ERK1/2 pathway (61, 75). Moreover, IL-33 directly targets colon cancer cells and breast cancer cells via JNK-cJun activation, which promotes cell proliferation and therefore tumor growth (65, 76).

The impact of IL-33 on the TME encompasses angiogenesis, matrix remodeling and cytokine/growth factor production by non-epithelial cell components. The IL-33/ST2 signaling pathway, favoring pro-angiogenic VEGF expression in tumor cells and reducing tumor necrosis, is highly involved in mammary tumor growth (77). Concerning matrix modeling, human subepithelial myofibroblasts stimulated *in vitro* with IL-33 induced the expression of extracellular matrix components and growth factors associated with intestinal tumor progression (78). IL-33-stimulated cancer cells produce cytokines, and TME infiltrating immune cells are also involved in the expression of IL-6 in response to IL-33/ST2 signaling. Likewise, IL-33 stimulates the secretion of cytokines and growth factors in bone marrow myeloid and non-hematopoietic cells, resulting in myeloproliferation of neoplasms (79, 80). Indeed, suppression of IL-33 or high expression of sST2 suppresses IL-33-induced angiogenesis, T_H2 responses, macrophage infiltration and M2 macrophage polarization. This negatively regulates tumor growth and metastatic spread of colorectal cancer, for instance through the modification of the TME (69). IL-33 in the TME recruits macrophages and stimulates their production of PGE_2, and in turn, macrophage-derived PGE_2 stimulates colon tumor development (76). The recruitment of macrophages in the TME might account for the stimulation of CCL2 expression by IL-33-stimulated cancer cells that express ST2, such as human colon cancer cells (81). After recruitment, macrophages are directly induced by IL-33 to be polarized in M2 tumor associated macrophages (TAM) in the TME. Such TAMs are then able to produce IL-10, VEGF, IL-6, and MMP9 which promote proliferation and invasiveness of cancer cells (82–84). Yang and collaborators showed that TAM are recruited by IL-33 in the TME, and IL-33-stimulated TAM can increase intravasation of tumor cells into the circulation at the early stages of metastasis (85). Even in the brain, IL-33 in the TME induces growth of glioma cells and facilitates microglia/macrophage infiltration (86). IL-33-stimulated macrophages are also activated to produce G-CSF, which in turn, boost myeloid-derived suppressor cells (MDSC) from the pro-tumoral TME (87). Indeed, MDSC contribute to tumor-mediated immune escape by suppressing antitumor immune responses. IL-33 released in tumor tissues in breast and colorectal cancer mouse models and in breast cancer patients, has been shown to facilitate MDSC expansion, recruitment and survival in the TME. This role could be due to the induction of an autocrine secretion of GM-CSF (88–90). Interestingly, another study showed that IL-33 does not affect the number of MDSC but can significantly reduce the differentiation of lineage-negative bone marrow progenitor cells into granulocytic MDSC in tumor-bearing mice. Moreover in the same study, IL-33-treated MDSC were shown to be less immunosuppressive, with a reduced capacity to inhibit T cell proliferation and IFN-γ production, production of reactive oxygen species and their capacity to induce T_{reg} differentiation and expansion (91). IL-33 has a direct effect on T_{reg} cells expressing surface ST2. Indeed, these lymphocytes are constitutively abundant in the intestine and able to prevent dysregulated inflammatory responses to self and environmental stimuli. IL-33 is constitutively expressed in epithelial cells at barrier sites. High levels of IL-33 were also observed in inflamed lesions of patients with inflammatory bowel disease, supporting its role in disease pathogenesis (92, 93). In inflammatory conditions, IL-33 signaling in T_{reg} cells enhances transforming growth factor (TGF)-β1-mediated differentiation. Alternatively, IL-33 may provide a signal necessary for inducing their accumulation and maintenance in inflamed tissues (94). Local accumulation of T_{reg} cells has been described in intestinal tumors preventing tumor clearance in mouse models and in patients. This role may be associated with a reduction of E-cadherin expression, increased β-catenin signaling and IL-33 production by malignant and injured epithelial cells (95). In tumors with low levels of infiltrating T_{reg} cells, administration of IL-33 accelerates tumor growth and occurrence of liver and lung metastasis in breast cancer mouse models, and these models display an intratumoral accumulation of MDSC and T_{reg} cells, as compared to untreated mice (90). Moreover, IL-33 blockade, in addition to abrogating the polarization of TAM, reduces the accumulation of T_{reg} cells in lung tumors of human lung preclinical mouse models (82). However, as inflammation contributes to tumorigenesis, the accumulation of T_{reg} in inflammatory zones must contain inflammation and therefore tumorigenesis. T_{reg} may promote or inhibit tumor development depending on the context, revealing the complex relationship between inflammation, and cancer development. Furthermore, mast cells which also express ST2 receptors and respond to cell injury via IL-33 released from necrotic cells, can secrete leukotrienes and cytokines to initiate pro-inflammatory responses (96). In a colorectal cancer mouse model, IL-33 deficiency reduced mast cell accumulation in tumors. This

deficiency further inhibited the expression of mast cell-derived proteases and cytokines that promote polyposis (78, 96–98). Generally, mast cells accumulate in inflamed gut and in colorectal tumors, and their presence is correlated with a poor prognosis and low overall survival (99, 100). In skin cancers, dermal mast cells are able to respond to UVB-induced IL-33 by releasing IL-10 to protect skin homeostasis after excessive UVB exposure. However, IL-10 may contribute to skin cancer development, as IL-10-deficient mice do not develop skin tumors upon UVB exposure (101, 102).

DIRECT OR INDIRECT EFFECTS OF IL-33 AS A TUMOR SUPPRESSOR

Alongside its pro-tumorigenic role, IL-33 can also behave as a tumor suppressor. Only one study has shown a direct anti-tumor effect of this cytokine with the *in vitro* inhibition of proliferation and induction of apoptosis of MIA PaCa-2, a pancreatic cancer cell line (103). However, its anti-tumor functions were largely associated with the activation of immune effector cells able to lead to tumor clearance. All immune cells express the ST2 receptor and are able to respond to IL-33 stimulation. IL-33 has a significant role in cancer immune-surveillance in primary prostate and lung tumors, which can be lost during the metastatic transition inducing immune escape. The correlation between IL-33 and HLA expression in human tumors using RNA-sequencing data of resected prostate tumors was recently shown. The down-regulation of IL-33 during the metastatic process ultimately decreases the functionality of HLA-I and reduces immune-surveillance favoring tumor development (104). In a multivariable analysis, the infiltration of human hepatocellular carcinomas (HCC) by cells expressing IL-33 and by $CD8^+$ T cells was associated with prolonged patient survival. These results led to propose an HCC immune score identifying high- vs. low-risk patients with different gene expression profiles (105). Injection of IL-33 into established murine melanoma or acute myeloid leukemia models inhibits tumor growth in a $CD8^+$ T cell-dependent manner prolonging the survival of mice. In the first model, the reduction of tumor growth delay was correlated with intratumoral accumulation of $CD8^+$ T cells, and a decrease in the number of immunosuppressive myeloid cells (106). In the second model, the anti-leukemia activity was associated with increased expansion and IFN-γ production of leukemia-reactive $CD8^+$ T cells (107). Moreover, the correlation between decreased IFN-γ secretion and colon cancer aggressiveness, suggests that IL-33 signaling defects may impair the generation of IFN-γ-mediated immunity (108). In soft tissue sarcoma, higher transcriptional levels of IL-33 were also associated with a good prognosis. The expression of IL-33 has also been negatively correlated with the expression of chemokines, such as TGF-β, recruiting T_{reg} and MDSC, and positively correlated with the expression of chemokines that recruit $CD8^+$ T cells which promote anti-tumor immune responses especially through INF-γ production (109).

It has been shown that IFN-γ-producing cells present in tumors associated with an IL-33 antitumor effect, were $CD8^+$ T cells and NK cells. Indeed, IL-33 expression in several cancers affects the number of $CD8^+$ T cells and NK cells in tumor tissues and the production of IFN-γ/TNF-α, thereby favoring tumor eradication through tumor cell cytolysis (110, 111). This was also shown with the reduction of tumor metastasis in B16 melanoma and Lewis lung carcinoma metastatic models thanks to the transgenic expression of IL-33. In these transgenic mice models, tumor infiltration and $CD8^+$ T lymphocyte and NK cell cytotoxicity was significantly increased compared to non-transgenic mice. Moreover, treatment with recombinant IL-33 increased $CD8^+$ T lymphocyte and NK cell cytotoxicity *in vitro* (112). $CD8^+$ T cells are also indirectly stimulated by IL-33 through DC. DC maturation is promoted by IL-33 which increases their cross presentation ability particularly during the anti-leukemia or anti-melanoma immune response (107, 113). As mentioned in the introduction, IL-33-activated DC are also able to promote the differentiation of T_H17 cells which play an important role in cancer development. T_H17 are T helper lymphocytes secreting IL-17 and other inflammatory cytokines, but can also display immunosuppressive activities, therefore mediating context dependent pro- or anti-tumor responses (114, 115). However, there are no published studies mentioning a direct relationship between T_H17 cells and IL-33 in cancers. T_H17 cells expressing the ST2 receptor were found to accumulate in the small intestine in bowel diseases where intestinal epithelial cells are the providing source of IL-33, we can therefore stipulate that these cells could play a role in digestive cancers. T_H17 cells could have an anti-tumor function with the production of pro-inflammatory cytokines (116) or a pro-tumor role when they can acquire a regulatory phenotype with immunosuppressive properties upon IL-33 activation (117). Furthermore, DC can also drive T_H9 cell dependent anti-tumor responses through the expression of Ox40L when activated by IL-33 and stimulated by dectin-1 signaling (118–120).

Finally, the ILC2s which support type 2 immune responses by producing IL-5 and IL-13 in response to IL-33 could also have an antitumor function. Indeed, their tissue-repair function can induce cholangiocarcinoma and liver metastasis (121). ILC2s can also be mobilized from the lung and other tissues thanks to IL-33, to penetrate tumors, mediate immune-surveillance with DC, and promote adaptive cytolytic T cell responses and attraction (122, 123).

CONCLUDING REMARKS

IL-33 therefore appears as a pro-tumorigenic cytokine that can also limit tumor growth through the activation of antitumor immunity. These opposing roles in tumorigenesis, as shown in this review, greatly depend on the IL-33/ST2 signaling in different immune cells. IL-33 is able to promote inflammatory events which contribute to tumorigenesis whilst activating anti-tumor immune responses. The different events promoted by IL-33 activation of various immune cells which can be found in the TME are summarized in the **Figure 1**. Depending on the tumor context, IL-33 produced in the TME can activate diverse

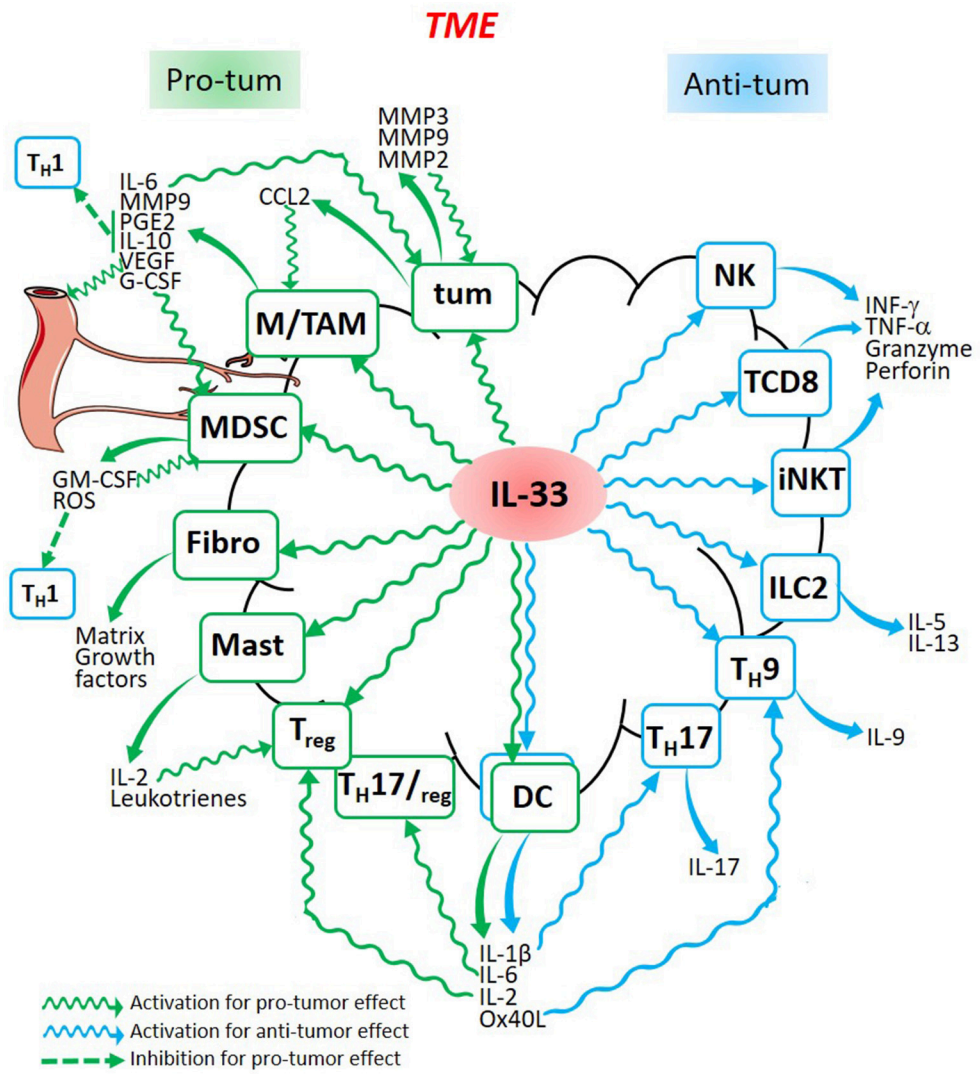


FIGURE 1 | Dual role of Il-33 in cancer**.** IL-33 released in the TME is able to stimulate cancer cells (tum), fibroblasts (Fibro), and different immune cells (Macrophages, TAM, MDSC, mast cells, T_{reg}, Dendritic Cells, T_H17, T_H9, ILC2, iNKT, $CD8^+$ T cells, and Natural Killer cells) which are activated to produce molecules involved in pro-tumor (green) or anti-tumor (blue) processes leading to the development or to the regression of the tumor. Some cytokines produced by pro-tumor cells such as MDSC or TAM, are also able to produce cytokines which inhibit anti-tumor cells such as all the T_H1 cells.

immune cells which are able to promote a pro-tumor effect such as TAM, MDSC, fibroblasts, mast cells, T_{reg} and DC, or to prevent tumor development such as NK cells, $CD8^+$ T cells, iNKT, ILC2, T_H9, and T_H17. All these cell types produce specific cytokines, chemokines, and other molecules. These conclusions are supported by Wasmer and Krebs' review who demonstrated the multiple functions of IL-33 in different cancer types (124). As many cytokines with immunomodulatory properties, IL-33 has been considered for anticancer immunotherapies. However, knowing its dual role, therapeutic manipulation of this cytokine should be considered with caution. The majority of the studies mentioned propose cancer immunotherapy strategies based on exogenous IL-33 administration. These IL-33 adjuvanted vaccines aim at activating the immune cells involved in the immune response (106, 107, 125–128). IL-33 could also indirectly activate effector T cells. For instance, a replicating viral vector system used in cancer immunotherapy which delivers tumor-associated antigens to DC for efficient cytotoxic T cells priming, depends on IL-33 signaling (129). IL-33 could likewise increase T cell activation to promote graft-vs.-leukemia (GVL) reactions while decreasing fatal graft-vs.-host- disease (GVHD) (130). Possibly however, ST2 blockade might preserve GVL activity by blocking T_{reg} controlling GVHD (131). Indeed, considering the immunosuppressive pro-tumorigenic role of IL-33, others have proposed to block IL-33 as a novel anticancer strategy (62, 65, 69, 76, 82, 88). In the future, IL-33 targeting in cancer immunotherapies should be considered with caution, especially taking into account the

intricate dual role of this cytokine in cancer as shown in this review.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This work was supported by institutional grants from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Université Toulouse 3, the Centre National de la Recherche Scientifique (CNRS) and the Laboratoire d'Excellence TOUCAN.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
2. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* (2012) 21:309–22. doi: 10.1016/j.ccr.2012.02.022
3. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* (2010) 140:883–99. doi: 10.1016/j.cell.2010.01.025
4. Leibovici J, Itzhaki O, Huszar M, Sinai J. The tumor microenvironment: part 1. *Immunotherapy* (2011) 3:1367–84. doi: 10.2217/imt.11.111
5. Candido J, Hagemann T. Cancer-Related Inflammation. *J Clin Immunol.* (2013) 33:79–84. doi: 10.1007/s10875-012-9847-0
6. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
7. Moussion C, Ortega N, Girard J-P. 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:e3331. doi: 10.1371/journal.pone.0003331
8. Carriere V, Roussel L, Ortega N, Lacorre D-A, Americh L, Aguilar L, 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 USA.* (2007) 104:282–7. doi: 10.1073/pnas.0606854104
9. Baekkevold ES, Roussigné M, Yamanaka T, Johansen F-E, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol.* (2003) 163:69–79. doi: 10.1016/S0002-9440(10)63631-0
10. Hardman CS, Panova V, McKenzie ANJ. IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation. *Eur J Immunol.* (2013) 43:488–98. doi: 10.1002/eji.201242863
11. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, 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) 188:3488–95. doi: 10.4049/jimmunol.1101977
12. Lingel A, Weiss TM, Niebuhr M, Pan B, Appleton BA, Wiesmann C, 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) 17:1398–410. doi: 10.1016/j.str.2009.08.009
13. Bonilla WV, Fröhlich A, Senn K, Kallert S, Fernandez M, Johnson S, et al. The alarmin interleukin-33 drives protective antiviral $CD8^+$ T cell responses. *Science* (2012) 335:984–9. doi: 10.1126/science.1215418
14. Cayrol C, Girard J-P. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA.* (2009) 106:9021–6. doi: 10.1073/pnas.0812690106
15. Gadani SP, Walsh JT, Smirnov I, Zheng J, Kipnis J. The glia-derived alarmin IL-33 orchestrates the immune response and promotes recovery following CNS injury. *Neuron* (2015) 85:703–9. doi: 10.1016/j.neuron.2015.01.013
16. Miller AM. Role of IL-33 in inflammation and disease. *J Inflamm.* (2011) 8:22. doi: 10.1186/1476-9255-8-22
17. Chen WY, Hong J, Gannon J, Kakkar R, Lee RT. Myocardial pressure overload induces systemic inflammation through endothelial cell IL-33. *Proc Natl Acad Sci USA.* (2015) 112:7249–54. doi: 10.1073/pnas.1424236112
18. Kakkar R, Hei H, Dobner S, Lee RT. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J Biol Chem.* (2012) 287:6941–8. doi: 10.1074/jbc.M111.298703
19. Rickard JA, O'Donnell JA, Evans JM, Lalaoui N, Poh AR, Rogers T, et al. RIPK1 regulates RIPK3-MLKL-driven systemic inflammation and emergency hematopoiesis. *Cell* (2014) 157:1175–88. doi: 10.1016/j.cell.2014.04.019
20. McHedlidze T, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, et al. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity* (2013) 39:357–71. doi: 10.1016/j.immuni.2013.07.018
21. Molofsky AB, Van Gool F, Liang H-E, Van Dyken SJ, Nussbaum JC, Lee J, et al. Interleukin-33 and interferon-γ counter-regulate group 2 innate lymphoid cell activation during immune perturbation. *Immunity* (2015) 43:161–74. doi: 10.1016/j.immuni.2015.05.019
22. Liew FY, Girard J-P, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
23. Lloyd CM. IL-33 family members and asthma - bridging innate and adaptive immune responses. *Curr Opin Immunol.* (2010) 22:800–6. doi: 10.1016/j.coi.2010.10.006
24. Mizutani N, Nabe T, Yoshino S. IL-17A promotes the exacerbation of IL-33-induced airway hyperresponsiveness by enhancing neutrophilic inflammation via CXCR2 signaling in mice. *J Immunol.* (2014) 192:1372–84. doi: 10.4049/jimmunol.1301538
25. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat Immunol.* (2018) 19:375–85. doi: 10.1038/s41590-018-0067-5
26. Talabot-Ayer D, McKee T, Gindre P, Bas S, Baeten DL, Gabay C, et al. Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Joint Bone Spine* (2012) 79:32–37. doi: 10.1016/j.jbspin.2011.02.011
27. Tajima S, Bando M, Ohno S, Sugiyama Y, Oshikawa K, Tominaga S, et al. ST2 gene induced by type 2 helper T cell (Th2) and proinflammatory cytokine stimuli may modulate lung injury and fibrosis. *Exp Lung Res.* (2007) 33:81–97. doi: 10.1080/01902140701198583
28. Cevikbas F, Steinhoff M. IL-33: a novel danger signal system in atopic dermatitis. *J Invest Dermatol.* (2012) 132:1326–9. doi: 10.1038/jid.2012.66
29. Hueber AJ, Alves-Filho JC, Asquith DL, Michels C, Millar NL, Reilly JH, et al. IL-33 induces skin inflammation with mast cell and neutrophil activation. *Eur J Immunol.* (2011) 41:2229–37. doi: 10.1002/eji.201041360
30. Gupta RK, Gupta K, Dwivedi PD. Pathophysiology of IL-33 and IL-17 in allergic disorders. *Cytokine Growth Factor Rev.* (2017) 38:22–36. doi: 10.1016/j.cytogfr.2017.09.005
31. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol.* (2017) 8:475. doi: 10.3389/fimmu.2017.00475
32. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity* (2013) 39:1003–18. doi: 10.1016/j.immuni.2013.11.010
33. Braun H, Afonina IS, Mueller C, Beyaert R. Dichotomous function of IL-33 in health and disease: from biology to clinical implications. *Biochem Pharmacol.* (2018) 148:238–52. doi: 10.1016/j.bcp.2018.01.010
34. Di Salvo E, Ventura-Spagnolo E, Casciaro M, Navarra M, Gangemi S. IL-33/IL-31 axis: a potential inflammatory pathway. *Mediators Inflamm.* (2018) 2018:3858032. doi: 10.1155/2018/3858032
35. Espinassous Q, Garcia-de-Paco E, Garcia-Verdugo I, Synguelakis M, von Aulock S, Sallenave J-M, et al. IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating

lipopolysaccharide receptor complex. *J Immunol.* (2009) 183:1446–55. doi: 10.4049/jimmunol.0803067
36. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem.* (2007) 282:26369–80. doi: 10.1074/jbc.M704916200
37. Iwahana H, Yanagisawa K, Ito-Kosaka A, Kuroiwa K, Tago K, Komatsu N, et al. Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. *Eur J Biochem.* (1999) 264:397–406.
38. Bergers G, Reikerstorfer A, Braselmann S, Graninger P, Busslinger M. Alternative promoter usage of the Fos-responsive gene Fit-1 generates mRNA isoforms coding for either secreted or membrane-bound proteins related to the IL-1 receptor. *EMBO J.* (1994) 13:1176–88.
39. Ali S, Huber M, Kollewe C, Bischoff SC, Falk W, Martin MU. IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proc Natl Acad Sci USA.* (2007) 104:18660–5. doi: 10.1073/pnas.0705939104
40. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* (2009) 113:1526–34. doi: 10.1182/blood-2008-05-157818
41. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TKA, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* (2010) 464:1367–70. doi: 10.1038/nature08900
42. Chang Y-J, Kim HY, Albacker LA, Baumgarth N, McKenzie ANJ, Smith DE, et al. Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nat Immunol.* (2011) 12:631–8. doi: 10.1038/ni.2045
43. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol.* (2009) 183:6469–77. doi: 10.4049/jimmunol.0901575
44. Turnquist HR, Sumpter TL, Tsung A, Zahorchak AF, Nakao A, Nau GJ, et al. IL-1beta-driven ST2L expression promotes maturation resistance in rapamycin-conditioned dendritic cells. *J Immunol.* (2008) 181:62–72. doi: 10.4049/jimmunol.181.1.62
45. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol.* (2009) 123:1047–54. doi: 10.1016/j.jaci.2009.02.026
46. Park SH, Kim MS, Lim HX, Cho D, Kim TS. IL-33-matured dendritic cells promote Th17 cell responses via IL-1β and IL-6. *Cytokine* (2017) 99:106–13. doi: 10.1016/j.cyto.2017.07.022
47. Vaccaro M, Cicero F, Mannucci C, Calapai G, Spatari G, Barbuzza O, et al. IL-33 circulating serum levels are increased in patients with non-segmental generalized vitiligo. *Arch Dermatol Res.* (2016) 308:527–30. doi: 10.1007/s00403-016-1675-2
48. Liang M, Liwen Z, Yun Z, Yanbo D, Jianping C. Serum levels of IL-33 and correlation with IL-4, IL-17A, and hypergammaglobulinemia in patients with autoimmune hepatitis. *Mediators Inflamm.* (2018) 2018:7964654. doi: 10.1155/2018/7964654
49. Theoharides TC, Petra AI, Taracanova A, Panagiotidou S, Conti P. Targeting IL-33 in autoimmunity and inflammation. *J Pharmacol Exp Ther.* (2015) 354:24–31. doi: 10.1124/jpet.114.222505
50. Han JM, Wu D, Denroche HC, Yao Y, Verchere CB, Levings MK. IL-33 Reverses an obesity-induced deficit in visceral adipose tissue ST2+ T regulatory cells and ameliorates adipose tissue inflammation and insulin resistance. *J Immunol.* (2015) 194:4777–83. doi: 10.4049/jimmunol.1500020
51. Hasnain SZ, Borg DJ, Harcourt BE, Tong H, Sheng YH, Ng CP, et al. Glycemic control in diabetes is restored by therapeutic manipulation of cytokines that regulate beta cell stress. *Nat Med.* (2014) 20:1417–26. doi: 10.1038/nm.3705
52. Baumann C, Bonilla WV, Fröhlich A, Helmstetter C, Peine M, Hegazy AN, et al. T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell responses. *Proc Natl Acad Sci USA.* (2015) 112:4056–61. doi: 10.1073/pnas.1418549112
53. Yang Q, Li G, Zhu Y, Liu L, Chen E, Turnquist H, et al. IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8+ T cells. *Eur J Immunol.* (2011) 41:3351–60. doi: 10.1002/eji.201141629
54. Smithgall MD, Comeau MR, Yoon B-RP, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int Immunol.* (2008) 20:1019–30. doi: 10.1093/intimm/dxn060
55. Bourgeois E, Van LP, Samson M, Diem S, Barra A, Roga S, et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol.* (2009) 39:1046–55. doi: 10.1002/eji.200838575
56. Zhang J, Wang P, Ji W, Ding Y, Lu X. Overexpression of interleukin-33 is associated with poor prognosis of patients with glioma. *Int J Neurosci.* (2017) 127:210–7. doi: 10.1080/00207454.2016.1175441
57. Sun P, Ben Q, Tu S, Dong W, Qi X, Wu Y. Serum interleukin-33 levels in patients with gastric cancer. *Dig Dis Sci.* (2011) 56:3596–601. doi: 10.1007/s10620-011-1760-5
58. Zhang P, Liu X-K, Chu Z, Ye JC, Li K-L, Zhuang W-L, et al. Detection of interleukin-33 in serum and carcinoma tissue from patients with hepatocellular carcinoma and its clinical implications. *J Int Med Res.* (2012) 40:1654–61. doi: 10.1177/030006051204000504
59. Santulli P, Even M, Chouzenoux S, Millischer AE, Borghese B, de Ziegler D, et al. Profibrotic interleukin-33 is correlated with uterine leiomyoma tumour burden. *Hum Reprod.* (2013) 28:2126–33. doi: 10.1093/humrep/det238
60. Wang C, Chen Z, Bu X, Han Y, Shan S, Ren T, et al. IL-33 signaling fuels outgrowth and metastasis of human lung cancer. *Biochem Biophys Res Commun.* (2016) 479:461–8. doi: 10.1016/j.bbrc.2016.09.081
61. Liu X, Zhu L, Lu X, Bian H, Wu X, Yang W, et al. IL-33/ST2 pathway contributes to metastasis of human colorectal cancer. *Biochem Biophys Res Commun.* (2014) 453:486–92. doi: 10.1016/j.bbrc.2014.09.106
62. Chen S-F, Nieh S, Jao S-W, Wu M-Z, Liu C-L, Chang Y-C, et al. The paracrine effect of cancer-associated fibroblast-induced interleukin-33 regulates the invasiveness of head and neck squamous cell carcinoma. *J Pathol.* (2013) 231:180–9. doi: 10.1002/path.4226
63. Liu J, Shen J-X, Hu J-L, Huang W-H, Zhang G-J. Significance of interleukin-33 and its related cytokines in patients with breast cancers. *Front Immunol.* (2014) 5:141. doi: 10.3389/fimmu.2014.00141
64. Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, Mills GB, Shaw KRM, Ozenberger BA, et al. The Cancer genome atlas pan-cancer analysis project. *Nat Genet.* (2013) 45:1113–20. doi: 10.1038/ng.2764
65. Kim JY, Lim S-C, Kim G, Yun HJ, Ahn S-G, Choi HS. Interleukin-33/ST2 axis promotes epithelial cell transformation and breast tumorigenesis via upregulation of COT activity. *Oncogene* (2015) 34:4928–38. doi: 10.1038/onc.2014.418
66. Tong X, Barbour M, Hou K, Gao C, Cao S, Zheng J, et al. Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways. *Mol Oncol.* (2016) 10:113–25. doi: 10.1016/j.molonc.2015.06.004
67. Wang Z, Xu L, Chang Y, Zhou L, Fu H, Zhang W, et al. IL-33 is associated with unfavorable postoperative survival of patients with clear-cell renal cell carcinoma. *Tumour Biol.* (2016) 37:11127–34. doi: 10.1007/s13277-016-4879-3
68. Bergis D, Kassis V, Ranglack A, Koeberle V, Piiper A, Kronenberger B, et al. High serum levels of the interleukin-33 receptor soluble ST2 as a negative prognostic factor in hepatocellular carcinoma. *Transl Oncol.* (2013) 6:311–8. doi: 10.1593/tlo.12418
69. Akimoto M, Maruyama R, Takamaru H, Ochiya T, Takenaga K. Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by modifying the tumour microenvironment. *Nat Commun.* (2016) 7:13589. doi: 10.1038/ncomms13589
70. Yang M, Feng Y, Yue C, Xu B, Chen L, Jiang J, et al. Lower expression level of IL-33 is associated with poor prognosis of pulmonary adenocarcinoma. *PLoS ONE* (2018) 13:e0193428. doi: 10.1371/journal.pone.0193428
71. Kim MS, Kim E, Heo J-S, Bae D-J, Lee J-UW, Lee T-H, et al. Circulating IL-33 level is associated with the progression of lung cancer. *Lung Cancer* (2015) 90:346–51. doi: 10.1016/j.lungcan.2015.08.011
72. Hsu Y-L, Hung J-Y, Lee Y-L, Chen F-W, Chang K-F, Chang W-A, Tsai Y-M, Chong I-W, Kuo P-L. Identification of novel gene expression signature in lung adenocarcinoma by using next-generation sequencing data and bioinformatics analysis. *Oncotarget* (2017) 8:104831–54. doi: 10.18632/oncotarget.21022

73. Koster R, Panagiotou OA, Wheeler WA, Karlins E, Gastier-Foster JM, Caminada de Toledo SR, et al. Genome-wide association study identifies the GLDC/IL33 locus associated with survival of osteosarcoma patients. *Int J Cancer* (2018) 142:1594–601. doi: 10.1002/ijc.31195
74. Ding L, Ren J, Zhang D, Li Y, Huang X, Hu Q, et al. A novel stromal lncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33. *Carcinogenesis* (2018) 39:397–406. doi: 10.1093/carcin/bgy006
75. Yu X-X, Hu Z, Shen X, Dong L-Y, Zhou W-Z, Hu W-H. IL-33 promotes gastric cancer cell invasion and migration via ST2-ERK1/2 pathway. *Dig Dis Sci.* (2015) 60:1265–72. doi: 10.1007/s10620-014-3463-1
76. Fang M, Li Y, Huang K, Qi S, Zhang J, Zgodzinski W, et al. IL33 promotes colon cancer cell stemness via JNK activation and macrophage recruitment. *Cancer Res.* (2017) 77:2735–45. doi: 10.1158/0008-5472.CAN-16-1602
77. Milosavljevic MZ, Jovanovic IP, Pejnovic NN, Mitrovic SLJ, Arsenijevic NN, Simovic Markovic BJ, et al. Deletion of IL-33R attenuates VEGF expression and enhances necrosis in mammary carcinoma. *Oncotarget* (2016) 7:18106–15. doi: 10.18632/oncotarget.7635
78. Maywald RL, Doerner SK, Pastorelli L, De Salvo C, Benton SM, Dawson EP, et al. IL-33 activates tumor stroma to promote intestinal polyposis. *Proc Natl Acad Sci USA.* (2015) 112:E2487–96. doi: 10.1073/pnas.1422445112
79. Mertz KD, Mager LF, Wasmer M-H, Thiesler T, Koelzer VH, Ruzzante G, et al. The IL-33/ST2 pathway contributes to intestinal tumorigenesis in humans and mice. *Oncoimmunology* (2016) 5:e1062966. doi: 10.1080/2162402X.2015.1062966
80. Mager LF, Riether C, Schürch CM, Banz Y, Wasmer M-H, Stuber R, et al. IL-33 signaling contributes to the pathogenesis of myeloproliferative neoplasms. *J Clin Invest.* (2015) 125:2579–91. doi: 10.1172/JCI77347
81. O'Donnell C, Mahmoud A, Keane J, Murphy C, White D, Carey S, et al. An antitumorigenic role for the IL-33 receptor, ST2L, in colon cancer. *Br J Cancer* (2016) 114:37–43. doi: 10.1038/bjc.2015.433
82. Wang K, Shan S, Yang Z, Gu X, Wang Y, Wang C, et al. IL-33 blockade suppresses tumor growth of human lung cancer through direct and indirect pathways in a preclinical model. *Oncotarget* (2017) 8:68571–82. doi: 10.18632/oncotarget.19786
83. Wan S, Zhao E, Kryczek I, Vatan L, Sadovskaya A, Ludema G, et al. Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. *Gastroenterology* (2014) 147:1393–404. doi: 10.1053/j.gastro.2014.08.039
84. Ariyoshi W, Okinaga T, Chaweewannakorn W, Akifusa S, Nisihara T. Mechanisms involved in enhancement of matrix metalloproteinase-9 expression in macrophages by interleukin-33. *J Cell Physiol.* (2017) 232:3481–95. doi: 10.1002/jcp.25809
85. Yang Y, Andersson P, Hosaka K, Zhang Y, Cao R, Iwamoto H, et al. The PDGF-BB-SOX7 axis-modulated IL-33 in pericytes and stromal cells promotes metastasis through tumour-associated macrophages. *Nat Commun.* (2016) 7:11385. doi: 10.1038/ncomms11385
86. Fang K-M, Yang C-S, Lin TC, Chan TC, Tzeng S-F. Induced interleukin-33 expression enhances the tumorigenic activity of rat glioma cells. *Neuro-Oncol* (2014) 16:552–66. doi: 10.1093/neuonc/not234
87. Huang J-R, Tsai Y-C, Chang YJ, Wu JC, Hung JT, Lin K-H, et al. α-Galactosylceramide but not phenyl-glycolipids induced NKT cell anergy and IL-33-mediated myeloid-derived suppressor cell accumulation via upregulation of egr2/3. *J Immunol.* (2014) 192:1972–81. doi: 10.4049/jimmunol.1302623
88. Zhang Y, Davis C, Shah S, Hughes D, Ryan JC, Altomare D, et al. IL-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis. *Mol Carcinog.* (2017) 56:272–87. doi: 10.1002/mc.22491
89. Xiao P, Wan X, Cui B, Liu Y, Qiu C, Rong J, et al. Interleukin 33 in tumor microenvironment is crucial for the accumulation and function of myeloid-derived suppressor cells. *Oncoimmunology* (2016) 5:e1063772. doi: 10.1080/2162402X.2015.1063772
90. Jovanovic IP, Pejnovic NN, Radosavljevic GD, Pantic JM, Milovanovic MZ, Arsenijevic NN, et al. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. *Int J Cancer* (2014) 134:1669–82. doi: 10.1002/ijc.28481
91. Lim HX, Choi S, Cho D, Kim TS. IL-33 inhibits the differentiation and immunosuppressive activity of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *Immunol Cell Biol.* (2017) 95:99–107. doi: 10.1038/icb.2016.72
92. Beltrán CJ, Núñez LE, Díaz-Jiménez D, Farfan N, Candia E, Heine C, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1097–107. doi: 10.1002/ibd.21175
93. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, 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 USA.* (2010) 107:8017–22. doi: 10.1073/pnas.0912678107
94. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* (2014) 513:564–8. doi: 10.1038/nature13577
95. Meinicke H, Bremser A, Brack M, Akeus P, Pearson C, Bullers S, et al. Tumour-associated changes in intestinal epithelial cells cause local accumulation of KLRG1+ GATA3+ regulatory T cells in mice. *Immunology* (2017) 152:74–88. doi: 10.1111/imm.12750
96. Enoksson M, Lyberg K, Möller-Westerberg C, Fallon PG, Nilsson G, Lunderius-Andersson C. Mast cells as sensors of cell injury through IL-33 recognition. *J Immunol.* (2011) 186:2523–2528. doi: 10.4049/jimmunol.1003383
97. Rigoni A, Bongiovanni L, Burocchi A, Sangaletti S, Danelli L, Guarnotta C, et al. Mast cells infiltrating inflamed or transformed gut alternatively sustain mucosal healing or tumor growth. *Cancer Res.* (2015) 75:3760–70. doi: 10.1158/0008-5472.CAN-14-3767
98. Moulin D, Donzé O, Talabot-Ayer D, Mézin F, Palmer G, Gabay C. Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine* (2007) 40:216–25. doi: 10.1016/j.cyto.2007.09.013
99. Gulubova M, Vlaykova T. Prognostic significance of mast cell number and microvascular density for the survival of patients with primary colorectal cancer. *J Gastroenterol Hepatol.* (2009) 24:1265–75. doi: 10.1111/j.1440-1746.2007.05009.x
100. Malfettone A, Silvestris N, Saponaro C, Ranieri G, Russo A, Caruso S, et al. High density of tryptase-positive mast cells in human colorectal cancer: a poor prognostic factor related to protease-activated receptor 2 expression. *J Cell Mol Med.* (2013) 17:1025–37. doi: 10.1111/jcmm.12073
101. Byrne SN, Beaugie C, O'Sullivan C, Leighton S, Halliday GM. The immune-modulating cytokine and endogenous alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation. *Am J Pathol.* (2011) 179:211–22. doi: 10.1016/j.ajpath.2011.03.010
102. Loser K, Apelt J, Voskort M, Mohaupt M, Balkow S, Schwarz T, et al. IL-10 controls ultraviolet-induced carcinogenesis in mice. *J Immunol.* (2007) 179:365–71.
103. Fang Y, Zhao L, Xiao H, Cook KM, Bai Q, Herrick EJ, et al. IL-33 acts as a foe to MIA PaCa-2 pancreatic cancer. *Med Oncol.* (2017) 34:23. doi: 10.1007/s12032-016-0880-3
104. Saranchova I, Han J, Huang H, Fenninger F, Choi KB, Munro L, et al. Discovery of a metastatic immune escape mechanism initiated by the loss of expression of the tumour biomarker interleukin-33. *Sci Rep.* (2016) 6:30555. doi: 10.1038/srep30555
105. Brunner SM, Rubner C, Kesselring R, Martin M, Griesshammer E, Ruemmele P, et al. Tumor-infiltrating, interleukin-33-producing effector-memory CD8(+) T cells in resected hepatocellular carcinoma prolong patient survival. *Hepatology* (2015) 61:1957–67. doi: 10.1002/hep.27728
106. Lucarini V, Zicchedda G, Macchia I, La Sorsa V, Peschiaroli F, Buccione C, et al. IL-33 restricts tumor growth and inhibits pulmonary metastasis in melanoma-bearing mice through eosinophils. *Oncoimmunology* (2017) 6:e1317420. doi: 10.1080/2162402X.2017.1317420
107. Qin L, Dominguez D, Chen S, Fan J, Long A, Zhang M, et al. Exogenous IL-33 overcomes T cell tolerance in murine acute myeloid leukemia. *Oncotarget* (2016) 7:61069–80. doi: 10.18632/oncotarget.11179
108. Eissmann MF, Dijkstra C, Wouters MA, Baloyan D, Mouradov D, Nguyen PM, et al. Interleukin 33 signaling restrains sporadic colon cancer in an interferon-γ-dependent manner. *Cancer Immunol Res.* (2018) 6:409–21. doi: 10.1158/2326-6066.CIR-17-0218

109. Chen H, Chen Y, Liu H, Que Y, Zhang X, Zheng F. Integrated expression profiles analysis reveals correlations between the IL-33/ST2 Axis and CD8+ T cells, regulatory T cells, and myeloid-derived suppressor cells in soft tissue sarcoma. *Front Immunol.* (2018) 9:1179. doi: 10.3389/fimmu.2018.01179
110. Jovanovic I, Radosavljevic G, Mitrovic M, Juranic VL, McKenzie ANJ, Arsenijevic N, et al. ST2 deletion enhances innate and acquired immunity to murine mammary carcinoma. *Eur J Immunol.* (2011) 41:1902–12. doi: 10.1002/eji.201141417
111. Gao X, Wang X, Yang Q, Zhao X, Wen W, Li G, et al. Tumoral expression of IL-33 inhibits tumor growth and modifies the tumor microenvironment through CD8+ T and NK cells. *J Immunol.* (2015) 194:438–45. doi: 10.4049/jimmunol.1401344
112. Gao K, Li X, Zhang L, Bai L, Dong W, Gao K, et al. Transgenic expression of IL-33 activates CD8(+) T cells and NK cells and inhibits tumor growth and metastasis in mice. *Cancer Lett.* (2013) 335:463–71. doi: 10.1016/j.canlet.2013.03.002
113. Dominguez D, Ye C, Geng Z, Chen S, Fan J, Qin L, et al. Exogenous IL-33 restores dendritic cell activation and maturation in established cancer. *J Immunol.* (2017) 198:1365–75. doi: 10.4049/jimmunol.1501399
114. Asadzadeh Z, Mohammadi H, Safarzadeh E, Hemmatzadeh M, Mahdian-Shakib A, Jadidi-Niaragh F, et al. The paradox of Th17 cell functions in tumor immunity. *Cell Immunol.* (2017) 322:15–25. doi: 10.1016/j.cellimm.2017.10.015
115. Song Y, Yang JM. Role of interleukin (IL)-17 and T-helper (Th)17 cells in cancer. *Biochem Biophys Res Commun.* (2017) 493:1–8. doi: 10.1016/j.bbrc.2017.08.109
116. Kempski J, Brockmann L, Gagliani N, Huber S. TH17 cell and epithelial cell crosstalk during inflammatory bowel disease and carcinogenesis. *Front Immunol.* (2017) 8:1373. doi: 10.3389/fimmu.2017.01373
117. Pascual-Reguant A, Bayat Sarmadi J, Baumann C, Noster R, Cirera-Salinas D, Curato C, et al. TH17 cells express ST2 and are controlled by the alarmin IL-33 in the small intestine. *Mucosal Immunol.* (2017) 10:1431–42. doi: 10.1038/mi.2017.5
118. de Kleer IM, Kool M, de Bruijn MJW, Willart M, van Moorleghem J, Schuijs MJ, et al. Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung. *Immunity* (2016) 45:1285–98. doi: 10.1016/j.immuni.2016.10.031
119. Zhao Y, Chu X, Chen J, Wang Y, Gao S, Jiang Y, et al. Dectin-1-activated dendritic cells trigger potent antitumour immunity through the induction of Th9 cells. *Nat Commun.* (2016) 7:12368. doi: 10.1038/ncomms12368
120. Chen J, Zhao Y, Jiang Y, Gao S, Wang Y, Wang D, , et al. Interleukin-33 contributes to the induction of Th9 cells and antitumor efficacy by dectin-1-activated dendritic cells. *Front Immunol.* (2018) 9:1787. doi: 10.3389/fimmu.2018.01787
121. Li J, Razumilava N, Gores GJ, Walters S, Mizuochi T, Mourya R, et al. Biliary repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation. *J Clin Invest.* (2014) 124:3241–51. doi: 10.1172/JCI 73742
122. Saranchova I, Han J, Zaman R, Arora H, Huang H, Fenninger F, et al. Type 2 innate lymphocytes actuate immunity against tumours and limit cancer metastasis. *Sci Rep.* (2018) 8:2924. doi: 10.1038/s41598-018-20 608-6
123. Kim J, Kim W, Moon UJ, Kim HJ, Choi H-J, Sin J-I, et al. Intratumorally establishing type 2 innate lymphoid cells blocks tumor growth. *J Immunol.* (2016) 196:2410–23. doi: 10.4049/jimmunol.1501730
124. Wasmer M-H, Krebs P. The role of IL-33-dependent inflammation in the tumor microenvironment. *Front Immunol.* (2016) 7:682. doi: 10.3389/fimmu.2016.00682
125. Villarreal DO, Wise MC, Walters JN, Reuschel EL, Choi MJ, Obeng-Adjei N, Yet al. Alarmin IL-33 acts as an immunoadjuvant to enhance antigen-specific tumor immunity. *Cancer Res.* (2014) 74:1789–800. doi: 10.1158/0008-5472.CAN-13-2729
126. Villarreal DO, Weiner DB. IL-33 isoforms: their future as vaccine adjuvants? *Expert Rev Vaccines* (2015) 14:489–92. doi: 10.1586/14760584.2015.1011135
127. Duault C, Franchini DM, Familliades J, Cayrol C, Roga S, Girard J-P, et al. TCRVγ9 γδ T cell response to IL-33: A CD4 T cell-dependent mechanism. *J Immunol.* (2016) 196:493–502. doi: 10.4049/jimmunol.1500260
128. Duault C, Betous D, Bezombes C, Roga S, Cayrol C, Girard J-P, et al. IL-33-expanded human Vγ9Vδ2 T cells have anti-lymphoma effect in a mouse tumor model. *Eur J Immunol.* (2017) 47:2137–41. doi: 10.1002/eji.201747093
129. Kallert SM, Darbre S, Bonilla WV, Kreutzfeldt M, Page N, Müller P, et al. Replicating viral vector platform exploits alarmin signals for potent CD8+ T cell-mediated tumour immunotherapy. *Nat Commun.* (2017) 8: 15327. doi: 10.1038/ncomms15327
130. Ramadan A, Griesenauer B, Adom D, Kapur R, Hanenberg H, Liu C, et al. Specifically differentiated T cell subset promotes tumor immunity over fatal immunity. *J Exp Med.* (2017) 214:3577–96. doi: 10.1084/jem.20170041
131. Zhang J, Ramadan AM, Griesenauer B, Li W, Turner MJ, Liu C, et al. ST2 blockade reduces sST2-producing T cells while maintaining protective mST2-expressing T cells during graft-versus-host disease. *Sci Transl Med.* (2015) 7:308ra160. doi: 10.1126/scitranslmed.aab0166

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Fournié and Poupot. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 13 November 2018
doi: 10.3389/fimmu.2018.02601

The Pleiotropic Immunomodulatory Functions of IL-33 and Its Implications in Tumor Immunity

Claudia Afferni[1], Carla Buccione[2], Sara Andreone[2], Maria Rosaria Galdiero[3,4], Gilda Varricchi[3,4], Gianni Marone[3,4,5], Fabrizio Mattei[2] and Giovanna Schiavoni[2]**

[1] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy, [2] Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy, [3] Department of Translational Medical Sciences and Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, Naples, Italy, [4] WAO Center of Excellence, Naples, Italy, [5] Institute of Experimental Endocrinology and Oncology "Gaetano Salvatore", National Research Council, Naples, Italy

Interleukin-33 (IL-33) is a IL-1 family member of cytokines exerting pleiotropic activities. In the steady-state, IL-33 is expressed in the nucleus of epithelial, endothelial, and fibroblast-like cells acting as a nuclear protein. In response to tissue damage, infections or necrosis IL-33 is released in the extracellular space, where it functions as an alarmin for the immune system. Its specific receptor ST2 is expressed by a variety of immune cell types, resulting in the stimulation of a wide range of immune reactions. Recent evidences suggest that different IL-33 isoforms exist, in virtue of proteolytic cleavage or alternative mRNA splicing, with potentially different biological activity and functions. Although initially studied in the context of allergy, infection, and inflammation, over the past decade IL-33 has gained much attention in cancer immunology. Increasing evidences indicate that IL-33 may have opposing functions, promoting, or dampening tumor immunity, depending on the tumor type, site of expression, and local concentration. In this review we will cover the biological functions of IL-33 on various immune cell subsets (e.g., T cells, NK, Treg cells, ILC2, eosinophils, neutrophils, basophils, mast cells, DCs, and macrophages) that affect anti-tumor immune responses in experimental and clinical cancers. We will also discuss the possible implications of diverse IL-33 mutations and isoforms in the anti-tumor activity of the cytokine and as possible clinical biomarkers.

Keywords: IL-33, cancer, immune cell subsets, tumor immunology, IL-33 isoforms

OPEN ACCESS

Edited by:
Hui-Rong Jiang,
University of Strathclyde,
United Kingdom

Reviewed by:
Xiao-Qing Wei,
Cardiff University, United Kingdom
Padraic Fallon,
Trinity College, Dublin, Ireland

***Correspondence:**
Fabrizio Mattei
fabrizio.mattei@iss.it
Giovanna Schiavoni
giovanna.schiavoni@iss.it

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *24 July 2018*
Accepted: *22 October 2018*
Published: *13 November 2018*

Citation:
Afferni C, Buccione C, Andreone S, Galdiero MR, Varricchi G, Marone G, Mattei F and Schiavoni G (2018) The Pleiotropic Immunomodulatory Functions of IL-33 and Its Implications in Tumor Immunity. Front. Immunol. 9:2601. doi: 10.3389/fimmu.2018.02601

INTRODUCTION TO IL-33 BIOLOGY

Interleukin-33 (IL-33) is a cytokine member of IL-1 family, including IL-1α, IL-1β, IL-18, and IL-1Ra that are related to each other by receptor structure and signal transduction pathways. These cytokines share a conserved structure of β-trefoil fold comprised of 12 anti-parallel β-strands that are arranged in a three-fold symmetric pattern. The β-barrel core motif is packed by various amounts of helices in each cytokine structure (1). IL-33 was initially described in 2003 by Girard's group as a nuclear protein abundantly expressed in high endothelial venules (HEVs), specialized blood vessels that mediate the entry of lymphocytes into lymphoid organs and therefore named "nuclear factor from high endothelial venules" (NF-HEV) (2).

It is now known that IL-33 is a chromatin-associated nuclear cytokine *in vivo* through chromatin-binding motif within its N-terminal nuclear domain, suggesting that nuclear localization and binding to histones are important for IL-33 function and regulation (3). Nuclear IL-33 can function as a transcriptional repressor when overexpressed in transfected cells, although there is still no direct evidence that endogenous nuclear IL-33 regulates gene or protein expression (4). IL-33 is constitutively expressed in different human and mouse tissues in the steady-state, including epithelial, endothelial, fibroblast-like cells, and myofibroblasts and its expression can be increased during inflammation (2, 5). After cell stress or necrosis, IL-33 is released into the extracellular space and functions as an endogenous danger signal that alerts the immune system of tissue damage during trauma or infection. Indeed, IL-33 is considered an "alarmin" able to activate different actors of the innate immune system, mediating a variety of immune reactions including anti-cancer immune responses (6). Here, we will review the biological role of IL-33 affecting immune responses with particular emphasis on anti-tumor immunity.

IL-33 Isoforms

Similar to IL-1β and IL-18, IL-33 is synthesized in a full-length form (amino acids 1–270) that is found in the nucleus, in the cytosol and outside the cell. As IL-1β and IL-18, IL-33 is cleaved intracellularly by the enzyme caspase-1 before release outside the cell. This process requires the NLRP3 inflammasome, which can be activated in response to endogenous and exogenous danger signals. This NLRP3 inflammasome leads to Caspase-1 activation and, in turn, to IL-33 processing and release (7). When cells undergo necrosis or injury, full-length IL-33 is released in the extracellular space where it is cleaved by inflammatory proteases. During apoptosis, a process that does not trigger inflammation *in vivo*, IL-33 is cleaved and inactivated by endogenous caspases (8–10). Processing by apoptotic caspases is an important regulatory mechanism that limits or suppresses the pro-inflammatory properties of IL-33 during homeostatic cell turnover. Another regulatory mechanism limiting IL-33 activity is oxidation. Extracellular IL-33 is susceptible to cysteine oxidation that leads to the formation of disulphide bridges, resulting in conformational changes that inhibit the binding to ST2 receptor, thus rapidly inactivating IL-33 following allergen exposure (11).

Recent studies have demonstrated the existence of several human full-length active mRNA splice variants dependent on both the cell type expressing IL-33 and the pathological condition and triggered by diverse stimulations during immune responses (12–14). Of note, inflammatory proteases from neutrophils (proteinase 3, elastase, and cathepsin G) (15), mast cells (chymase, tryptase, and granzyme B) (16), and environmental allergens (17) can process full-length IL-33 into shorter mature forms (18–21 kDa) whose biological activity is 10- to 30-fold more potent than the full-length form (see **Figure 1**). The mature form does not translocate into the nucleus because it lacks the nuclear localization signal found in full-length IL-33 (18, 19). Proteolytic cleavage of IL-33 was shown to induce allergic inflammation *in vivo* (17) highlighting a novel mechanism by which inflammatory and environmental proteases can amplify allergic inflammation. Of interest, isoform variants as well as cleavage by endogenous and exogenous proteases has been described also for other epithelial-derived cytokines, such as thymic stromal lymphopoietin (TSLP), resulting in pleiotropic functions in health and disease (20). Although both isoforms are biologically active the relative importance of full length and mature IL-33 forms *in vivo* remains unclear (2, 21). In a mouse model of lung delivery of recombinant adenoviruses encoding IL-33 isoforms the full-length IL-33 induced inflammation in an ST2-independent fashion, but not pulmonary eosinophilia, goblet cell hyperplasia, or Th2 skewing, whereas mature IL-33 induced ST2-dependent Th2-associated effects. Both isoforms had similar effects on gene expression, suggesting that the different effects are due to differential utilization of the ST2 receptor (22). In addition, in a mouse model of DNA cancer vaccine, delivery of either full-length or mature IL-33 as an immunoadjuvant induced potent Th1 and cytotoxic T cell (CTL)-associated anti-tumor immunity and complete regression of established TC-1 tumor in mice. Interestingly, the full-length IL-33 was more potent than mature IL-33 in expanding the humoral immune response (23).

The IL-33/ST2 Axis

IL-33 exert its cytokine activity through binding to its primary specific receptor ST2, which is dependent on the co-receptor, IL-1 receptor accessory protein (IL-1RAcP), and the adaptor protein MyD88 for signaling (24). The crystal structure of IL-33 with ST2 has revealed that surface charge complementarity is crucial for specific binding (25). The gene that encodes for ST2 produces its transmembrane receptor but also produces a soluble form of ST2 (sST2), which acts as a binding decoy for IL-33 and thus down-modulates IL-33 activity during inflammatory responses, such as in experimental allergic asthma (26) and collagen-induced arthritis (27). Most hematopoietic cells express ST2. ILC2s, some Treg cells, and mast cells are the primary tissue-resident cells that constitutively express high levels of ST2, implying that these cells are initial targets of IL-33 (3). Non-hematopoietic cells, including endothelial cells, epithelial cells, and fibroblasts, are reported to express ST2 and respond to IL-33, although the *in vivo* consequences of signaling in these populations are less well-characterized.

In hematopoietic cells, IL-33 acts primarily on immune cells associated with type 2 and regulatory immune responses, including ILC2s, Th2 cells, eosinophils, mast cells, and basophils, as well as subsets of dendritic cells, myeloid-derived suppressor cells, and Tregs (28). However, it is now clear that the action of IL-33 is not limited to the activation of type-2 immune responses. Indeed, recent studies have revealed important roles of IL-33 in the activation of immune cells involved in type-1 immunity, such as Th1 cells, NK cells, $CD8^+$ T cells, neutrophils, macrophages, B cells, and NKT cells (19, 29, 30). This pleiotropic nature of IL-33 (**Figure 2**) is likely to explain why IL-33 has been implicated in a wide variety of non-allergic diseases, including infectious diseases (fungal, helminth, protozoa, bacterial, and viral infection), cardiovascular diseases, chronic obstructive pulmonary disease (COPD), fibrotic diseases, musculoskeletal diseases, inflammatory bowel diseases, diseases of the central

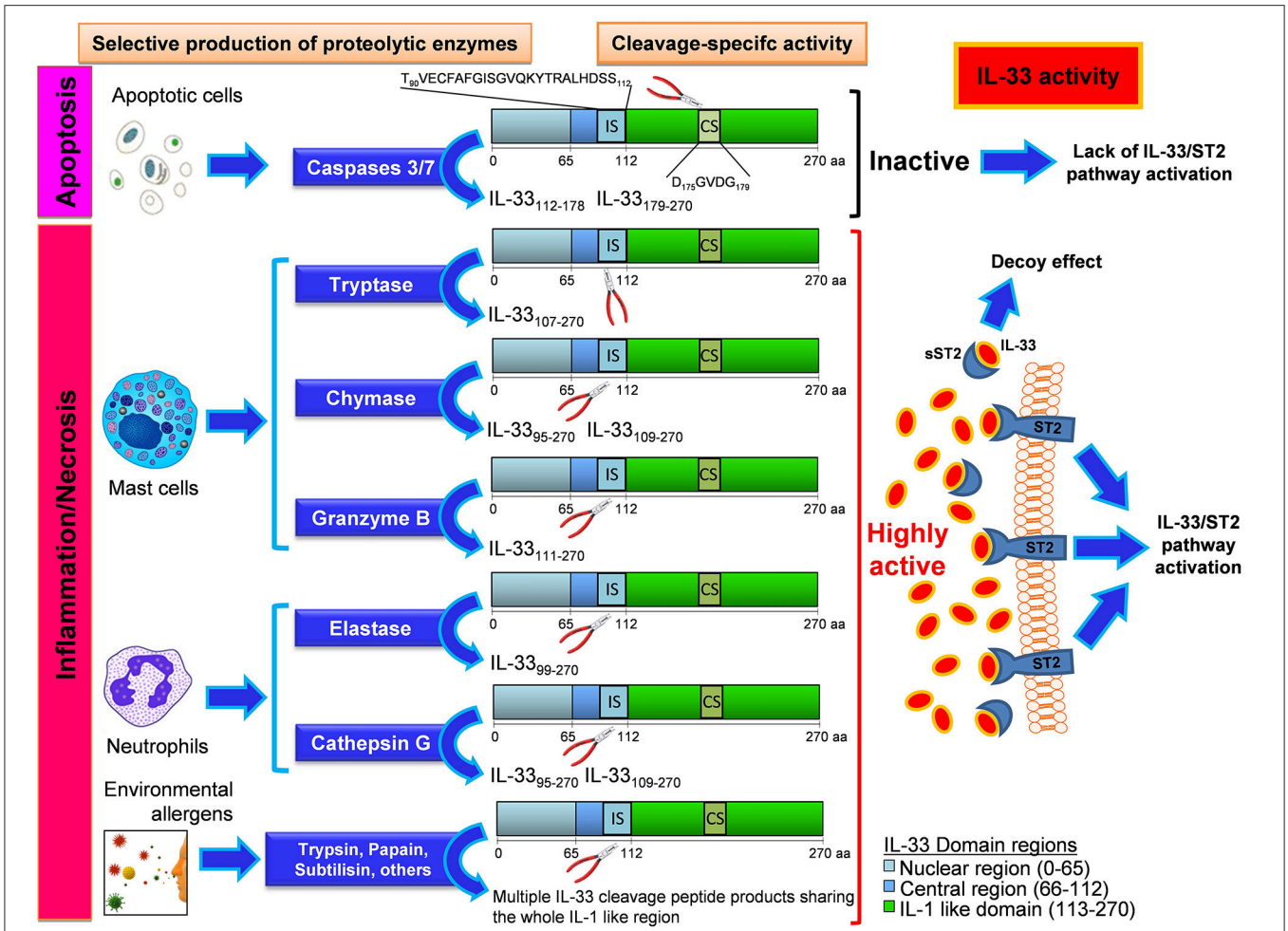


FIGURE 1 | Mechanisms and effects of the enzyme-specific IL-33 cleavage. Biological events such as apoptotic stress, Inflammation, and necrosis can differentially generate various IL-33 protein variants with high biological activity or no activity depending on the enzyme produced by the cells. Apoptotic cells enable the production and release of caspases 3 and 7, that cleaves IL-33 in the caspase site (CS) generating inactive fragments of IL-33 by disruption of some IL-1 like functional domains close to CS. The insurgence of inflammation or necrosis process leads to the local recruitment of mast cells and neutrophils, the main effectors of inflammatory processes. When these cells reach the inflammed site, they produce, and release enzymes that cleave the IL-33 protein at the Inflammatory site (IS) inside the central region. These cleavage-specific enzymes allow the production and release of highly active forms of IL-33, capable to stably bind the ST2 receptor (ST2) on a plethora of ST2-expressing cells. In other cases this active IL-33 does bind to the soluble form of its receptor (sST2), which acts as a decoy receptor. In this latter event the effect of IL-33 will be suppressed by the formation of an sST2/IL-33 decoy complex. When no apoptotic nor inflammatory enzymes are produced, an uncleaved form of IL-33 is released, with a very low biological activity compared to that showed by IL-33 cleavage products originated by neutrophil and mast cell-derived enzymatic cutting. When the IL-33 is cleaved by certain environmental allergens, their enzymatic activity at the IS site gives rise to multiple peptide products sharing the whole IL-1 like region of IL-33, that does retain the ability to bind ST2. The IL-33 cleavage products herein shown are all equipped with the ST2 binding sequence (inside the IL-1 like region). The secondary fragments lacking the ST2 binding sequence and generated during the cleavage reaction are not depicted and have no effect on IL-33/ST2 binding.

nervous system (Alzheimer), graft vs. host disease (GVHD), obesity, diabetes, and cancer (3).

IMMUNE CELL TARGETS OF IL-33 AFFECTING TUMOR-IMMUNE RESPONSES

CD4$^+$ Th Cells

Naïve CD4$^+$ T helper cells constitutively express ST2 and stimulation with IL-33 skews their differentiation toward a Th2 phenotype. CD4$^+$ T cells are needed in the effector phase of a protective antitumor immune response against tumors lacking MHC class II (31). However, human CD4$^+$ T cells can suppress tumors expressing adequate levels of MHC class II and self-antigens on their surface, through secretion of IFN-γ or direct tumor killing (32). Interestingly, Villareal et al. demonstrated that IL-33 can be an effective adjuvant when combined with an HPV16 E6/E7-encoded DNA vaccine, enhancing both antigen specific CD4$^+$ and CD8$^+$ IFN-γ$^+$ T cells, and antigen specific IgG concentration in the serum, leading to regression of

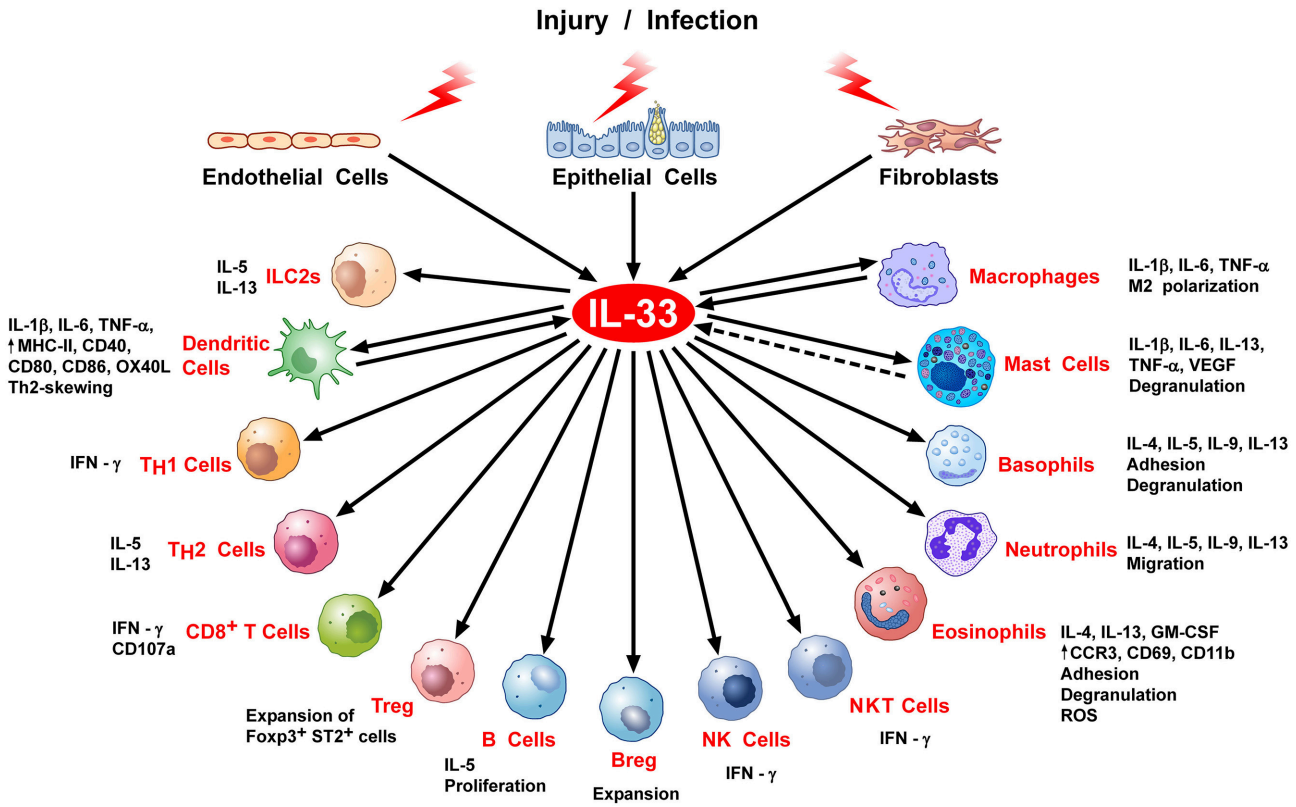


FIGURE 2 | Stimulation of immune cells by IL-33. IL-33 is secreted by epithelial, endothelial, or fibroblasts in response to various stimuli, including infection or cell stress causing injury. IL-33 exerts its biological activities through binding to its specific receptor ST2 expressed by most hematopoietic cells. IL-33/ST2 can stimulate a variety of immune cell reactions, including: an atypical Th2-type of immune response through production of IL-5 and IL-13 by ILC2 and Th2 cells; IFNγ production by NK, NKT, and Th1 cells; CD107a exposure and IFNγ production by activated CD8 T cells; *in vivo* expansion of ST2$^+$ Treg cells B and Breg cells; release of inflammatory cytokines (e.g., IL-1β, IL-6, TNFα) by DC, macrophages and mast cells; M2 macrophage polarization; degranulation of mast cells, basophils, and eosinophils; neutrophil migration; phenotypic, and functional activation of DC and eosinophils. There is some evidence that activated DCs, macrophages, and perhaps mast cells can produce IL-33.

established TC-1 tumor in mice (23). In accordance with this study, Mousa Komai-Koma et al. showed that IL-33 may promote CD4$^+$ T helper 1 (Th1) differentiation by a mechanism depending on IL-12 and ST2. IL-33 and IL-12 synergistically increase both ST2 and IL-12R expression in early activated CD4$^+$ T cells. These data indicate that IL-33 promotes Th1 cell development, while it is ineffective on mature Th1 cells (33). A possible explanation for such differences is that ST2 expression is induced only in early-TCR activated naïve CD4$^+$ T cells and is then gradually inhibited when Th1 cells fully mature. Although the signaling pathway by which IL-33 enhances Th1 polarization is still unknown, it is likely that IL-33 inducing Th1 or Th2response depends on the cytokine milieu, in particular the balance of IL-12 and IL-4 levels *in vivo* (33). Of note, IL-33 also promotes the differentiation of IL-9-producing Th cells (34) which exert potent antitumor immune responses *in vivo* (35, 36).

CD4$^+$ Treg Cells

ST2/IL-33 signaling is known to expand suppressive CD4$^+$ Foxp3$^+$ GATA3$^+$ Treg cells *in vivo* and *in vitro* (37). IL-33-expanded Tregs express ST2 and can be found in several immune and non-immune tissues exerting potent suppressor function in a variety of pathological conditions, such as autoimmunity, inflammation, transplantation, and allergy (38). ST2$^+$ Treg expansion can be mediated by IL-33 signaling in DCs, through production of IL-2, which selectively expands ST2$^+$ Tregs (39). In the intestine, particularly rich in ST2$^+$ Treg cells, IL-33 signaling stimulates transforming growth factor (TGF)-β1-mediated differentiation of Treg cells and provides a signal for Treg-cell accumulation and maintenance in inflamed tissues (40). In Apc$^{Min/+}$ mice, epithelial-derived IL-33 promoted the expansion of ST2$^+$ Treg cells in the colon correlating with increased tumor burden (41, 42). A similar observation was recently reported in the CT26 adenocarcinoma model, where rIL-33 administration to tumor-bearing mice promoted, while IL-33 blockade reduced, the expansion of ST2$^+$ Treg cells in tumor tissue and spleen (43). Moreover, IL-33 blockade reduced accumulation of Treg cells in tumor microenvironment and inhibited tumor growth in a preclinical model of human non-small-cell lung cancer (NSCLC) xenografts (44). In contrast, some studies have reported inhibitory effects of IL-33/ST2 on Treg cells expansion. In a melanoma mouse

model, IL-33 was shown to inhibit Treg infiltration in the tumor microenvironment indirectly, through stimulation of MDSCs, which had reduced capacity to induce the differentiation or expansion of Treg cells *in vitro* (45). A recent study using reciprocal bone marrow chimeras in a mouse model of sporadic colon cancer, genetic ablation of ST2 in both hematopoietic and non-hematopoietic compartments leads to increased tumor-infiltrating ST2$^+$ Foxp3$^+$ Tregs and enhanced colon tumor development (46).

CD8$^+$ T Cells

Unlike CD4$^+$ T cells, only effector CD8$^+$ T cells or polarized Tc1 cells, but not naïve and early activated CD8$^+$ T cells, express ST2 (47). High expression of ST2 in CD8$^+$ T cells cultured in Tc1 polarizing conditions is regulated by T-bet, a master transcription regulator of Th1 effector functions. Moreover, it was shown that IL-12 and IL-33 synergistically increased T-bet and Blimp1, transcription factors critical for effector fate of CD8$^+$ T cell (47). Recent studies from transplantable solid tumor models have indicated a direct role of exogenous IL-33 in promoting antitumor CD8$^+$ T cell immunity using either IL-33 transgenic mice (48), IL-33 DNA as vaccine adjuvant (23), or IL-33 expressing tumor cells (49). Systemic administration of rIL-33 in melanoma tumor bearing mice, promoted expansion, increased tumor infiltration and effector function of antigen-specific CD8$^+$ IFN-γ^+ T cells by both a direct or DCs-mediated effect (50). In the aggressive C1498 acute myeloid leukemia (AML) model, IL-33 treatment significantly increased the percentage of effector memory liver CD8$^+$ T cells leading to delayed leukemia development and improved overall survival (51). This finding suggest a role of exogenous IL-33 in promoting rapid expansion of the effector memory CD8$^+$ T cell pool, consistent with the results from solid tumor models (23, 48). In this study the increased CD8$^+$ T cells activation level up-regulates PD-1/PD-L1 expression *in vivo*, therefore combination of PD-1 blockade and IL-33 treatment further improves survival of leukemia-bearing mice (51).

Type-2 Innate Lymphoid Cells

Innate lymphoid cells (ILCs), belonging to the family of innate cells, are characterized by classic lymphoid cell morphology, but lack lineage-specific markers and somatically rearranged antigen receptors. Based on the expression of transcription factors, phenotypic markers, and effector cytokine production profiles, ILCs have been divided into three distinct subclasses: group 1 ILCs, group 2 ILCs, and group 3 ILCs (52). ILC are derived from a common lymphoid progenitor and possess a wide range of cell surface markers, many of which have only recently been elucidated (53). ILC2, originally identified in the mouse and human mesenteric lymph nodes as lineage marker negative, c-kit$^+$, Sca-1$^+$, IL-7Ra$^+$, and ST2$^+$ cells (54), were also found in lung, skin, and gut, while only a small number of circulating ILC2s can be detected in blood (55). They are involved in tissue repair (56), anti-helminth immunity (57), and allergic inflammation (58).These cells are dependent on transcription factor GATA-binding protein 3 for their development and maintenance (59). Activation of ILC2s by alarmins (IL-25, IL-33, and TSLP) secreted by epithelial cells upon cellular stress and tissue damage (55, 60), produce IL-5, IL-13 (54), IL-4, IL-6, IL-9, and amphiregulin which induce Th2 differentiation (61). This group of innate cells was often observed to infiltrate tumors in humans, but their role seems more frequently associated with cancer progression than restriction. Clinical studies suggested that increased numbers of ILC2s in peripheral blood of patients with gastric cancer, could contribute by cytokines they secrete to the immunosuppressive environment maintained by CD4$^+$ T helper 2 (Th2), myeloid-derived suppressor cells (MDSC), and macrophages (62). ILC2s might also induce immune suppression *via* secretion of amphiregulin (63), which enhances Treg activity *in vivo* and can thereby inhibit antitumor immune responses induced by DC vaccination (64). The anti-tumoral activity of ILC2 was described for the first time by Ikutani et al. in a mouse model of lung metastatic melanoma. Following tumor induction, administration of rIL-33 induced the development of IL-5-producing ILC2, which recruited and maintained eosinophils responsible for tumor cell death and tumor metastasis prevention (65). Overexpression of IL-33 in several tumor cell lines induced high numbers of ILC2s, when transplanted in mice, with potent anti-tumoral activity. The latter study suggests that local production of IL-33 induces ILC2 to release CXCR2 ligands able to sustain the expression of CXCR2 on tumor cells and induce their apoptosis (66).

NK and NKT Cells

IL-33 directly activates both human (19) and mouse (30) NKT and NK cells inducing IFN-γ production via cooperation with IL-12, thus contributing to establish Th-1 immunity. During viral infection, IL-33/ST2 axis amplifies the expansion of NK cells and enhances host defense (67, 68). The role of IL-33 on NKT in cancer immunity is unknown. In contrast, a number of reports have analyzed the effects of IL-33 on NK cell expansion and/or activation in tumor-bearing mice. In mouse experimental metastasis models of B16 melanoma and Lewis lung carcinoma, transgenic expression of IL-33 in the host promoted the recruitment of cytotoxic NK cells to the pulmonary site that inhibited metastasis formation (48). *In vitro*, IL-33 directly activated NK cell cytotoxicity, stimulated NF-κB and up-regulated CD69 expression (48). Furthermore, B16 and 4T1 tumor cells overexpressing IL-33 implanted into syngeneic mice induced IFN-γ^+ NK cells in tumor tissue that mediated IL-33 anti-tumoral effect (49). Similarly, increased frequencies of CD107a$^+$IFN-γ^+ NK cells were observed following exogenous administration of IL-33 in spleens and tumors of B16 melanoma-bearing mice (69). In contrast with these reports, previous studies in 4T1 breast cancer model showed that IL-33/ST2 signaling impairs NK cell activation. ST2-deficient mice bearing 4T1 tumors exhibited increased numbers of activated NK cells (IFN-γ^+ CD27^{high} CD11b^{high}, CD69$^+$ KLRG$^-$) and NK cytotoxic activity, with respect to wild-type (WT) counterparts. *In vivo* depletion of NK cells accelerated 4T1 tumor growth in ST2$^{-/-}$ mice (70). Moreover, exogenous administration of IL-33 to WT 4T1 tumor-bearing mice decreased NK cell activation and cytotoxicity and promoted tumor progression (71), thus suggesting a detrimental role for IL-33 in NK cell-dependent

anti-tumor responses. These contrasting results suggest that IL-33 may exert opposing effects on NK cells within the tumor microenvironment depending on the levels of IL-33 expressed and on the primary target cells.

Macrophages

Several lines of evidence indicate that IL-33 amplifies the expression of M2 markers on macrophages *in vitro* and *in vivo*, thus promoting the suppressor function of tumor-associated macrophages (TAMs). Blockade of IL-33 abrogates the polarization of TAMs into (alternatively activated) M2 macrophages in a model of human non-small-cell lung cancer (44). Expression of IL-33 was found to stimulate the recruitment of M2-like macrophages into the cancer microenvironment in mouse models of colon (41, 72–74) and breast cancer (71) correlating with tumor progression. Of note, IL-33 stimulated macrophages to produce prostaglandin E_2, which supported colon cancer stemness (73). Furthermore, in mouse tumor xenografts IL-33 was shown to promote metastasis through recruitment of M2-like TAMs (75). Recently, it was shown in the mouse monocyte/macrophage line RAW264.7 that IL-33 directly induces MMP-9 expression, which facilitates tumor progression, invasion, and angiogenesis (76). These observations indicate that IL-33/ST2 signaling on macrophages promotes M2 polarization, immunosuppression, and tumor progression.

Dendritic Cells

Although dendritic cells (DCs) express low levels of ST2 on their cell surfaces, they respond to IL-33 by up-regulating MHC-II, CD40, CD80, CD86, OX40L, CCR7, and by increasing production of several cytokines (IL-4, IL-5, IL-13, TNF-α, and IL-1β) and chemokines (CCL17 and CCL22) (77–80). In addition, IL-33-activated DCs promote an atypical Th2-type of immune response inducing IL-5- and IL-13-producing $CD4^+$ T cells *in vitro* and *in vivo* (77, 79), which can be further amplified during allergic inflammatory response *via* ST2 (78, 81). *In vivo*, IL-33 exposure induces DC recruitment and activation in the lung (78, 79). IL-33 promotes the expansion of DCs from bone marrow (BM), by stimulating the secretion of basophils-derived GM-CSF. However, such IL-33 differentiated BM-DCs expressed low levels of MHC-II but high PD-L1 and PD-L2 immune checkpoints on the surface, and displayed reduced capacity to prime naïve T cells (82). In support of this potential tolerogenic effect, IL-33 has been shown to promote IL-2 secretion by murine DCs, thus supporting the *in vitro* and *in vivo* expansion of ST2-expressing Treg cells (39). In mice bearing 4T1 breast cancer IL-33 administration increased the percentage of splenic $CD11c^+$ DCs expressing IL-10 (71). In contrast, in a murine AML model, systemic IL-33 administration promoted DC activation and "licensing" for cross-priming of tumor-reactive $CD8^+$ T cells (51). Likewise, in EG7 lymphoma, B16, and inducible $Braf^{V600E}$PTEN melanoma models exogenous IL-33 activated myeloid DCs within the tumor microenvironment increasing antigen cross-presentation and restoring anti-tumor T cell activity in a ST2, MyD88, and STAT1-dependent manner (50). On the whole, these results suggest that IL-33 depending on the context can stimulate DC antigen presentation and, thus, anti-tumor immune responses, or induce tolerogenic features, thus supporting tumor growth.

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are closely related to granulocytes and monocytes but differ from them in that they are absent in healthy individuals but expand under pathological conditions, such as cancer, exerting potent immune suppressive role (83). Several reports described the ability of IL-33 to expand MDSCs *in vivo* during tumorigenesis. In 4T1 breast cancer model, IL-33 has been reported to promote MDSC expansion (71). Exogenous administration of IL-33 increased intratumoral and systemic accumulation of $CD11b^+$ $Gr\text{-}1^+$ MDSCs that expressed TGF-β1 and IL-13α1R with higher incidence of monocytic vs. granulocytic MDSCs. Fittingly, absence of IL-33/ST2 signaling reduced the accumulation, proliferation, and immunosuppressive ability of MDSCs in tumor-bearing mice (71, 84). Moreover, IL-33 upregulated in MDSCs the expression and activity of arginase-1 *in vitro* and activated NF-κB and MAPK signaling *in vivo*, which augment their immunosuppressive ability (84). Conversely, *in vitro* IL-33 was shown to negatively regulate MDSC development from BM progenitor cells inhibiting the differentiation of granulocytic MDSCs (G-MDSCs), but not of monocytic MDSCs (M-MDSCs). In addition, IL-33-treated BM-derived MDSCs exhibited diminished immunosuppressive capacity, reduced inhibition on T-cell proliferation and IFN-γ production, and diminished production of ROS (45). In B16 melanoma mouse models, IL-33 administration was shown to decrease MDSCs accumulation in the spleen and tumor microenvironment (45, 69). These evidences indicate that IL-33 may promote or halt MDSCs expansion depending on the tumor type.

Neutrophils

IL-33 directly acts on murine neutrophils in the lungs (85). Indeed, IL-33-treated neutrophils produced IL-4, IL-5, IL-9, and IL-13 and displayed a distinct gene expression profile in contrast to resting and lipopolysaccharide (LPS)-treated neutrophils. These neutrophils were found in the lungs of ovalbumin (OVA)-induced mouse model of asthma. Adoptive transfer of IL-33-driven neutrophils significantly worsened the severity of the disease in this model (86). *In vitro*, IL-33 promoted IL-4, IL-5, IL-9, and IL-13 expression in murine neutrophils in time- and dose- dependent manner. IL-33-induced neutrophils expressed high levels of CXCR1, CCR1, IL-1R2, and CXCR2 mRNAs compared with LPS-induced neutrophils. In a mouse model of choriomeningitis virus-induced hepatitis, IL-33 promoted neutrophil recruitment in the liver and dampened liver injury by limiting T-cell activation. Liver neutrophils displayed an immunosuppressive phenotype, characterized by high levels of arginase-1, iNOS and IL-10 (87). Thus, IL-33 may promote inflammatory or regulatory neutrophils, depending on the pathological condition. Little is known about the effects of IL-33 on neutrophils in tumor immunity. In a mouse model of ectopic CT26 colon carcinoma, systemic chemotherapy with irinotecan induced intestinal mucositis, associated with the induction of IL-33, and increased neutrophil accumulation

in the intestine. Supernatants from intestine explants treated with irinotecan enhanced migration of neutrophils *in vitro* in an IL-33/CXCL1/2/CXCR2-dependent manner. Importantly, IL-33 blockade reduced mucositis and enabled prolonged irinotecan treatment of ectopic colon carcinoma leading to a beneficial outcome of the chemotherapy. These results suggest that inhibition of the IL-33/ST2 pathway may represent a novel approach to limit mucositis and improve the effectiveness of chemotherapy (88).

Eosinophils

Administration of IL-33 in mice causes massive tissue infiltration of eosinophils and elevations of typical type 2 cytokines such as IL-5, IL-9, and IL-13, contributing to allergy and fibrosis (89). These responses also occurred in *RAG* knockout mice, suggesting that innate cells, particularly IL-5 producing ILC2s, were the direct target of IL-33 (54). However, IL-33 is now known to act directly on eosinophils leading to upregulation of CCR3, CD69, and CD11b, production of chemokines (CCL17, CXCL2, CXCL3, and CXCL10), and cytokines (IL-6, IL-13, GM-CSF) (90). IL-33 sustained eosinophil survival *in vivo* in a ST2-dependent manner and *via* autocrinous production of GM-CSF. Moreover, IL-33 and GM-CSF promoted the production of IL-4 and IL-13 by eosinophils, which in turn favored macrophage polarization toward M2 phenotype (91). In a murine model of melanoma, *in vivo* expansion of innate IL-5–producing ILC2 cells following systemic IL-33 injection played an important role for eosinophil recruitment and metastasis control. Innate IL-5-producing cells were increased in response to tumor invasion, and their regulation of eosinophils is critical to halt tumor metastasis (65). We reported that in transplantable B16 melanoma models, IL-33 restricted tumor growth and inhibited lung metastasis through recruitment and activation of eosinophils. Indeed, ST2-deficient mice presented an increased metastatic load and reduced lung eosinophilia compared to wild type mice. Depletion of eosinophils completely abolished the anti-tumor effects of IL-33 administration (69). Kim et al. also reported substantial expansion of intratumoral eosinophils in mice transplanted with IL-33-expressing tumor cells (EL4, CT26, and B16); however, their role in IL-33-induced anti-tumor effects was not addressed (66). In our study, IL-33 expanded eosinophils expressed T cell-attracting chemokines and induced NK and $CD8^+$ T cell-recruitment at the subcutaneous tumor site, but not at the lung metastatic site. In addition, IL-33 activated eosinophils *in vitro* enhancing CD11b, CD69, and granzyme B expression and activating cytotoxic functions against melanoma cells (69). These findings suggest that depending on the tumor site, eosinophils may play an accessory role, supporting the recruitment of tumor-reactive $CD8^+$ T cells (92), or a direct cytotoxic effect against tumor cells. Indeed, IL-33 can potently activate human eosinophils, enhancing adhesion, promoting survival, and inducing ROS production and degranulation (93, 94). Of note, degranulation of eosinophils has been shown *in vivo* in proximity of tumors (95, 96) and after *in vitro* stimulation, resulting in efficient killing of target mouse (97) and human (98) tumor cells, highlighting the tumoricidal properties of eosinophils (99).

Mast Cells

IL-33 activates its receptor complex (ST2: IL-1RAcP) on human mast cells (100) and basophils (19). IL-33 synergizes with IgE- and non-IgE-dependent stimuli to release cytokines from human mast cells (101). Similarly, IL-33 augments substance P-induced vascular endothelial growth factor (VEGF) production from human mast cell lines (102). The latter findings are clinically relevant because VEGFs produced by human mast cells (103) and basophils (104) play an important role in chronic inflammation and in tumor growth (105). We have demonstrated that IL-33 up-regulated the Fcγ receptor type IIa and synergistically enhanced immune complex-triggered activation of human mast cells (106). Collectively, these findings demonstrate that IL-33 can synergistically potentiate the immunologic and non-immunologic release of mediators from human and rodent mast cells. Single cell analysis demonstrated that IL-33 increased both the number of degranulating and chemokine-producing mast cells and the magnitude of individual mast cell response (107). The relevance of IL-33-mediated mast cell response has been found also *in vivo* in several pathological conditions, including cancer. Mast cells and basophils are known to infiltrate several types of tumors but, due to the wide range of mediators they release, it is difficult to define their specific pro- or anti-tumoral activity (103). Mast cell activation by IL-33 may occur in a number of tumor types. In skin cancers, mast cells accumulated with IL-33 expressing fibroblasts in UV-exposed murine skin samples (108). In the $Apc^{Min/+}$ mouse model, IL-33 deficiency reduced tumor burden (109, 110) and decreased mast cell density in polyps as well as suppressed the gene expression of mast cell-derived proteases and cytokines that promote angiogenesis, Treg function, and MDSC recruitment within the tumor microenvironment (111–113).

Basophils

Human basophils constitutively express ST2 which is induced by IL-3 (114, 115). Although IL-33 alone failed to directly induce degranulation of human basophils, it exerted priming effects. It enhanced degranulation and IL-4 production in response to IgE cross-linking (114). By contrast, IL-33 alone activated unprimed murine basophils *in vitro* (116). Recently, Rivellese and collaborators, using highly purified human basophils, elegantly demonstrated that IL-33 alone induces the release of low but detectable amounts of IL-4 (117). IL-33 synergistically potentiated IL-4 production induced by IL-3 or anti-IgE. Interestingly, IL-33 did not induce basophil degranulation, as evaluated by the membrane expression of CD63, but significantly enhanced IgE-mediated histamine release. Collectively, these findings indicate that IL-33 can activate human basophils presumably through the engagement of ST2. Furthermore, IL-33 can enhance IL-3- and anti-IgE-mediated basophil degranulation, histamine secretion, and cytokine production. The role of basophils in anti-cancer immunity is poorly characterized and the effects of IL-33 in stimulating or regulating basophils responses in cancer are unknown. In mouse models of melanoma, Sektioglu et al. reported that intratumoral basophils enhanced $CD8^+$ T cell infiltration *via* production of the chemokines CCL3 and CCL4, contributing to tumor rejection

following Treg cell depletion (118). Recently, low circulating eosinophils and basophils were associated with poor prognosis in CRC patients (119). Moreover, basophils in tumor-draining lymph nodes of both mice and pancreatic ductal adenocarcinoma patients correlated with Th2 responses in tumors and poor prognosis (120).

ROLE OF IL-33 IN CANCER: EVIDENCES FROM EXPERIMENTAL TUMOR MODELS

Accumulating evidences indicate that the axis IL-33/ST2 plays a role in tumor immunity. However, both pro-tumoral and anti-tumoral functions have been reported and the current literature suggest that IL-33 may differently affect tumor immunity depending on the tumor type, immune cell targets and on cooperating microenvironmental factors (121).

Breast Cancer

The majority of reports point to a pro-tumoral role of IL-33 in breast cancer models. In mice, the IL-33/ST2 axis was shown to promote the growth and lung and liver metastases of 4T1 mammary tumors facilitating the intratumoral accumulation of immunosuppressive myeloid (Gr-1$^+$ TGF-β1$^+$ MDSC, IL-10-expressing CD11c$^+$ DCs, and alternatively activated M2 macrophages), ILC2, and Treg cells, while dampening the expansion of activated NK cells (70, 71, 84). Lukic group demonstrated that IL-33/ST2 axis promotes the expression of pro-angiogenic VEGF in tumor cells and attenuates tumor necrosis, thus facilitating mammary tumor growth (122). In contrast, another study reported that transgenic overexpression of IL-33 in 4T1 breast cancer cells reduces tumor growth and metastasis *in vivo* (49). *In vitro*, IL-33 acts as a critical tumor promoter during epithelial cell transformation and sustains breast cancer tumorigenesis (123). Recently, it has been reported that IL-33 overexpression in human ER-positive breast cancer cells results in resistance to tamoxifen-induced tumor growth inhibition, by promoting cancer stem cell properties (124). These findings indicate that in breast cancer IL-33/ST2 exert both intrinsic and extrinsic pro-tumoral function favoring tumorigenesis and stemness and reducing anti-tumor immunity.

Colorectal Cancer

A number of evidences from mouse models point to an important role of the IL-33/ST2 axis in promoting colorectal cancer (CRC) tumorigenesis, progression and malignancy (125). In the Apc$^{Min/+}$ mouse model for human familial adenomatous polyposis, abrogation of the IL-33/ST2 axis by knockout of IL-33 (109) or ST2 (41, 110) inhibits proliferation, induces apoptosis, and suppresses angiogenesis, thus decreasing tumor number and size. Accordingly, overexpression of IL-33 in MC-38 mouse CRC cells results in increased *in vitro* proliferation and enhanced tumor growth and liver metastasis after orthotopic transplant in syngeneic mice through tumor-derived IL-33-induced recruitment of CD11b$^+$ GR1$^+$ and CD11b$^+$ F4/80$^+$ myeloid cells and angiogenesis (126). In addition, expression of IL-33 in human CRC cells promoted their growth and metastasis *in vivo* and reduced the survival of recipient nude mice (127). Likewise, IL-33/ST2 signaling in CRC tissues promoted the malignant growth and metastatic spread of CRC through modification of the tumor microenvironment (72). In a recent study, it was shown that IL-33 overexpression or exogenous administration of IL-33 to human or murine colon cancer cells enhanced cell growth *in vivo* and promoted colon cancer cell stemness through an immune-associated mechanism (73). In contrast, some studies have reported an anti-tumoral function of IL-33 in CRC models. Abrogation of ST2 signaling in CT26 mouse adenocarcinoma cells enhanced tumor development after subcutaneous transplant into syngeneic BALB/c mice (74). In the azoxymethane (AOM)/dextran sodium sulfate (DSS) model, IL-33-deficient mice were shown to be highly susceptible to cancer-associated colitis showing increased tumor number, size, and grade, due to a protective function of IL-33 in regulating an IgA-microbiota axis in the intestine (128). Fittingly, in a mouse model of sporadic colon cancer tumorigenesis in the absence of preexisting inflammation lack of IL-33 signaling enhanced colon tumorigenesis, while IL-33 treatment reduced tumor growth in the transplantable MC38 model, via IFN-γ-mediated antitumor immune response (46). These evidences suggest that in the absence of preexisting chronic or acute inflammation the homeostatic release of IL-33 by dying colon epithelial cells protects against the initiation and development of sporadic colon cancer.

Lung Cancer

The IL-33/ST2 axis plays a critical role in various inflammatory lung diseases, including asthma and fibrosis (129). However, few studies have investigated the contribution of this pathway to lung cancer. Akimoto et al. reported that ST2 expression in human and murine lung cancer is inversely correlated with metastatic potential (130). Exposure to IL-33 enhanced oncotic cell death of ST2$^+$ low-metastatic Lewis lung carcinoma cells, but not of ST2$^-$ high-metastatic cells, thus suggesting that IL-33 enhances lung cancer progression by selecting for malignant cells. Moreover, *in vitro* stimulation with IL-33 promoted the migration and invasiveness of human lung A549 cells (131) and enhanced the growth and metastasis of primary non-small-cell lung cancer (NSCLC) cells after xenotransplant into immunodeficient mice (132). *Vice versa*, IL-33 blockade efficiently inhibited tumor growth of patient-derived NSCLC xenografts, abrogating M2 polarization of macrophages, and reducing the accumulation of Treg cells within the tumor microenvironment (44). In mouse lung tumor models stable transfection of *IL-33* gene into tumor cells inhibited tumor growth and metastatic spread *in vivo* (49, 133). Of note, IL-33 expressing metastatic A9 lung cancer cells, a TC1-derived cell line with spontaneous down-regulation of MHC-I, restored MHC-I expression and immune recognition in mice (133). Although these evidences indicate that IL-33/ST2 expression in lung tissue or cancer cells fuels tumor growth, the contribution of IL-33 to anti-tumor adaptive immune responses against this cancer remains to be established.

Melanoma

Accumulating evidences indicate that in melanoma models IL-33 exert anti-tumoral and anti-metastatic effects by conditioning

the local immune environment. Systemic injections of IL-33 significantly inhibit tumor growth in mice bearing subcutaneous B16.F10 melanomas (50, 69) and in $BRAF^{V600E}$PTEN-inducible melanoma model (50). Dominguez et al. reported that IL-33 both directly stimulated $CD8^+$ T cell expansion and IFN-γ production and activated myeloid dendritic cells (mDCs) increasing antigen cross-presentation. Of note, combination therapy with rIL-33 and agonistic anti-CD40 antibodies demonstrated synergistic anti-tumoral activity in this model (50). Using subcutaneous and experimental metastasis B16 melanoma models, we recently demonstrated that IL-33 inhibits melanoma tumor growth and pulmonary metastasis *in vivo* in an eosinophil-dependent manner (69). In a previous study, it was shown that IL-33 transgenic mice inhibit tumor metastasis in the B16 melanoma model (48). Likewise, transgenic expression of a secretable mature form of IL-33 by plasmid DNA in B16 tumor cells reduced tumor growth and metastasis *in vivo* (49). In these models, both NK and $CD8^+$ T cells were required for the anti-tumoral effect of IL-33. Instead, Kim et al. showed that subcutaneous injection of adenoviral vector-transfected B16 melanoma cells engineered to secrete IL-33 did not develop palpable tumors in mice, in a $CD8^+$ T- and NK-independent manner, through expansion of CXCR2 ligands-secreting intratumoral ILC2 (66). *Ex vivo* explanted IL-33-expressing B16 tumors exhibited higher reactive oxygen species (ROS) levels and increased CXCR2 expression and apoptosis thus suggesting a role for IL-33 in creating a hypoxic tumor microenvironment supporting ILC2-induced apoptosis (66). The explanation for such diverse immune-mediated anti-tumor mechanisms needs further investigations, although it is plausible that different local concentrations of IL-33 within the tumor microenvironment may stimulate different responses.

Other Tumors

IL-33 has been reported to exert an anti-tumoral role in other tumor models. In a human papilloma virus (HPV)-associated model for cancer immunotherapy IL-33 was shown to act as a potent vaccine adjuvant augmenting Th1 and $CD8^+$ T-cell responses, inducing anti-tumor immunity *in vivo* (23). Enforced expression of IL-33 in a large collection of murine tumor cell lines, including CT26 colon carcinoma, EL4 lymphoma, B16 melanoma, Lewis lung carcinoma (LLC), A9, and 4T1 lung cancer, results in reduced tumor growth *in vivo* (49, 66, 133). In a murine acute myeloid leukemia (AML) model administration of IL-33 significantly inhibited leukemia growth and improved mice survival rate in a $CD8^+$ T cell dependent manner (51). Notably, combination of PD-1 checkpoint blockade with IL-33 further prolonged mice survival, and induced complete leukemia regression in 50% of animals. The latter report represents the first evidence for combining IL-33 with immunotherapy targeting immune checkpoint inhibitors. Recently, administration of rhIL-33 was shown to expand Vγ9 T cells improving the therapeutic response to phosphoantigen in preventing tumor growth in a humanized mouse lymphoma model (134). Pro-tumoral effects were observed in several other types of experimental cancers (135). In an organotypic culture model, carcinoma-associated fibroblasts (CAFs) were found to express high levels of IL-33 which promoted cancer invasive behavior of head and neck squamous cell carcinoma (HNSCC) cells (136). In gastric cancer, IL-33 exerted a pro-tumorigenic function inducing cancer cell invasion by stimulating the secretion of MMP-3 and IL-6 via ST2-ERK1/2 pathway (137) and activation of the JNK pathway (138) conferring chemotherapy resistance *in vitro*. Finally, in mouse models of cholangiocarcinoma (CCA), a malignant neoplasm of the biliary-duct system, administration of IL-33 was found to increase biliary tumorigenesis through an increase of IL-6 expression in tumor tissue (139) and to enhance cholangiocyte proliferation, by increasing the numbers of IL-13-producing ILC2s (140). The effects of IL-33 in experimental cancers are summarized in **Table 1**.

IL-33/ST2 AS A BIOMARKER PREDICTIVE OF CANCER PROGRESSION AND PATIENTS SURVIVAL

The recent literature regarding IL-33 involvement in tumorigenesis is controversial, since IL-33 seems to have dual, pro-inflammatory or protective, roles depending on the cellular and cytokine context. Some studies have shown a positive correlation between IL-33 expression in tumor tissue and a favorable prognosis in cancer patients. For example expression levels of IL-33 and ST2 were significantly down-regulated in both adenocarcinoma and squamous cell carcinoma of lung tissues when compared to adjacent normal lung controls (141). Furthermore, plasma IL-33 levels were elevated during the early stage of lung cancer and decreased with advanced cancer stages, probably due to lung volume reduction containing bronchial epithelium and vascular endothelium as sources of IL-33 (142). The observed decreases indicate that the expression levels of IL-33 are inversely associated with lung cancer progression (143). Of interest, in hepatocellular carcinoma resected tissues expression of IL-33 by intratumoral effector memory $CD62L^-KLRG1^+CD107a^+$ $CD8^+$ T cells was shown to be a prognostic marker for increased patients survival (144). Serum levels of IL-33 were significantly higher in patients with breast cancer compared to patients with benign breast diseases, so the local expression of IL-33 may be a marker for differentiating malignant from normal/benign tissues (145, 146). IL-33 expression in adjacent tissues also tends to be higher compared to normal tissues, suggesting that adjacent non-cancerous tissues may be similarly relevant to cancers in terms of anti-tumor immunity. Local IL-33 expression may also increase intratumoral accumulation of immunosuppressive lymphoid cells in patients with breast cancer (145, 146). Plasma sST2 levels and nuclear IL-33 expression were found to be increased in endothelial cells in bone marrow biopsies from patients with myeloproliferative neoplasms, whereas low/undetectable levels were found in healthy donors (147). It has been proposed that IL-33 induces the production of cytokines and growth factors that promote myelopoiesis and facilitates the development of leukemia by inducing and/or enhancing the proliferation of hematopoietic progenitors in the

TABLE 1 | Role of IL-33 in experimental tumors.

Role	Cancer type	Species	Reported effects	References
Pro-tumoral	Breast	Mouse	IL-33/ST2 favors accumulation of immunosuppressive myeloid cells, Tregs, and ILCs. Increases tumor pro-angiogenic VEGF.	(70, 71, 84, 122)
		Human	IL-33/ST2 promotes epithelial cell transformation and breast tumorigenesis. Confers breast cancer endocrine resistance and cancer stem cell properties.	(123, 124)
	CRC	Mouse	IL-33/ST2 increases polyposis in $Apc^{Min/+}$ mice. Enhances tumor proliferation, growth, and angiogenesis. Promotes accumulaion of immunosuppressive myeloid cells.	(41, 72, 73, 109, 110, 126)
		Human	IL-33/ST2 expression increases tumor growth and metastasis in nude mice, tumor recruitment of prostaglandin E_2-producing macrophages, and M2 polarization. Promotes colon cancer cell stemness.	(72, 73, 127)
	Lung (NSCLC)	Human	ST2 increases tumor invasiveness and metastatic potential by selection of malignant cells. IL-33 blockade suppresses tumor growth and metastasis in nude mice, M2 polarization, and Treg accumulation.	(44, 130–132)
	Gastric	Human	IL-33 induces cancer cell invasion and confers chemotherapy resistance *in vitro*.	(137, 138)
	HNSCC	Human	IL-33 expression in CAFs promotes cancer invasive behavior.	(136)
	CCA	Mouse	IL-33 increases biliary tumorigenesis in mice via IL-6. It induces cholangiocyte proliferation mediated by ILC2s.	(139, 140)
Anti-tumoral	Melanoma	Mouse	Transgenic host or tumoral expression or IL-33 administration inhibits tumor growth and pulmonary metastasis, via stimulation of NK, $CD8^+$ T cells, DCs, ILC2s, and eosinophils.	(48–50, 66, 69)
	Lung	Mouse	IL-33 expression in lung cancer cells reduces tumor growth *in vivo*, restores MHC-I expression and immunovisibility.	(49, 133)
	CRC	Mouse	IL-33/ST2 signaling negatively correlates with tumor progression of CT26 and MC38 tumors. IL-33 induced IFNγ-mediated antitumor immune response. In a model of DSS-induced colitis IL-33 exerts a protective function regulating in testinal IgA-microbiota axis.	(46, 74, 128)
	AML	Mouse	IL-33 inhibits leukemia growth, improves mice survival rate through $CD8^+$ T cells and synergizes with PD-1 blockade to promote tumor rejection.	(51)
	Lymphoma	Mouse	IL-33 expands anti-lymphoma Vγ9 T cells and improves the therapeutic response to phosphoantigen.	(134)

CRC, colorectal cancer; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; CCA, cholangiocarcinoma; AML, acute myeloid leukemia; CAF, cancer associated fibroblasts.

bone marrow microenvironment from patients with chronic myeloid leukemia (147).

In other clinical conditions, increased IL-33 expression was inversely correlated with the overall survival of cancer patients. Serum IL-33 levels were increased in renal cell carcinoma (RCC) patients compared to healthy volunteers (148). Such over-expression was associated with advanced tumor-lymph node-metastasis (TNM) stage, resulting in reduced survival and increased risk of recurrence in patients. Mechanistically, it has been shown that IL-33 enhances RCC cell growth *in vivo* and prevents chemotherapy-induced tumor apoptosis *in vitro* via JNK signaling activation in tumor cells (149). Similarly, both IL-33 and ST2 were up-regulated in ovarian tumors compared to normal ovary and ovarian benign tumors, and the expression levels were further increased in tumor tissues at the metastatic site (150). It has been proposed that IL-33/ST2 axis promotes ovarian cancer migration and metastasis through regulation of ERK and JNK signaling pathways (151). Furthermore, most head and neck squamous cell carcinoma (HNSCC) cases with a low invasion pattern grading score (IPGS) showed low or no expression of IL-33, whereas most HNSCC cases with high IPGS displayed abundant expression of IL-33 in CAFs and in cancer cells. This observation suggests a paracrine effect of IL-33 as a result of the crosstalk between tumor cells and surrounding stromal cells. Hence, CAFs overexpressing IL-33 promote the induction of epithelial-to-mesenchymal trans-differentiation, eventually leading to tumor progression and poor prognosis (136). Higher IL-33 expression was described in glioma tissue compared to normal brain tissues at both transcriptional and translational levels (152, 153). It has been proposed that IL-33 stimulates cell migration through the expression of matrix metalloproteinases (MMP2/MMP9) *via* the ST2/ NF-κB pathway, thereby promoting cell invasion and tumor growth (154). Higher expression of IL-33 and total ST2 (ST2L and sST2) have also been reported in colorectal cancer (CRC) tissues compared to adjacent normal tissues (127). This observation could be explained considering that inflammatory cytokines are important components of the CRC microenvironment and colon cancer progression is closely related to chronic inflammation. Increased IL-33 expression is observed in poorly differentiated human CRC cells, which is associated with poor survival in patients with metastatic colon cancer (73).

The relation between IL-33 and sST2 serum levels and the survival of patients suffering from liver cirrhosis (LC) and hepatocellular carcinoma (HCC) has not been determined yet. No significant difference in IL-33 serum levels was found in HCC compared to LC. IL-33 levels did not correlate with overall survival or liver function parameters, whereas sST2 levels were significantly elevated in LC and HCC patients, compared to healthy subjects, and were associated with overall survival of

HCC. Therefore, its function remains to be clarified (155). Similarly, IL-33 protein levels were significantly lower in gastric cancer tissues than adjacent tissues. These levels were associated to the depth of tumor invasion and the morphology of the tumor, suggesting that IL-33 is involved in the process of inflammatory reaction in the development of gastric cancer, while it is not significantly associated with the overall survival of these patients (156).

ANALYSIS OF IL-33 MUTATIONS AND ISOFORMS IN CANCER

IL-33 gene undergoes a certain number of somatic mutations during tumorigenesis. Depending on the exact location in the gene (and protein), the mutation may be putative of some key functional aberrancies that influence the IL-33 global functional properties (**Figure 3**). In general, IL-33 mutations occurred with very low frequencies in all tumors examined (0.072–1.391%). However, some mutations may putatively have a key impact on the functionality of IL-33. IL-33 protein is equipped with three main regions (Nuclear, Central, IL-1-like) with three specific binding and cleavage sequences in each of these domains (**Figure 3**). For example, there are 15 unique mutations in skin cancer patients, with a missense mutation (M52I at nucleotide 6250538 of the chromosome 9) targeting the chromatin binding site (R1). We hypothesize that this amino acid change potentially breaks the ability of IL-33 to bind DNA and thus to exert its regulatory functions. There are additionally 11 unique somatic missense mutations all affecting the IL-1-like domain of the protein (but outside the R3 sequence), that can putatively compromise the IL-33 binding ability to its specific receptor ST2 (3, 157). Another tumor that displays a mutation affecting the ability of IL-33 to bind the

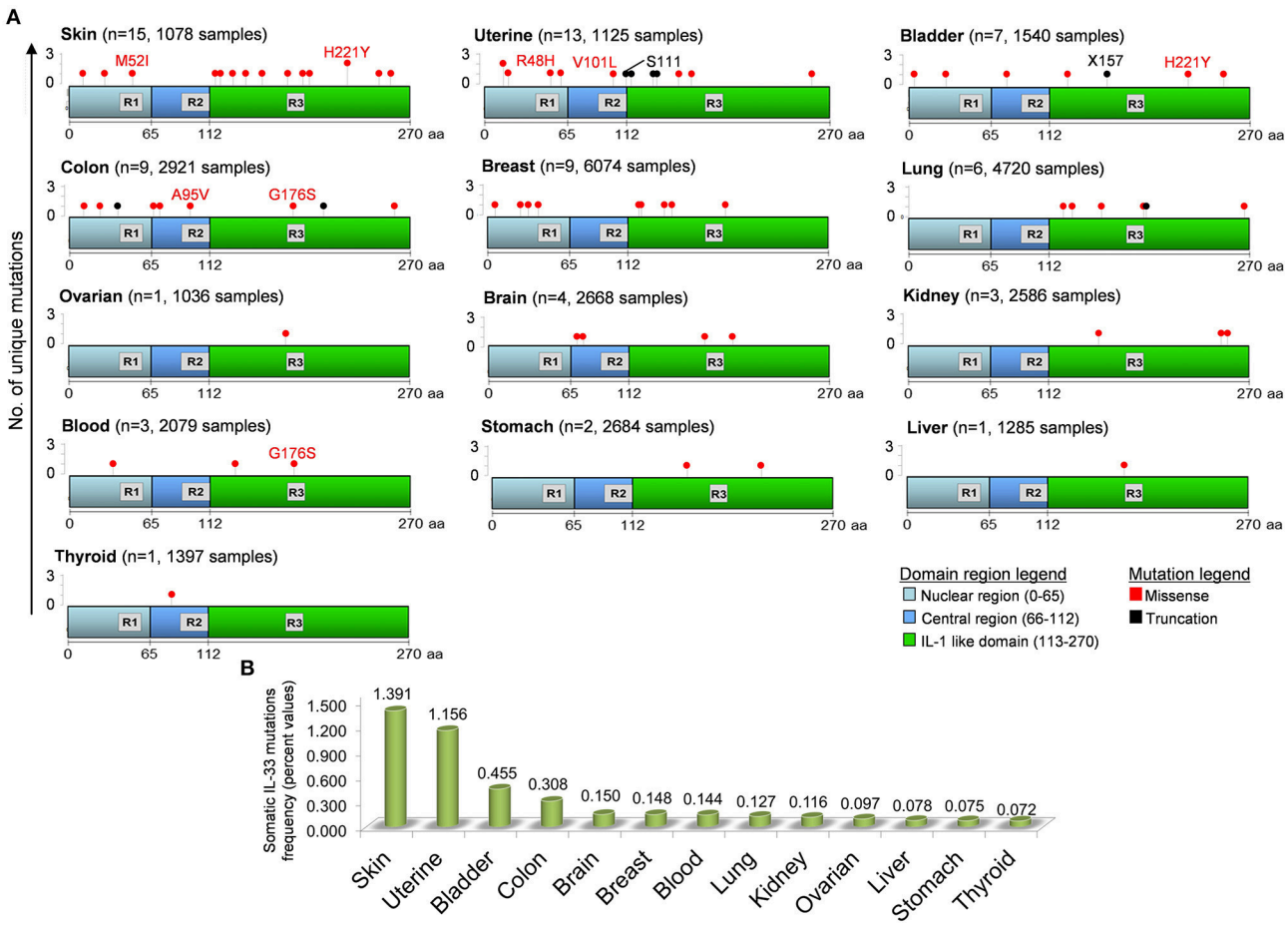


FIGURE 3 | Distribution of unique somatic IL-33 gene mutations in tumors and in the domain regions of the IL-33 protein. **(A)** IL-33 gene mutations in each tumor have been obtained by inferring the publicly available cBioPortal database for analysis of large-scale cancer genomics data sets (http://www.cbioportal.org/). For each tumor type the number of mutations (*n*) and the total number of sample patients is depicted. The IL-33 gene mutation positions are aligned to the three principal regions of the IL-33 protein. R1, Chromatin binding domain (M45XLRSGXXI53); R2, Cleavage site for inflammatory Proteases (T90VECFAFGISGVQKYTRALHDSS112); R3, Cleavage site for caspase 3 and caspase 7 (D175GVDG179). Some missense (red text) and truncation (black text) mutations are depicted. **(B)** Somatic IL-33 gene mutation frequencies calculated by data analyzed in **(A)** and indicated for each tumor type.

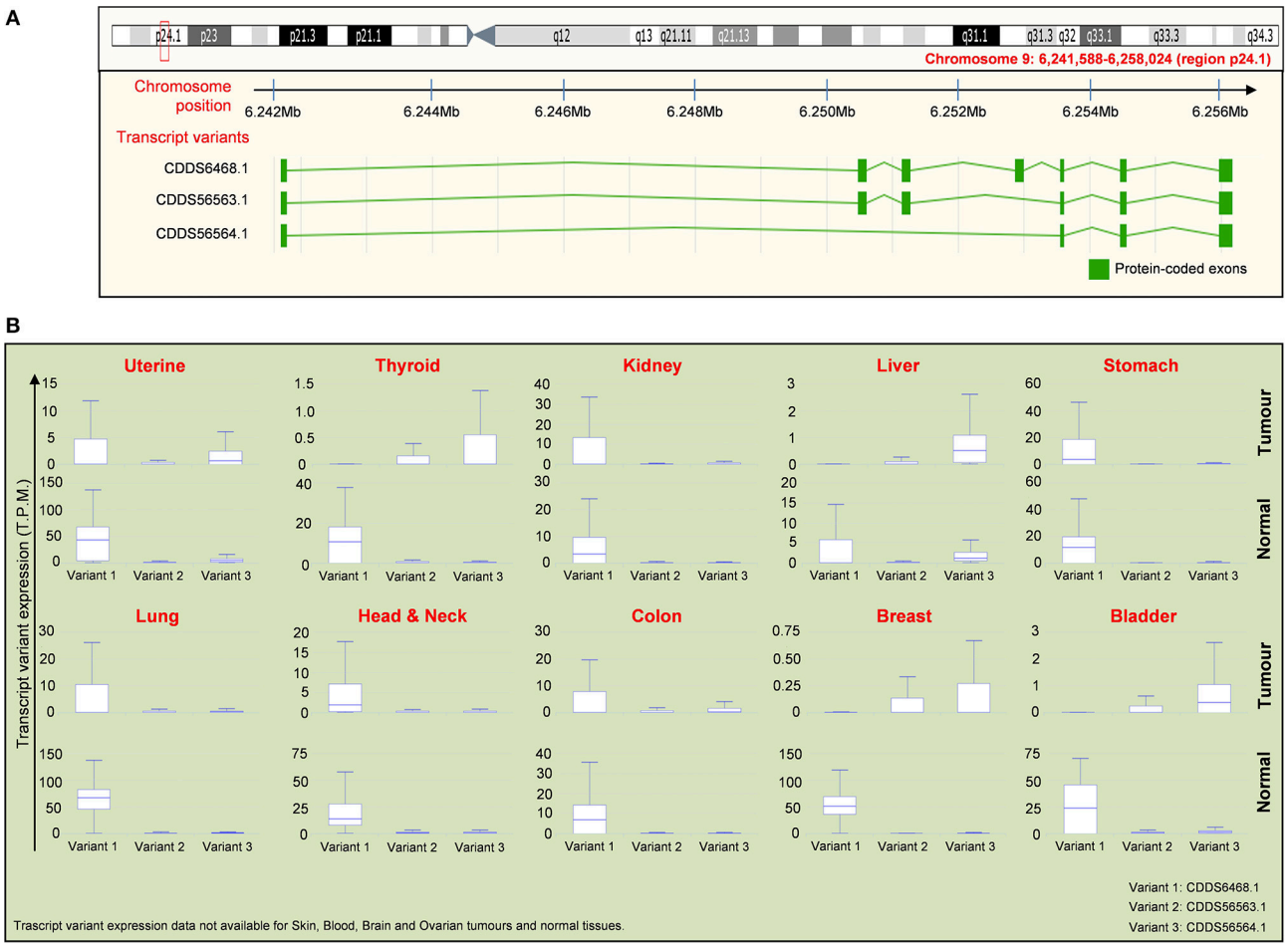


FIGURE 4 | Distribution of IL-33 RNA transcript variants in tumor samples compared to normal tissues. **(A)** Schematic representation of the chromosome location (red box and text) of the IL-33 gene (upper panel) with the location of exons present in each indicated transcript variant (lower panel). Data are obtained by ENSEMBL genome browser (https://www.ensembl.org/). There are 3 isoforms of IL-33 gene, which are: NM_001199640 (CDDS56563.1), NM_001199641 (CDDS56564.1), NM_033439 (CDDS6468.1). **(B)** Expression of the IL-33 transcript variants indicated in **(A)** for each tumor compared to the normal tissues. Expression data (T.P.M, transcript per million) are obtained by interrogating the public database for Isoform expression resource analysis (Isoexpresso, http://wiki.tgilab.org/ISOexpresso/main.php). IL-33 transcript variant expression data are not available for skin, blood, brain, and ovarian cancers/normal tissues within Isoexpresso database. The predominant forms for brain and ovarian tumors are variants 1 whereas the predominant form expressed in skin tumors is the transcript variant 3 (resource: Mammalian Transcriptomic Database, http://mtd.cbi.ac.cn/).

chromatin is the uterine cancer with the R48H amino acid change (**Figure 3**). Interestingly, the mutations E121K, H221Y, S225F, and H246Y, all occurring in the IL-1-like domain, are classified as deleterious in terms of IL-33 functional changes for skin cancers as evidenced by the cBioPortal database. Some of these mutations, like the amino acid change H221Y, are also shared with other tumors such as bladder cancer, thus denoting their significance. There is only one somatic missense alteration (G176S) occurring inside the R3 region, shared by colon and blood cancer patients (**Figure 3**). These mutations can putatively disrupt the cleavage site for caspase 3 and 7, thus increasing the IL-33 resistance to these proteases with key consequences in skin tumor survival and progression. Mutations in the cleavage site for inflammatory proteases (R2) occur only in colon and uterine tumors, with the A95V amino acid change (colon) and two mutations in uterine tumor (missense V101L and truncation S111).

As mentioned above, the IL-33 gene is equipped with a splicing system generating three different variants of the IL-33 protein (**Figure 4A**). The first isoform variant is composed by 7 exons, whereas the variants 2 and 3 contain 6 and 4 exons, respectively. The role and the extent of expression of each isoform in cancer is still a field of debate. Nevertheless, public databases such as IsoExpresso are now available, containing expression data of isoforms associated to thousands of human genes involved in cancer progression (158). We then extracted data about the expression of IL-33 isoform variants in different types of tumors and the normal counterparts (**Figure 4B**). The IsoExpresso database revealed a differential expression across the lesions and normal tissues or across the isoform variants

of IL-33. Thyroid, liver, breast, and bladder cancers display a change in isoform expression when compared to normal counterparts. Indeed, variants 2 and 3 are highly expressed in these tumors compared to expression levels observed in normal tissues (**Figure 4B**). Of note, bladder cancer presents a splice site mutation (X157, **Figure 3**), and the presence of this mutation may probably be associated to the observed expression shift of the IL-33 isoform variants in this type of cancer (**Figure 4**). Indeed, in the tumor lesions the mainly expressed isoforms are variant 2 and 3, whereas the normal tissues express the variant 1 only (**Figure 4B**). This strongly suggests that this mutation affects the disappearing of IL-33 exon 4 (**Figure 4A**). In contrast, uterine tumor has been associated with a splice mutation (X115, close to the S111 truncation, **Figure 3**) not affecting the expression of IL-33 splice variants (**Figure 4B**).

CONCLUDING REMARKS

The role of IL-33 in tumorigenesis and cancer immunity remains controversial. The current literature indicate that IL-33 expression can be regulated during the progression of distinct types of cancers and that IL-33/ST2 signaling within the tumor microenvironment may differently contribute to tumorigenesis, promoting antitumor responses or mediating tumor growth or metastasis, depending on the nature of the malignant tissue. Further studies are needed to elucidate the role of this pathway at specific time points during cancer development as a possible diagnostic/prognostic marker for patients and to clarify whether IL-33/ST2 blockade may represent a valid approach for adjuvant therapies of established IL-33-dependent tumors.

It has been suggested that IL-33 has opposing effects in tumor immunity depending on its local concentration (121). In this respect, the tumor histotype may crucially determine the amount of IL-33 expressed. In addition, the presence of different IL-33 isoforms may play a crucial role. Thus, it may be possible that under steady-state conditions the homeostatic release of IL-33 by apoptotic epithelial cells may be regulated by caspase cleavage, thus limiting excessive inflammation. We envisage that, as in several chronic inflammatory diseases, also in tumors IL-33 could exist in several different forms as a result of post-translational processes, including intracellular and extracellular modification, or as a result of mRNA alternative splicing. There is compelling evidence that different forms and cleavage products of IL-33 can exert dissimilar biological activities. Unfortunately, the identification and the concentrations of the different forms of IL-33 in different tumors is largely unknown. Future studies should characterize the different forms of IL-33 in human and experimental tumors. Moreover, the mutation level of IL-33 gene observed in human solid tumors (**Figure 3**) may be associated with different isoforms and distinct stimulated antitumor immune responses. Lastly, since the new frontier of cancer immunotherapy is the employment of immune checkpoint inhibitors (i.e., against PD-1, PD-L1, CTLA-4) further studies are needed to clarify whether IL-33 conditioning may increase the therapeutic response to checkpoint blockade in cancer patients.

AUTHOR CONTRIBUTIONS

GS, CA, and FM have conceived and designed the review. CA, CB, SA, MG, GV, GM, FM, and GS contributed intellectually and to the writing of the submitted version of the manuscript.

FUNDING

This work was supported in part by grants from AIRC IG 14297 to GS; Italian Ministry of Health RF-2011-02347120 to FM; Regione Campania CISI-Lab Project, CRèME Project, and TIMING Project to GM.

ACKNOWLEDGMENTS

The authors wish to thank all colleagues that have indirectly supported this work with ideas, discussion and encouragement. Special thanks to Fabrizio Fiorbianco for artwork.

REFERENCES

1. Krumm B, Xiang Y, Deng J. Structural biology of the IL-1 superfamily: key cytokines in the regulation of immune and inflammatory responses. *Protein Sci.* (2014) 23:526–38. doi: 10.1002/pro.2441
2. Cayrol C, Girard J-P. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. *Immunol Rev.* (2018) 281:154–68. doi: 10.1111/imr.12619
3. Liew FY, Girard J-P, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
4. Carriere V, Roussel L, Ortega N, Lacorre D-A, Americh L, Aguilar L, 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 USA.* (2007) 104:282–7. doi: 10.1073/pnas.0606854104
5. Moussion C, Ortega N, Girard J-P. 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:e3331. doi: 10.1371/journal.pone.0003331
6. Gajardo Carrasco T, Morales RA, Perez F, Terraza C, Yanez L, Campos-Mora M, et al. Alarmin' immunologists: IL-33 as a putative target for modulating T cell-dependent responses. *Front Immunol.* (2015) 6:232. doi: 10.3389/fimmu.2015.00232
7. Cassel SL, Joly S, Sutterwala FS. The NLRP3 inflammasome: a sensor of immune danger signals. *Semin Immunol.* (2009) 21:194–8. doi: 10.1016/j.smim.2009.05.002
8. Ali S, Nguyen DQ, Falk W, Martin MU. Caspase 3 inactivates biologically active full length interleukin-33 as a classical cytokine but does not prohibit nuclear translocation. *Biochem Biophys Res Commun.* (2010) 391:1512–6. doi: 10.1016/j.bbrc.2009.12.107
9. Cayrol C, Girard J-P. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA.* (2009) 106:9021–6. doi: 10.1073/pnas.0812690106
10. Lüthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* (2009) 31:84–98. doi: 10.1016/j.immuni.2009.05.007
11. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun.* (2015) 6:8327. doi: 10.1038/ncomms9327

12. Gordon ED, Simpson LJ, Rios CL, Ringel L, Lachowicz-Scroggins ME, Peters MC, et al. Alternative splicing of interleukin-33 and type 2 inflammation in asthma. *Proc Natl Acad Sci USA*. (2016) 113:8765–70. doi: 10.1073/pnas.1601914113
13. Hong J, Bae S, Jhun H, Lee S, Choi J, Kang T, et al. Identification of constitutively active interleukin 33 (IL-33) splice variant. *J Biol Chem.* (2011) 286:20078–86. doi: 10.1074/jbc.M111.219089
14. Tsuda H, Komine M, Karakawa M, Etoh T, Tominaga S-I, Ohtsuki M. Novel splice variants of IL-33: differential expression in normal and transformed cells. *J Invest Dermatol.* (2012) 132:2661–4. doi: 10.1038/jid.2012.180
15. Lefrançais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, Girard J-P, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc Natl Acad Sci USA*. (2012) 109:1673–8. doi: 10.1073/pnas.1115884109
16. Lefrançais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc Natl Acad Sci USA*. (2014) 111:15502–7. doi: 10.1073/pnas.1410700111
17. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat Immunol.* (2018) 19:375–85. doi: 10.1038/s41590-018-0067-5
18. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage–CD25+CD44hi lymphoid cells mediate innate type-2 immunity and allergic inflammation in the lungs. *J Immunol.* (2012) 188:1503–13. doi: 10.4049/jimmunol.1102832
19. Smithgall MD, Comeau MR, Park Yoon B-R, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK Cells. *Int Immunol.* (2008) 20:1019–30. doi: 10.1093/intimm/dxn060
20. Varricchi G, Pecoraro A, Marone G, Criscuolo G, Spadaro G, Genovese A, et al. Thymic stromal lymphopoietin isoforms, inflammatory disorders, and cancer. *Front Immunol.* (2018) 9:1595. doi: 10.3389/fimmu.2018.01595
21. Villarreal DO, Weiner DB. IL-33 isoforms: their future as vaccine adjuvants? *Expert Rev Vaccines* (2015) 14:489–92. doi: 10.1586/14760584.2015.1011135
22. Luzina IG, Pickering EM, Kopach P, Kang PH, Lockatell V, Todd NW, et al. Full-length IL-33 promotes inflammation but not Th2 response *in vivo* in an ST2-independent fashion. *J Immunol.* (2012) 189:403–10. doi: 10.4049/jimmunol.1200259
23. Villarreal DO, Wise MC, Walters JN, Reuschel EL, Choi MJ, Obeng-Adjei N, et al. Alarmin IL-33 acts as an immunoadjuvant to enhance antigen-specific tumor immunity. *Cancer Res.* (2014) 74:1789–800. doi: 10.1158/0008-5472.CAN-13-2729
24. Martin MU. Special aspects of interleukin-33 and the IL-33 receptor complex. *Semin Immunol.* (2013) 25:449–57. doi: 10.1016/j.smim.2013.10.006
25. Liu X, Hammel M, He Y, Tainer JA, Jeng US, Zhang L, et al. Structural insights into the interaction of IL-33 with its receptors. *Proc Natl Acad Sci USA*. (2013) 110:14918–23. doi: 10.1073/pnas.1308651110
26. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem.* (2007) 282:26369–80. doi: 10.1074/jbc.M704916200
27. Leung BP, Xu D, Culshaw S, McInnes IB, Liew FY. A novel therapy of murine collagen-induced arthritis with soluble T1/ST2. *J Immunol.* (2004) 173:145–50. doi: 10.4049/jimmunol.173.1.145
28. Cayrol C, Girard J-P. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* (2014) 31:31–7. doi: 10.1016/j.coi.2014.09.004
29. Bonilla WV, Fröhlich A, Senn K, Kallert S, Fernandez M, Johnson S, et al. The alarmin interleukin-33 drives protective antiviral $CD8^+$ T cell responses. *Science* (2012) 335:984–9. doi: 10.1126/science.1215418
30. Bourgeois E, Van Linh P, Samson M, Diem Sv, Barra A, Roga Sp, et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol.* (2009) 39:1046–55. doi: 10.1002/eji.200838575
31. Xie Y, Akpinarli A, Maris C, Hipkiss EL, Lane M, Kwon EK, et al. Naive tumor-specific CD4(+) T cells differentiated *in vivo* eradicate established melanoma. *J Exp Med.* (2010) 207:651–67. doi: 10.1084/jem.20091921
32. Hombach A, Kohler H, Rappl G, Abken H. Human CD4+ T cells lyse target cells via granzyme/perforin upon circumvention of MHC class II restriction by an antibody-like immunoreceptor. *J Immunol.* (2006) 177:5668–75. doi: 10.4049/jimmunol.177.8.5668
33. Komai-Koma M, Wang E, Kurowska-Stolarska M, Li D, McSharry C, Xu D. Interleukin-33 promoting Th1 lymphocyte differentiation dependents on IL-12. *Immunobiology* (2016) 221:412–7. doi: 10.1016/j.imbio.2015.11.013
34. Blom L, Poulsen BC, Jensen BM, Hansen A, Poulsen LK. IL-33 induces IL-9 production in human $CD4^+$ T cells and basophils. *PLoS ONE* (2011) 6:e21695. doi: 10.1371/journal.pone.0021695
35. Lu Y, Hong S, Li H, Park J, Hong B, Wang L, et al. Th9 cells promote antitumor immune responses *in vivo*. *J Clin Invest.* (2012) 122:4160–71. doi: 10.1172/JCI65459
36. Ramadan A, Griesenauer B, Adom D, Kapur R, Hanenberg H, Liu C, et al. Specifically differentiated T cell subset promotes tumor immunity over fatal immunity. *J Exp Med.* (2017) 214:3577–96. doi: 10.1084/jem.20170041
37. Peine M, Marek RM, Löhning M. IL-33 in T cell differentiation, function, and immune homeostasis. *Trends Immunol.* (2016) 37:321–33. doi: 10.1016/j.it.2016.03.007
38. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol.* (2017) 8:475. doi: 10.3389/fimmu.2017.00475
39. Matta BM, Lott JM, Mathews LR, Liu Q, Rosborough BR, Blazar BR, 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) 193:4010–20. doi: 10.4049/jimmunol.1400481
40. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T cell function in the intestine. *Nature* (2014) 513:564–8. doi: 10.1038/nature13577
41. He Z, Chen L, Souto FO, Canasto-Chibuque C, Bongers G, Deshpande M, et al. Epithelial-derived IL-33 promotes intestinal tumorigenesis in Apc(Min/+) mice. *Sci Rep.* (2017) 7:5520. doi: 10.1038/s41598-017-05716-z
42. Meinicke H, Bremser A, Brack M, Akeus P, Pearson C, Bullers S, et al. Tumour-associated changes in intestinal epithelial cells cause local accumulation of KLRG1+ GATA3+ regulatory T cells in mice. *Immunology* (2017) 152:74–88. doi: 10.1111/imm.12750
43. Zhou Y, Ji Y, Wang H, Zhang H, Zhou H. IL-33 promotes the development of colorectal cancer through inducing tumor-infiltrating ST2L+ regulatory T Cells in mice. *Technol Cancer Res Treat.* (2018) 17:1533033818780091. doi: 10.1177/1533033818780091
44. Wang K, Shan S, Yang Z, Gu X, Wang Y, Wang C, et al. IL-33 blockade suppresses tumor growth of human lung cancer through direct and indirect pathways in a preclinical model. *Oncotarget* (2017) 8:68571–82. doi: 10.18632/oncotarget.19786
45. Lim Hui X, Choi S, Cho D, Kim Tae S. IL-33 inhibits the differentiation and immunosuppressive activity of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *Immunol Cell Biol.* (2017) 95:99–107. doi: 10.1038/icb.2016.72
46. Eissmann MF, Dijkstra C, Wouters MA, Baloyan D, Mouradov D, Nguyen PM, et al. Interleukin 33 signaling restrains sporadic colon cancer in an interferon-γ-dependent manner. *Cancer Immunol Res.* (2018) 6:409–21. doi: 10.1158/2326-6066.CIR-17-0218
47. Yang Q, Li G, Zhu Y, Liu L, Chen E, Turnquist H, et al. IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8+ T cells. *Eur J Immunol.* (2011) 41:3351–60. doi: 10.1002/eji.201141629
48. Gao K, Li X, Zhang L, Bai L, Dong W, Shi G, et al. Transgenic expression of IL-33 activates $CD8^+$ T cells and NK cells and inhibits tumor growth and metastasis in mice. *Cancer Lett.* (2013) 335:463–71. doi: 10.1016/j.canlet.2013.03.002
49. Gao X, Wang X, Yang Q, Zhao X, Wen W, Li G, et al. Tumoral expression of IL-33 inhibits tumor growth and modifies the tumor microenvironment through CD8+ T and NK cells. *J Immunol.* (2015) 194:438–45. doi: 10.4049/jimmunol.1401344
50. Dominguez D, Ye C, Geng Z, Chen S, Fan J, Qin L, et al. Exogenous IL-33 restores dendritic cell activation and maturation in established cancer. *J Immunol.* (2017) 198:1365–75. doi: 10.4049/jimmunol.1501399

51. Qin L, Dominguez D, Chen S, Fan J, Long A, Zhang M, et al. Exogenous IL-33 overcomes T cell tolerance in murine acute myeloid leukemia. *Oncotarget* (2016) 7:61069–80. doi: 10.18632/oncotarget.11179
52. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells–a proposal for uniform nomenclature. *Nat Rev Immunol.* (2013) 13:145–9. doi: 10.1038/nri3365
53. Harly C, Cam M, Kaye J, Bhandoola A. Development and differentiation of early innate lymphoid progenitors. *J Exp Med.* (2018) 215:249–62. doi: 10.1084/jem.20170832
54. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* (2010) 463:540–4. doi: 10.1038/nature08636
55. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, 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) 12:1055–62. doi: 10.1038/ni.2104
56. Monticelli LA, Osborne LC, Noti M, Tran SV, Zaiss DM, Artis D. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci USA.* (2015) 112:10762–7. doi: 10.1073/pnas.1509070112
57. Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, Hewett DR, 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) 203:1105–16. doi: 10.1084/jem.20051615
58. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* (2014) 40:425–35. doi: 10.1016/j.immuni.2014.01.011
59. Hoyler T, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity* (2012) 37:634–48. doi: 10.1016/j.immuni.2012.06.020
60. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med.* (2013) 5:170ra16. doi: 10.1126/scitranslmed.3005374
61. Dancescu M, Rubio-Trujillo M, Biron G, Bron D, Delespesse G, Sarfati M. Interleukin 4 protects chronic lymphocytic leukemic B cells from death by apoptosis and upregulates Bcl-2 expression. *J Exp Med.* (1992) 176:1319–26. doi: 10.1084/jem.176.5.1319
62. Bie Q, Zhang P, Su Z, Zheng D, Ying X, Wu Y, et al. Polarization of ILC2s in peripheral blood might contribute to immunosuppressive microenvironment in patients with gastric cancer. *J Immunol Res.* (2014) 2014:923135. doi: 10.1155/2014/923135
63. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol.* (2011) 12:1045–54. doi: 10.1038/ni.2131
64. Zaiss DM, van Loosdregt J, Gorlani A, Bekker CP, Grone A, Sibilia M, et al. Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity* (2013) 38:275–84. doi: 10.1016/j.immuni.2012.09.023
65. Ikutani M, Yanagibashi T, Ogasawara M, Tsuneyama K, Yamamoto S, Hattori Y, et al. Identification of innate IL-5-producing cells and their role in lung eosinophil regulation and antitumor immunity. *J Immunol.* (2012) 188:703–13. doi: 10.4049/jimmunol.1101270
66. Kim J, Kim W, Moon UJ, Kim HJ, Choi HJ, Sin JI, et al. Intratumorally establishing type 2 innate lymphoid cells blocks tumor growth. *J Immunol.* (2016) 196:2410–23. doi: 10.4049/jimmunol.1501730
67. Liang Y, Jie Z, Hou L, Yi P, Wang W, Kwota Z, et al. IL-33 promotes innate IFN-g production and modulates dendritic cell response in LCMV-induced hepatitis in mice. *Eur J Immunol.* (2015) 45:3052–63. doi: 10.1002/eji.201545696
68. Nabekura T, Girard J-P, Lanier LL. IL-33 receptor ST2 amplifies the expansion of natural killer cells and enhances host defense during mouse cytomegalovirus infection. *J Immunol.* (2015) 194:5948–52. doi: 10.4049/jimmunol.1500424
69. Lucarini V, Zicchedda G, Macchia I, La Sorsa V, Peschiaroli F, Buccione C, et al. IL-33 restricts tumor growth and inhibits pulmonary metastasis in melanoma-bearing mice through eosinophils. *Oncoimmunology* (2017) 6:e1317420. doi: 10.1080/2162402X.2017.1317420
70. Jovanovic I, Radosavljevic G, Mitrovic M, Lisnic Juranic V, McKenzie ANJ, Arsenijevic N, et al. ST2 deletion enhances innate and acquired immunity to murine mammary carcinoma. *Eur J Immunol.* (2011) 41:1902–12. doi: 10.1002/eji.201141417
71. Jovanovic IP, Pejnovic NN, Radosavljevic GD, Pantic JM, Milovanovic MZ, Arsenijevic NN, et al. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. *Int J Cancer* (2014) 134:1669–82. doi: 10.1002/ijc.28481
72. Akimoto M, Maruyama R, Takamaru H, Ochiya T, Takenaga K. Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by modifying the tumour microenvironment. *Nat Commun.* (2016) 7:13589. doi: 10.1038/ncomms13589
73. Fang M, Li Y, Huang K, Qi S, Zhang J, Zgodzinski W, et al. IL33 Promotes colon cancer cell stemness via JNK activation and macrophage recruitment. *Cancer Res.* (2017) 77:2735–45. doi: 10.1158/0008-5472.CAN-16-1602
74. O'Donnell C, Mahmoud A, Keane J, Murphy C, White D, Carey S, et al. An antitumorigenic role for the IL-33 receptor, ST2L, in colon cancer. *Br J Cancer* (2016) 114:37–43. doi: 10.1038/bjc.2015.433
75. Yang Y, Andersson P, Hosaka K, Zhang Y, Cao R, Iwamoto H, et al. The PDGF-BB-SOX7 axis-modulated IL-33 in pericytes and stromal cells promotes metastasis through tumour-associated macrophages. *Nat Commun.* (2016) 7:11385. doi: 10.1038/ncomms11385
76. Ariyoshi W, Okinaga T, Chaweewannakorn W, Akifusa S, Nisihara T. Mechanisms involved in enhancement of matrix metalloproteinase-9 expression in macrophages by interleukin-33. *J Cell Physiol.* (2017) 232:3481–95. doi: 10.1002/jcp.25809
77. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol.* (2009) 123:1047–54. doi: 10.1016/j.jaci.2009.02.026
78. Gabriele L, Schiavoni G, Mattei F, Sanchez M, Sestili P, Butteroni C, et al. Novel allergic asthma model demonstrates ST2-dependent dendritic cell targeting by cypress pollen. *J Allergy Clin Immunol.* (2013) 132:686–95. doi: 10.1016/j.jaci.2013.02.037
79. Besnard A-G, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol.* (2011) 41:1675–86. doi: 10.1002/eji.201041033
80. Kurokawa M, Matsukura S, Kawaguchi M, Ieki K, Suzuki S, Watanabe S, et al. Interleukin-33-activated dendritic cells induce the production of thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Arch Allergy Immunol.* (2013) 161 (Suppl. 2):52–7. doi: 10.1159/000350363
81. Su Z, Lin J, Lu F, Zhang X, Zhang L, Gandhi NB, et al. Potential autocrine regulation of interleukin-33/ST2 signaling of dendritic cells in allergic inflammation. *Mucosal Immunol.* (2013) 6:921–30. doi: 10.1038/mi.2012.130
82. Mayuzumi N, Matsushima H, Takashima A. IL-33 promotes DC development in BM culture by triggering GM-CSF production. *Eur J Immunol.* (2009) 39:3331–42. doi: 10.1002/eji.200939472
83. Tcyganov E, Mastio J, Chen E, Gabrilovich DI. Plasticity of myeloid-derived suppressor cells in cancer. *Curr Opin Immunol.* (2018) 51:76–82. doi: 10.1016/j.coi.2018.03.009
84. Xiao P, Wan X, Cui B, Liu Y, Qiu C, Rong J, et al. Interleukin 33 in tumor microenvironment is crucial for the accumulation and function of myeloid-derived suppressor cells. *Oncoimmunology* (2015) 5:e1063772. doi: 10.1080/2162402X.2015.1063772
85. Foley SC, Hamid Q. Images in allergy and immunology: neutrophils in asthma. *J Allergy Clin Immunol.* (2007) 119:1282–6. doi: 10.1016/j.jaci.2007.02.006
86. Sun B, Zhu L, Tao Y, Sun H-X, Li Y, Wang P, et al. Characterization and allergic role of IL-33-induced neutrophil polarization. *Cell Mol Immunol.* (2018) 15:782–93. doi: 10.1038/cmi.2017.163
87. Liang Y, Yi P, Yuan DMK, Jie Z, Kwota Z, Soong L, et al. IL-33 induces immunosuppressive neutrophils via a type 2 innate lymphoid cell/IL-13/STAT6 axis and protects the liver against injury

in LCMV infection-induced viral hepatitis. *Cell Mol Immunol.* (2018). doi: 10.1038/cmi.2017.147. [Epub ahead of print].
88. Guabiraba R, Besnard AG, Menezes GB, Secher T, Jabir MS, Amaral SS, et al. IL-33 targeting attenuates intestinal mucositis and enhances effective tumour chemotherapy in mice. *Mucosal Immunol.* (2014) 7:1079–93. doi: 10.1038/mi.2013.124
89. Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N, et al. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int Immunol.* (2008) 20:791–800. doi: 10.1093/intimm/dxn037
90. Johnston LK, Bryce PJ. Understanding interleukin 33 and its roles in eosinophil development. *Front Med (Lausanne)* (2017) 4:51. doi: 10.3389/fmed.2017.00051
91. Willebrand R, Voehringer D. IL-33-Induced cytokine secretion and survival of mouse eosinophils is promoted by autocrine GM-CSF. *PLoS ONE* (2016) 11:e0163751. doi: 10.1371/journal.pone.0163751
92. Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, Hammerling GJ. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol.* (2015) 16:609–17. doi: 10.1038/ni.3159
93. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol.* (2008) 121:1484. doi: 10.1016/j.jaci.2008.04.005
94. Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H, et al. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest.* (2008) 88:1245–53. doi: 10.1038/labinvest.2008.82
95. Cormier SA, Taranova AG, Bedient C, Nguyen T, Protheroe C, Pero R, et al. Pivotal advance: eosinophil infiltration of solid tumors is an early and persistent inflammatory host response. *J Leukoc Biol.* (2006) 79:1131–9. doi: 10.1189/jlb.0106027
96. Caruso RA, Parisi A, Quattrocchi E, Scardigno M, Branca G, Parisi C, et al. Ultrastructural descriptions of heterotypic aggregation between eosinophils and tumor cells in human gastric carcinomas. *Ultrastruct Pathol.* (2011) 35:145–9. doi: 10.3109/01913123.2011.578233
97. Costain DJ, Guha AK, Liwski RS, Lee TDG. Murine hypodense eosinophils induce tumour cell apoptosis by a granzyme B-dependent mechanism. *Cancer Immunol Immunother.* (2001) 50:293–9. doi: 10.1007/PL00006690
98. Legrand F, Driss V, Delbeke M, Loiseau S, Hermann E, Dombrowicz D, et al. Human eosinophils exert TNF-alpha and granzyme A-mediated tumoricidal activity toward colon carcinoma cells. *J Immunol.* (2010) 185:7443–51. doi: 10.4049/jimmunol.1000446
99. Varricchi G, Galdiero MR, Loffredo S, Lucarini V, Marone G, Mattei F, et al. Eosinophils: the unsung heroes in cancer? *Oncoimmunology* (2018) 7:e1393134. doi: 10.1080/2162402X.2017.1393134
100. Iikura M, Suto H, Kajiwara N, Oboki K, Ohno T, Okayama Y, et al. IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest.* (2007) 87:971–8. doi: 10.1038/labinvest.3700663
101. Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res.* (2010) 59:207–18. doi: 10.1007/s00011-009-0088-5
102. Theoharides TC, Zhang B, Kempuraj D, Tagen M, Vasiadi M, Angelidou A, et al. IL-33 augments substance P-induced VEGF secretion from human mast cells and is increased in psoriatic skin. *Proc Natl Acad Sci USA.* (2010) 107:4448–53. doi: 10.1073/pnas.1000803107
103. Varricchi G, Galdiero MR, Loffredo S, Marone G, Iannone R, Marone G, et al. Are mast cells MASTers in cancer? *Front Immunol.* (2017) 8:424. doi: 10.3389/fimmu.2017.00424
104. de Paulis A, Prevete N, Fiorentino I, Rossi FW, Staibano S, Montuori N, et al. Expression and functions of the vascular endothelial growth factors and their receptors in human basophils. *J Immunol.* (2006) 177:7322–31. doi: 10.4049/jimmunol.177.10.7322
105. Marone G, Varricchi G, Loffredo S, Granata F. Mast cells and basophils in inflammatory and tumor angiogenesis and lymphangiogenesis. *Eur J Immunol.* (2016) 778:146–51. doi: 10.1016/j.ejphar.2015.03.088
106. Rivellese F, Suurmond J, Habets K, Dorjée Annemarie L, Ramamoorthi N, Townsend Michael J, et al. Ability of interleukin-33- and immune complex-triggered activation of human mast cells to down-regulate monocyte-mediated immune responses. *Arthritis Rheumatol.* (2015) 67:2343–53. doi: 10.1002/art.39192
107. Joulia R, L'Faqihi F-E, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. *J Allergy Clin Immunol.* (2017) 140:497–509.e10. doi: 10.1016/j.jaci.2016.09.049
108. Byrne SN, Beaugie C, O'Sullivan C, Leighton S, Halliday GM. The immune-modulating cytokine and endogenous alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation. *Am J Pathol.* (2011) 179:211–22. doi: 10.1016/j.ajpath.2011.03.010
109. Maywald RL, Doerner SK, Pastorelli L, De Salvo C, Benton SM, Dawson EP, et al. IL-33 activates tumor stroma to promote intestinal polyposis. *Proc Natl Acad Sci USA.* (2015) 112:E2487–96. doi: 10.1073/pnas.1422445112
110. Mertz KD, Mager LF, Wasmer MH. The IL-33/ST2 pathway contributes to intestinal tumorigenesis in humans and mice. *Oncoimmunology* (2015) 26:e1062966. doi: 10.1080/2162402X.2015.1062966
111. Gounaris E, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, et al. Mast cells are an essential hematopoietic component for polyp development. *Proc Natl Acad Sci USA.* (2007) 104:19977. doi: 10.1073/pnas.0704620104
112. Blatner NR, Bonertz A, Beckhove P, Cheon EC, Krantz SB, Strouch M, et al. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. *Proc Natl Acad Sci USA.* (2010) 107:6430–5. doi: 10.1073/pnas.0913683107
113. Cheon EC, Khazaie K, Khan MW, Strouch MJ, Krantz SB, Phillips J, et al. Mast cell 5-lipoxygenase activity promotes intestinal polyposis in APCΔ468 mice. *Cancer Res.* (2011) 71:1627–36. doi: 10.1158/0008-5472.CAN-10-1923
114. Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A, et al. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol.* (2008) 181:5981–9. doi: 10.4049/jimmunol.181.9.5981
115. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* (2009) 113:1526–34. doi: 10.1182/blood-2008-05-157818
116. Schneider E, Petit-Bertron A-F, Bricard R, Levasseur Ml, Ramadan A, Girard J-P, et al. IL-33 activates unprimed murine basophils directly *in vitro* and induces their *in vivo* expansion indirectly by promoting hematopoietic growth factor production. *J Immunol.* (2009) 183:3591–7. doi: 10.4049/jimmunol.0900328
117. Rivellese F, Suurmond J, de Paulis A, Marone G, Huizinga Tom WJ, Toes René EM. IgE and IL-33-mediated triggering of human basophils inhibits TLR4-induced monocyte activation. *Eur J Immunol.* (2014) 44:3045–55. doi: 10.1002/eji.201444731
118. Sektioglu IM, Carretero R, Bulbuc N, Bald T, Tüting T, Rudensky AY, et al. Basophils promote tumor rejection via chemotaxis and infiltration of CD8+ T cells. *Cancer Res.* (2017) 77:291–302. doi: 10.1158/0008-5472.CAN-16-0993
119. Wei Y, Zhang X, Wang G, Zhou Y, Luo M, Wang S, et al. The impacts of pretreatment circulating eosinophils and basophils on prognosis of stage I-III colorectal cancer. *Asia Pac J Clin Oncol.* (2018). 14:e243–51. doi: 10.1111/ajco.12871
120. De Monte L, Wörmann S, Brunetto E, Heltai S, Magliacane G, Reni M, et al. Basophil recruitment into tumor-draining lymph nodes correlates with Th2 inflammation and reduced survival in pancreatic cancer patients. *Cancer Res.* (2016) 76:1792–803. doi: 10.1158/0008-5472.CAN-15-1801-T
121. Lu B, Yang M, Wang Q. Interleukin-33 in tumorigenesis, tumor immune evasion, and cancer immunotherapy. *J Mol Med (Berl).* (2016) 94:535–43. doi: 10.1007/s00109-016-1397-0
122. Milosavljevic MZ, Jovanovic IP, Pejnovic NN, Mitrovic SLJ, Arsenijevic NN, Simovic Markovic BJ, et al. Deletion of IL-33R attenuates VEGF expression and enhances necrosis in mammary carcinoma. *Oncotarget* (2016) 7:18106–15. doi: 10.18632/oncotarget.7635
123. Kim JY, Lim SC, Kim G, Yun HJ, Ahn SG, Choi HS. Interleukin-33/ST2 axis promotes epithelial cell transformation and breast tumorigenesis via upregulation of COT activity. *Oncogene* (2015) 34:4928–38. doi: 10.1038/onc.2014.418

124. Hu H, Sun J, Wang C, Bu X, Liu X-p, Mao YP, et al. IL-33 facilitates endocrine resistance of breast cancer by inducing cancer stem cell properties. *Biochem Biophys Res Commun.* (2017) 485:643–50. doi: 10.1016/j.bbrc.2017.02.080
125. Akimoto M, Takenaga K. Role of the IL-33/ST2L axis in colorectal cancer progression. *Cell Immunol.* (2018). doi: 10.1016/j.cellimm.2017.12.014. [Epub ahead of print].
126. Zhang Y, Davis C, Shah S, Hughes D, Ryan JC, Altomare D, et al. IL-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis. *Mol Carcinogen.* (2017) 56:272–87. doi: 10.1002/mc.22491
127. Liu X, Zhu L, Lu X, Bian H, Wu X, Yang W, et al. IL-33/ST2 pathway contributes to metastasis of human colorectal cancer. *Biochem Biophys Res Commun.* (2014) 453:486–92. doi: 10.1016/j.bbrc.2014.09.106
128. Malik A, Sharma D, Zhu Q, Karki R, Guy CS, Vogel P, et al. IL-33 regulates the IgA-microbiota axis to restrain IL-1a-dependent colitis and tumorigenesis. *J Clin Invest.* (2016) 126:4469–81. doi: 10.1172/JCI88625
129. Drake LY, Kita H. IL-33: Biological properties, functions and roles in airway disease. *Immunol Rev.* (2017) 278:173–84. doi: 10.1111/imr.12552
130. Akimoto M, Hayashi JI, Nakae S, Saito H, Takenaga K. Interleukin-33 enhances programmed oncosis of ST2L-positive low-metastatic cells in the tumour microenvironment of lung cancer. *Cell Death Dis.* (2016) 7:e2057. doi: 10.1038/cddis.2015.418
131. Yang Z, Gao X, Wang J, Xu L, Zheng Y, Xu Y. Interleukin-33 enhanced the migration and invasiveness of human lung cancer cells. *Onco Targets Ther.* (2018) 11:843–9. doi: 10.2147/OTT.S155905
132. Wang C, Chen Z, Bu X, Han Y, Shan S, Ren T, et al. IL-33 signaling fuels outgrowth and metastasis of human lung cancer. *Biochem Biophys Res Commun.* (2016) 479:461–8. doi: 10.1016/j.bbrc.2016.09.081
133. Saranchova I, Han J, Huang H, Fenninger F, Choi KB, Munro L, et al. Discovery of a metastatic immune escape mechanism initiated by the loss of expression of the tumour biomarker interleukin-33. *Sci Rep.* (2016) 6:30555. doi: 10.1038/srep30555
134. Duault C, Betous D, Bezombes C, Roga S, Cayrol C, Girard J-P, et al. IL-33-expanded human Vγ9Vδ2 cells have anti-lymphoma effect in a mouse tumor model. *Eur J Immunol.* (2017) 47:2137–41. doi: 10.1002/eji.201747093
135. Wasmer M-H, Krebs P. The role of IL-33-dependent inflammation in the tumor microenvironment. *Front Immunol.* (2017) 7:682. doi: 10.3389/fimmu.2016.00682
136. Chen S-F, Nieh S, Jao S-W, Wu M-Z, Liu C-L, Chang Y-C, et al. The paracrine effect of cancerassociated fibroblast-induced interleukin-33 regulates the invasiveness of head and neck squamous cell carcinoma. *J Pathol.* (2013) 231:180–9. doi: 10.1002/path.4226
137. Yu X, Hu Z, Shen X, Dong L, Zhou W, Hu W. IL-33 promotes gastric cancer cell invasion and migration via ST2-ERK1/2 pathway. *Dig Dis Sci.* (2015) 60:1265–72. doi: 10.1007/s10620-014-3463-1
138. Ye X, Zhao Y, Weng G, Chen Y, Wei X, Shao J, et al. IL-33-induced JNK pathway activation confers gastric cancer chemotherapy resistance. *Oncol Rep.* (2015) 33:2746–52. doi: 10.3892/or.2015.3898
139. Yamada D, Rizvi S, Razumilava N, Bronk SF, Davila JI, Champion MD, et al. IL-33 facilitates oncogene induced cholangiocarcinoma in mice by an IL-6 sensitive mechanism. *Hepatology* (2015) 61:1627–42. doi: 10.1002/hep.27687
140. Li J, Razumilava N, Gores GJ, Walters S, Mizuochi T, Mourya R, et al. Biliary repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation. *J Clin Invest.* (2014) 124:3241–51. doi: 10.1172/JCI73742
141. Yang M, Feng Y, Yue C, Xu B, Chen L, Jiang J, et al. Lower expression level of IL-33 is associated with poor prognosis of pulmonary adenocarcinoma. *PLoS ONE* (2018) 13:e0193428. doi: 10.1371/journal.pone.0193428
142. Kim MS, Kim E, Heo J-S, Bae D-J, Lee J-UW, Lee T-H, et al. Circulating IL-33 level is associated with the progression of lung cancer. *Lung Cancer* (2015) 90:346–51. doi: 10.1016/j.lungcan.2015.08.011
143. Hsu Y-L, Hung J-Y, Lee Y-L, Chen F-W, Chang K-F, Chang W-A, et al. Identification of novel gene expression signature in lung adenocarcinoma by using next-generation sequencing data and bioinformatics analysis. *Oncotarget* (2017) 8:104831–54. doi: 10.18632/oncotarget.21022
144. Brunner SM, Rubner C, Kesselring R, Martin M, Griesshammer E, Ruemmele P, et al. Tumor-infiltrating, interleukin-33-producing effector-memory CD8+ T cells in resected hepatocellular carcinoma prolong patient survival. *Hepatology* (2015) 61:1957–67. doi: 10.1002/hep.27728
145. Jafarzadeh A, Minaee K, Farsinejad A-R, Nemati M, Khosravimashizi A, Daneshvar H, et al. Evaluation of the circulating levels of IL-12 and IL-33 in patients with breast cancer: influences of the tumor stages and cytokine gene polymorphisms. *Iran J Basic Med Sci.* (2015) 18:1189–98. doi: 10.22038/IJBMS.2015.6271
146. Liu J, Shen J-X, Hu J-L, Huang W-H, Zhang G-J. Significance of interleukin-33 and its related cytokines in patients with breast cancers. *Front Immunol.* (2014) 5:141. doi: 10.3389/fimmu.2014.00141
147. Levescot A, Flamant S, Basbous S, Jacomet F, Féraud O, Anne Bourgeois E, et al. BCR-ABL-induced deregulation of the IL-33/ST2 pathway in CD34(+) progenitors from chronic myeloid leukemia patients. *Cancer Res.* (2014) 74:2669–76. doi: 10.1158/0008-5472.CAN-13-2797
148. Wang Z, Xu L, Chang Y, Zhou L, Fu H, Zhang W, et al. IL-33 is associated with unfavorable postoperative survival of patients with clear-cell renal cell carcinoma. *Tumor Biol.* (2016) 37:11127–34. doi: 10.1007/s13277-016-4879-3
149. Wu C, Wu Y, Cheng C, Hong Z, Shi Z, Lin S, et al. Interleukin-33 predicts poor prognosis and promotes renal cell carcinoma cell growth through its receptor ST2 and the JNK signaling pathway. *Cell Physiol Biochem.* (2018) 47:191–200. doi: 10.1159/000489766
150. Saied EM, El-Etreby NM. The role and prognostic value of inducible nitric oxide synthase (iNOS) and interleukin-33 (IL-33) in serous and mucinous epithelial ovarian tumours. *Ann Diagn Pathol.* (2017) 27:62–8. doi: 10.1016/j.anndiagpath.2017.01.006
151. Tong X, Barbour M, Hou K, Gao C, Cao S, Zheng J, et al. Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways. *Mol Oncol.* (2016) 10:113–25. doi: 10.1016/j.molonc.2015.06.004
152. Gramatzki D, Frei K, Cathomas G, Moch H, Weller M, Mertz KD. Interleukin-33 in human gliomas: expression and prognostic significance. *Oncol Lett.* (2016) 12:445–52. doi: 10.3892/ol.2016.4626
153. Zhang J, Wang P, Ji W, Ding Y, Lu X. Overexpression of interleukin-33 is associated with poor prognosis of patients with glioma. *Int J Neurosci.* (2017) 127:210–7. doi: 10.1080/00207454.2016.1175441
154. Zhang J-F, Wang P, Yan Y-J, Li Y, Guan M-W, Yu J-J, et al. IL-33 enhances glioma cell migration and invasion by upregulation of MMP2 and MMP9 via the ST2-NF-κB pathway. *Oncol Rep.* (2017) 38:2033–42. doi: 10.3892/or.2017.5926
155. Bergis D, Kassis V, Ranglack A, Koeberle V, Piiper A, Kronenberger B, et al. High serum levels of the interleukin-33 receptor soluble ST2 as a negative prognostic factor in hepatocellular carcinoma. *Transl Oncol.* (2013) 6:311–8. doi: 10.1593/tlo.12418
156. Hu W, Li X, Li Q, Tan Y, Xu B, Xie Q, et al. Interleukin-33 expression does not correlate with survival of gastric cancer patients. *Pathol Oncol Res.* (2017) 23:615–9. doi: 10.1007/s12253-016-0167-1
157. De la Fuente M, MacDonald TT, Hermoso MA. The IL-33/ST2 axis: role in health and disease. *Cytokine Growth Factor Rev.* (2015) 26:615–23. doi: 10.1016/j.cytogfr.2015.07.017
158. Yang IS, Son H, Kim S, Kim S. ISOexpresso: a web-based platform for isoform-level expression analysis in human cancer. *BMC Genomics* (2016) 17:631. doi: 10.1186/s12864-016-2852-6

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Afferni, Buccione, Andreone, Galdiero, Varricchi, Marone, Mattei and Schiavoni. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 15 November 2018
doi: 10.3389/fimmu.2018.02663

Interleukin-33 in Systemic Sclerosis: Expression and Pathogenesis

Liya Li[1,2], *Honglin Zhu*[1,2*] *and Xiaoxia Zuo*[1,2*]

[1] Department of Rheumatology and immunology, Xiangya Hospital, Central South University, Changsha, China, [2] The Institute of Rheumatology and Immunology, Central South University, Changsha, China

Interleukin-33 (IL-33), a member of the IL-1 superfamily, functions as a traditional cytokine and nuclear factor. It is proposed to have an "alarmin" role. IL-33 mediates biological effects by interacting with the ST2 receptor and IL-1 receptor accessory protein, particularly in innate immune cells and T helper 2 cells. Recent articles have described IL-33 as an emerging pro-fibrotic cytokine in the immune system as well as a novel potential target for systemic sclerosis. Here, we review the available information and focus on the pleiotropic expression and pathogenesis of IL-33 in systemic sclerosis, as well as the feasibility of using IL-33 in clinical applications.

Keywords: interleukin-33, ST2, systemic sclerosis, pathogenesis, fibrosis

OPEN ACCESS

Edited by:
Fang-Ping Huang,
University of Hong Kong, Hong Kong

Reviewed by:
Piergiuseppe De Berardinis,
Istituto di Biochimica delle Proteine (IBP), Italy
Angela Bonura,
Consiglio Nazionale Delle Ricerche (CNR), Italy

***Correspondence:**
Honglin Zhu
honglinzhu@csu.edu.cn
Xiaoxia Zuo
susanzuo@csu.edu.cn

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *06 January 2018*
Accepted: *29 October 2018*
Published: *15 November 2018*

Citation:
Li L, Zhu H and Zuo X (2018) Interleukin-33 in Systemic Sclerosis: Expression and Pathogenesis. Front. Immunol. 9:2663. doi: 10.3389/fimmu.2018.02663

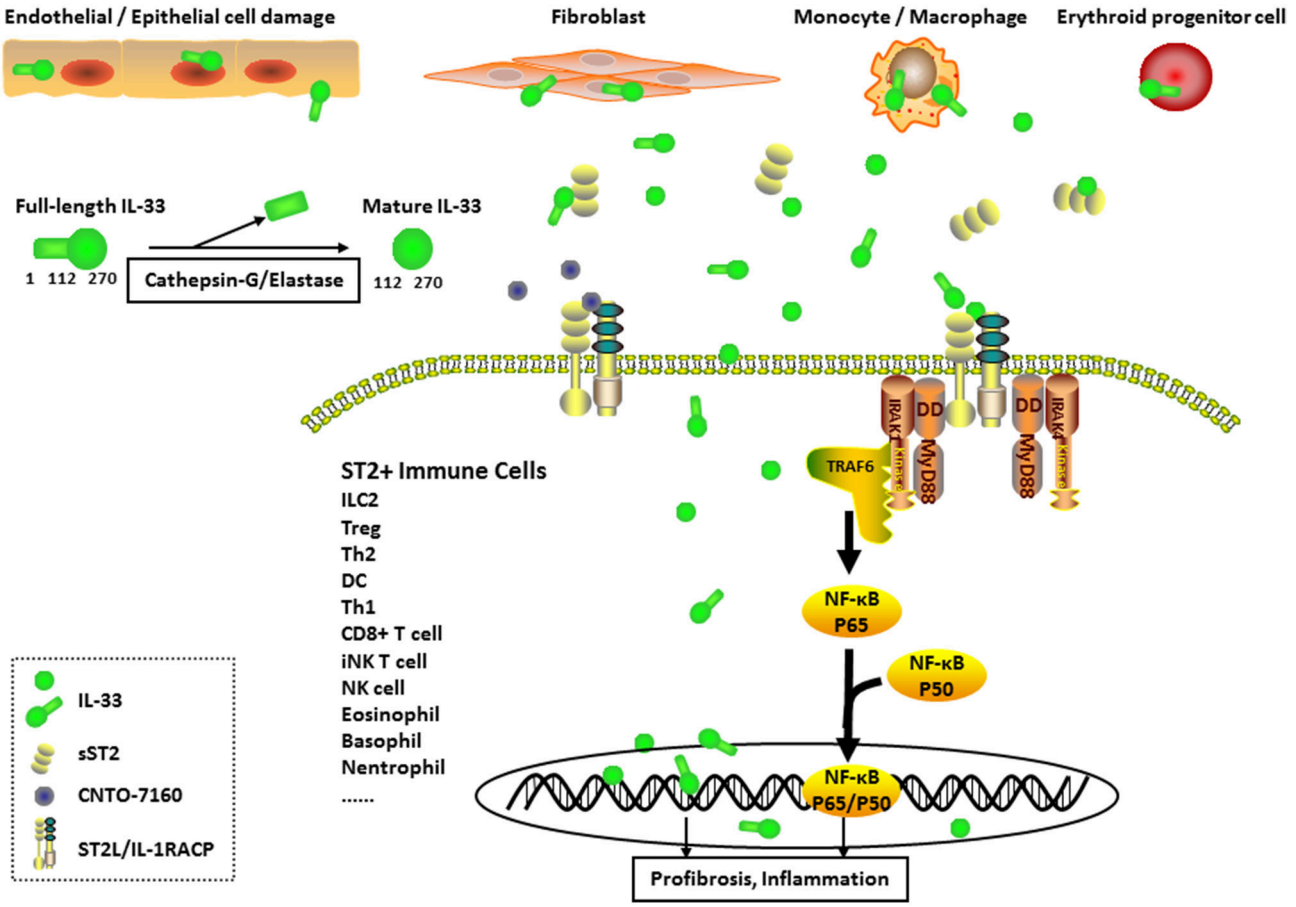
INTRODUCTION

Systemic sclerosis (scleroderma, SSc) is a heterogeneous autoimmune disease of unknown etiology, clinically characterized with obliterative microvasculopathy, inflammation, and extensive fibrosis of the skin and multiple organ systems and serologically characterized by the presence of circulating specific autoantibodies. SSc has the highest cause-specific mortality among connective tissue diseases (1, 2), and pulmonary artery hypertension and interstitial lung disease (ILD) are the leading causes of death (3, 4). Therapeutic interventions for SSc mainly involve the comprehensive administration of glucocorticoids and immunosuppressants and targeted treatment. To date, no effective medical intervention has been developed to control and reverse the progression of this fibrotic disease (5). Thus, effective and safe targeted therapies for SSc-related fibrosis are urgently needed. In the pathogenesis of SSc, endothelial damage may be a primary event. SSc also exhibits complex interactions during the transition from fibroblasts to myofibroblasts and non-infective inflammation or autoimmunity.

Interleukin (IL)-33 belongs to the IL-1 superfamily and is widely expressed throughout the human body. During cell damage or tissue injury, IL-33 is released into the extracellular space, wherein it produces endogenous danger signals to alert adjacent cells. This function deems IL-33 as an alarmin. IL-33 also functions as a nuclear factor regulating gene transcription in cytokine-expressing or cytokine-responsive cells (6). IL-33 is known to play crucial roles in inflammation. However, recent studies indicated that IL-33 participates in the development and progression of fibrotic diseases and SSc. Here, we review the profibrotic roles of IL-33 and its related mechanisms and discuss its potential application in the treatment of SSc.

BIOLOGICAL CHARACTERISTICS OF IL-33

IL-33, also known as IL-1F11, is a member of the IL-1 superfamily (7) and exhibits dual functionality (8). This cytokine was first identified as a nuclear factor in high endothelial venules in 2003 (9) but was renamed as IL-33 when a study in 2005 demonstrated its role as a specific



Graphical Abstract | Expression and Pathogenesis of interleukin (IL)-33 in Systemic sclerosis (SSc). IL-33-producing cells (mainly endothelial cells, epithelial cells, fibroblasts, monocytes, macrophages, and erythroid progenitor cells) secrete IL-33 into the extracellular environment. Full-length IL-33 is transformed into mature IL-33 through cleavage by inflammatory proteases (such as cathepsin-G and elastase). The formation of the IL-33/ST2 longer transmembrane form (ST2L)/IL-1 receptor accessory protein complex in ST2+ immune cell membranes results in the activation of nuclear factor-κB transcription factors through the MyD88, IL-1R-associated kinase (IRAK), and tumor necrosis factor receptor associated factor 6 (TRAF6) signaling pathways, leading to the induction of inflammation and profibrosis in pathological cells. IL-33 also functions as a nuclear factor to regulate gene transcription in cytokine-expressing or cytokine-responsive cells. Moreover, sST2 acts as a decoy receptor for IL-33 (full-length IL-33 or mature IL-33), and CNTO-7160 (the first monoclonal anti-ST2 antibody) was designed as a new IL-33 inhibitor. Both of these molecules block the downstream signaling of IL-33.

extracellular ligand for the orphan IL-1 receptor family member ST2 (also known as IL-1RL1, DER4, T1, and FIT-1). ST2 is a member of the Toll-like receptor (TLR)/IL-1 receptor superfamily (10), which has two main isoforms, namely, a short soluble form (sST2) and longer transmembrane form (ST2L), with four isoforms in total, including ST2V and ST2LV (11). The mRNA encoding sST2 is a secretory sequence that is generated by alternative splicing and lacks the sequence encoding the transmembrane domain of ST2L (12).

Abbreviations: ST2L, ST2 longer transmembrane form; sST2, soluble ST2; ILC2, type 2 innate lymphoid cell; Treg, regulatory T cell; DC, dendritic cell; Th1, T helper 1 cell; CD8+ T cell, CD8-positive T cell; iNK T cell, invariant natural killer T cell; NK cell, natural killer cell; SSc, systemic sclerosis; IRAK, IL-1R-associated kinase; TRAF6, tumor necrosis factor receptor associated factor 6; IL, interleukin; ILD, interstitial lung disease; IL-1RAcP, IL-1 receptor accessory protein; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; IPF, idiopathic pulmonary fibrosis; TGF, transforming growth factor; TLR, Toll-like receptor; IFN, interferon; IRF-7, IFN regulatory factor 7.

The IL-33 gene is located on human chromosome 9 (or chromosome 19 in mice) and is transcribed from seven coding exons. The protein is synthesized as a 31-kDa pro-IL-33_{1--270} (full-length IL-33). Following synthesis, IL-33 is transported into the nucleus as a nuclear factor. Similar to the IL-1 family members IL-1β and IL-18, IL-33 lacks the classic signal sequence necessary for the transport by the endoplasmic reticulum/Golgi secretion pathway (13). Upon natural secretion from pathological cells undergoing necrosis or necroptosis, the full-length IL-33 is cleaved by caspase-3 and caspase-7 to activate apoptotic pathways in the cytoplasm, followed by its release into the extracellular environment (14). Once released into the extracellular matrix, full-length IL-33 is further processed by serine proteases (such as cathepsin-G and elastase) into the 18-kDa IL-$33_{112--270}$ (mature IL-33) with increased activity (15, 16), forming a soluble recombinant cytokine in circulation. However, both full-length and mature IL-33 bind to ST2L in $ST2^{+}$ immune cell membranes

and interact with IL-1 receptor accessory protein (IL-1RAcP), eventually leading to the formation of an IL-33/ST2L/IL-1RAcP complex. This complex induces signaling pathways through MyD88, IL-1R-associated kinase (IRAK), and tumor necrosis factor receptor associated factor 6 (TRAF6) and activates the canonical nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways (17).

IL-33 is principally produced by stromal cells, including epithelial cells, endothelial cells, fibroblast-like cells, and myofibroblasts of lymphoid as well as non-lymphoid organs, under both steady state and inflammation conditions (18–20). In erythroid progenitor cells, IL-33 is produced during the maturation of red blood cells and released upon haemolysis (21). Innate immune cells expressing ST2 mainly include dendritic cells (DCs), natural killer (NK) cells, eosinophils, basophils, macrophages, and neutrophils (12). Full-length IL-33 predominantly remains inside the cell and regulates the expression of genes, which induce pulmonary inflammation and fibrosis. In contrast, mature IL-33 promotes asthma as well as allergic and anti-parasitic responses through the ST2 receptor and Th2 mechanisms (22).

Mechanisms such as inactivation by oxidation of cysteine residues, nuclear localization or sequestration, and proteolytic processing, and receptor antagonists as well as sST2 have evolved to regulate the expression and activities of IL-33 (12, 14, 15, 23, 24). sST2 is constitutively expressed in the human serum, wherein it acts as a decoy receptor for IL-33 and is not involved in signaling (25, 26). sST2, induced during tissue damage, may restrict the deleterious effects of increased IL-33 level. A novel mechanism for the rapid inactivation of IL-33 protein released from the cell *in vivo* was reported, wherein an oxidation-driven conformational change involving the formation of two disulphide bonds was observed, resulting in the elimination of ST2-dependent activity and reduction of inflammation, consistent with the mechanism of many other IL-1 family members (23).

EXPRESSION OF IL-33 AND ST2 IN SSC

According to recent studies, increased IL-33 and sST2 levels have been observed in patients with infections, cardiovascular disorders, allergic diseases, and rheumatic diseases such as systemic lupus erythematosus, rheumatoid arthritis (RA), Wegener's granulomatosis, and Behcet's disease (27–31). The serum levels of sST2 and synovial fluid of IL-33 were higher in patients with RA than in healthy controls and patients with osteoarthritis (27). Furthermore, serum sST2 levels were higher in patients with active, newly diagnosed, anti-neutrophil cytoplasmic antibody-associated vasculitis than in patients in remission, indicative of the marker role for sST2 (32). Furthermore, ST2 and IL-33 were highly expressed around ectopic germinal centers in salivary glands from patients with IgG4-related disease, whereas IL-33 was expressed only in epithelial cells in patients with Sjögren's syndrome and controls (33). Interestingly, the exposure of mice *in vivo* or human skin samples *ex vivo* to inflammatory doses of ultraviolet B irradiation induced IL-33 expression within the epidermal and dermal skin layers (34). Proteomic analysis used to determine the extracellular and intracellular roles of IL-33 in primary human endothelial cells revealed the induction of inflammation-related protein expression of the exogenous extracellular IL-33, whereas the knockdown of the endogenous nuclear IL-33 expression had no reproducible effect on the endothelial cell proteome (35).

The results described above support that the expression level and biological role of IL-33 are similar to those of ST2. In general, IL-33 expression is upregulated in inflamed tissues following pro-inflammatory stimulation, and the role of IL-33 in cells may vary under different pathophysiological conditions. In SSc, with an exception during tissue inflammation, the authors proposed that IL-33 commonly responds to tissue injury and typically affects rapid tissue repair and regeneration (36–38).

In patients with SSc, serum levels of IL-33 and sST2 were elevated (39) and positively correlated with the extent of skin sclerosis (higher in diffused cutaneous SSc than in limited cutaneous SSc), severity of pulmonary interstitial fibrosis, and vascular involvement in SSc development (40–44). In the lesion skin tissues, IL-33 expression is altered depending on the disease stage. IL-33 is downregulated in most endothelial cells in early SSc but not in late SSc (45). IL-33 produced by activated dermal fibroblasts/myofibroblasts has been implicated in the fibrotic pathology associated with SSc, which is profoundly increased by hypertrophic and mechanical stress (46, 47).

The expression of IL-33 mRNA is reported to increase in the primary pulmonary fibroblasts from patients with SSc-ILD as well as in those from patients with idiopathic pulmonary fibrosis (IPF). The elevated levels of IL-33 in bronchoalveolar lavage fluids may be useful in differentiating IPF from other chronic ILDs (48). In patients with IPF and SSc-ILD, the expression of full-length IL-33 was elevated in the affected lungs, consistent with the observation reported in a bleomycin-induced mouse model. Under the conditions of ST2 gene deficiency, the full-length IL-33 could stimulate the expression of several non-Th2 cytokines and heat shock protein 70. On the other hand, the matured form of IL-33 was unaffected and instead activated Th2 responses (49). In contrast, the expression of the matured form of IL-33 was enhanced but that of the full-length counterpart reduced in the macrophages of bleomycin-induced mouse lung tissues (50). These findings suggest that the full-length IL-33 may serve as a synergistic pro-inflammatory and pro-fibrotic regulator in the lungs.

PATHOGENESIS OF IL-33/ST2 IN SSC

Fibrosis, a prominent pathological characteristic of SSc (38), is characterized with a deregulated and uncontrolled repair process. Many molecular and signaling pathways involved in the fibrosis of SSc (51, 52), including transforming growth factor (TGF)-β, TLR4, and interferons (IFNs), are well-studied. TGF-β is responsible for both physiological and pathological matrix remodeling (53) as well as fibroblast-myofibroblast transformation (54). TLR4 induces pro-fibrotic responses by activating NF-κB signaling through MyD88, IRAK,

and TRAF6. The TLR/NF-κB signaling pathways enhance the TGF-β-dependent fibrotic process (55, 56). IFNs generally act as negative regulators of collagen synthesis and TGF-β-mediated fibrotic responses, while the mechanism of type I IFN signaling in SSc-promoted fibrosis remains unclear (37, 57). The role of IL-33 in SSc was recently evaluated. In pediatric patients with limited cutaneous SSc, high levels of IL-33 and IFN-γ positively correlated with anti-histone and anti-ssDNA antibodies, indicating that the co-expression of IL-33 and IFN-γ may contribute to the pathogenesis of SSc (58). Subcutaneous injection of IL-33 in mice resulted in the development of cutaneous fibrosis, similar to that observed in patients with SSc, including dermal mast cells and skin-infiltrating neutrophils through the suppression of Th1-mediated contact hypersensitivity responses (59). This observation highlights the important roles of IL-33 in SSc. However, the exact mechanisms require further investigation.

Known as a master regulator of pathological fibrosis, TGF-β may be produced by IL-33-induced cells. During the amplification of the alternatively activated M2 macrophage polarization, the IL-33/ST2 pathway was shown to play a significant role (60). IL-33 polarized M2 macrophages to produce IL-13 and TGF-β1 and induced the expansion of type 2 innate lymphoid cells (ILC2s) for the production of IL-13 *in vitro* and *in vivo*. ST2 may protect ILC2s from IL-33 stimulation by reducing the production of IL-5 and IL-13 (61). IL-13 is a well-known profibrotic cytokine downstream of IL-33 in the immune system (51).

IFN-γ may play regulatory roles in physiological processes involving IL-33. In type 2 immune responses, IL-33 and ILC2s are central mediators that promote tissue and metabolic homeostasis, whereas IFN-γ suppresses this pathway and promotes inflammatory responses (62). *In vivo*, the co-expression of IL-33 and IFN-γ in pulmonary fibroblast culture and lungs resulted in the attenuation of IL-33 protein levels (63). IFN-regulated genes may regulate IL-33 gene expression. In both human monocytes and macrophages from C57BL/6 mice, transcriptional activation of the IL-33 gene stimulated by the acute-phase protein serum amyloid A, a TLR2 ligand, may be regulated by IFN regulatory factor 7 (IRF-7) recruited to the IL-33 promoter. Silencing of IRF-7 expression may result in the abrogation of the expression of IL-33 induced by serum amyloid A (64).

In fibrosis, DCs elevated the expression of IL-33 via TLR/NF-κB signaling pathways in response to allergic inflammation, resulting in an increase in the expression levels of MyD88, NF-κB1, NF-κB2, and RelA accompanied with NF-κB p65 nuclear translocation, possibly through a potential autocrine regulation. These elevations may be blocked with a TLR5 antibody or NF-κB inhibitor quinazoline and thought to be decreased in DCs from MyD88-knockout mice (65). The deficiency in the NF-κB negative feedback regulator A20 in hyperactive mast cells may result in amplified pro-inflammatory responses downstream of IgE/FcεRI, TLRs, IL-1R, and IL-33R (ST2), thereby exacerbating inflammatory disorders (66). In addition, Th2-stimulated (allergen-specific IgG immune complexes and house dust mites) signaling occurs through FcRγ-associated receptors on DCs to upregulate IL-33 production and induce Th2-mediated allergic airway inflammation (67).

In conclusion, IL-33 functions as a pro-fibrogenic cytokine in the development of SSc. IL-33 may enhance the TGF-β-dependent fibrotic process by increasing the production of TGF-β and activate TLR/NF-κB-dependent fibrosis signaling pathways, which are regulated by IFN-γ (**Table 1**).

To determine whether IL-33 is a useful therapeutic target, Locksley et al. described the complexity of using IL-33 and therapeutic strategies for altering IL-33 activities *in vivo* (68). The framework of IL-33 biology was described as a stepwise process. First, the focal cellular necrosis or other signals induce the release of IL-33 from the nucleus to maintain homeostasis; IL-33 acts on tissue-resident ST2-expressing effector cells such as ILC2s, regulatory T cells (Tregs), and mast cells to create a tissue environment that limits inflammation and promotes a reparative state characterized by tolerance. Second, amplification occurs upon exposure to chronic stimuli such as allergens and repetitive tissue damage, wherein excess extracellular IL-33 leads to multiple self-stimulating cycles of release to promote chronic allergic pathology, fibrosis, and excess stores of IL-33 in the circulation and tissues. The third step is conversion, wherein the activated inflammatory cells and cytokines responsive to the IL-33/ST2 axis play various roles such as killing pathogens, mounting anticancer immune responses, increasing tissue damage, and repressing the type 2-associated immune regulation responses. In patients with SSc, repetitive tissue damage by other pro-fibrotic mediators in fibroblasts and endothelial cells likely suppresses the IL-33 pool increases and regulatory mechanisms. Next, inflammation is amplified, fibrosis occurs, and tissue IL-33 levels increase, ultimately contributing to tissue fibrosis and sclerosis.

Therefore, IL-33 from different sources can be up- or downregulated to exert pleiotropic roles in SSc. Zhao et al. proposed that these apparently contradictory results indicate the presence of an extremely complex process of IL-33 processing and secretion (69). The functional properties of recombinant IL-33 used in previous studies are becoming well-characterized, whereas the cellular sources of IL-33 in natural and stimulated expression remain largely unknown. Additional studies are warranted to explain the differences between *in vitro* and *in vivo* results.

CLINICAL APPLICATIONS OF IL-33 IN SSC

Various aspects of the clinical applications of IL-33 have been examined. However, few studies have evaluated these effects in patients with SSc. Thus, information may be obtained from studies of other diseases that may be applicable to SSc.

IL-33-responsive ILC2s may promote the restoration of injured skin, lung, and gut cells (70). During the regeneration of injured muscles, fibro-adipogenic progenitor cells are the only known source of IL-33 in muscles. The low level of IL-33 expression in older, injured muscle reduces the recruitment and proliferation effects of non-increased muscle-resident Tregs; after the administration of IL-33, the Treg population increases and

TABLE 1 | Targets/pathways involved in IL-33-dependent fibrosis process.

Targets/Pathways	Effector cells	Mediators	Role of IL-33	References
TGF-β	M2 macrophages and ILC2s	IL-13, IL-5	IL-33 induced cells to produce TGF-β	(61)
IFN-γ	ILC2s, pulmonary fibroblast, and lungs		IL-33 was inhibited by IFN-γ	(62, 63)
	Monocytes and macrophages	IRF-7	IRF-7 promoted the expression of IL-33	(64)
TLR/NF-κB signaling pathways	Dendritic cells	MyD88, NF-κB1, NF-κB2, and RelA	IL-33 or ST2 was regulated by TLR/NF-κB signaling pathways	(65, 67)
	Mast cells	NF-κB negative feedback regulator A20		(66)

TGF-β, transforming growth factor-β; ILC2s, type-2 innate lymphoid cells; IFN, interferon; IRF-7, IFN regulatory factor 7; TLR, Toll-like receptor; NF-κB, nuclear factor-κB; IL, interleukin.

regeneration is enhanced (71, 72). Furthermore, the upstream and downstream regulation of the IL-33 gene may promote the remodeling of tissues such as nerves and tendons (73, 74).

In general, studies of IL-33 in patients with SSc have indicated that IL-33 is a novel and important pro-fibrogenic cytokine and a potential biomarker for monitoring disease activity (40–45). Genetic polymorphisms in the IL-33 gene may be useful for the prediction of the risk of various diseases. The IL-33 rs7044343 CC genotype was suggested to be associated with an increased risk of developing SSc and a decreased risk of developing RA; the T allele may be a susceptibility marker for premature coronary artery disease and central obesity and possibly involved in the regulation of IL-33 production (53, 75–77). The first monoclonal anti-ST2 antibody, CNTO-7160, was recently designed as a new IL-33 inhibitor; this antibody is being evaluated in phase I clinical trials for the treatment of severe asthma and atopic dermatitis, but no data have been published to date (78).

PROSPECTS

The alarmin IL-33 has dual functions of a cytokine and nuclear factor. However, differences in the levels of IL-33 and systemic sST2 indicate intra-individual and inter-individual biological variation, reference changes, and sex-specific differences (79). Moreover, the evaluation of the circulating concentrations of sST2, full-length IL-33, mature IL-33, and complexes of sST2 and IL-33 in the same patients is interesting; measurement of these four analytes and their ratios may increase the understanding of IL-33-related pathophysiology in various diseases (80).

Recent investigations suggested that IL-33 is a novel pro-fibrogenic cytokine in the development of SSc, mainly because it affects the TLR/NF-κB signaling pathways, and TGF-β1 expression is also regulated by IFN-γ. These effects are crucial for the early diagnosis of pulmonary fibrosis. Whether IL-33 is involved in fibroblast activation alone or in combination with other factors is unclear; however, this molecule is likely a potential biomarker and novel therapy target for managing fibrosis in patients with SSc. Furthermore, the inhibitor of IL-33 (CNTO-7160), currently being examined in clinical trials, may possibly be developed as a new therapy for fibrosis in patients with SSc (78).

AUTHOR CONTRIBUTIONS

LL devised and wrote the manuscript. HZ and XZ revised the manuscript.

FUNDING

This work was supported by The National Key Research and Development Program of China (2016YFC0903900), grants from National Natural Science Foundation of China (81671622, 81701621) and Independent Innovation Projects of Central South University (2018zzst290).

REFERENCES

1. Li L, Zuo X, Luo H, Li Y, You Y, Duan L, et al. Mortality trend of inpatients with connective tissue diseases: 2005-2014. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* (2017) 42:927–33. doi: 10.11817/j.issn.1672-7347.2017.08.009
2. Elhai M, Meune C, Avouac J, Kahan A, Allanore Y. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. *Rheumatology* (2012) 51:1017–26. doi: 10.1093/rheumatology/ker269
3. Elhai M, Avouac J, Kahan A, Allanore Y. Systemic sclerosis: recent insights. *Joint Bone Spine* (2015) 82:148–53. doi: 10.1016/j.jbspin.2014.10.010
4. Tyndall A J, Bannert B, Vonk M, Airo P, Cozzi F, Carreira PE, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis.* (2010) 69:1809–15. doi: 10.1136/ard.2009.114264
5. Allanore Y, Distler O. Systemic sclerosis in 2014: advances in cohort enrichment shape future of trial design. *Nat Rev Rheumatol.* (2015) 11:72–4. doi: 10.1038/nrrheum.2014.222
6. Bertheloot D, Latz E. HMGB1, IL-1alpha, IL-33 and S100 proteins: dual-function alarmins. *Cell Mol Immunol.* (2017) 14:43–64. doi: 10.1038/cmi.2016.34
7. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity* (2013) 39:1003–18. doi: 10.1016/j.immuni.2013.11.010
8. Luheshi NM, Rothwell NJ, Brough D. Dual functionality of interleukin-1 family cytokines: implications for anti-interleukin-1 therapy. *Br J Pharmacol.* (2009) 157:1318–29. doi: 10.1111/j.1476-5381.2009.00331.x

9. Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol.* (2003) 163:69–79. doi: 10.1016/S0002-9440(10)63631-0
10. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. *Immunol Rev.* (2008) 226:10–8. doi: 10.1111/j.1600-065X.2008.00701.x
11. Yanagisawa K, Takagi T, Tsukamoto T, Tetsuka T, Tominaga S. Presence of a novel primary response gene ST2L, encoding a product highly similar to the interleukin 1 receptor type 1. *FEBS Lett.* (1993) 318:83–7.
12. Cayrol C, Girard JP. Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. *Immunol Rev.* (2018) 281:154–68. doi: 10.1111/imr.12619
13. Arend WP, Palmer G, Gabay, C. IL-1, IL-18, and IL-33 families of cytokines. *Immunol Rev.* (2008) 223:20–38. doi: 10.1111/j.1600-065X.2008.00624.x
14. Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* (2009) 31:84–98. doi: 10.1016/j.immuni.2009.05.007
15. Lefrancais E, Roga S, Gautier VA, Gonzalez-de-Peredo, Monsarrat B, Girard JP, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc Natl Acad Sci USA.* (2012) 109:1673–8. doi: 10.1073/pnas.1115884109
16. Lefrancais E, Cayrol C. Mechanisms of IL-33 processing and secretion: differences and similarities between IL-1 family members. *Eur Cytokine Netw.* (2012) 23:120–7. doi: 10.1684/ecn.2012.0320
17. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
18. Moussion C, Ortega N, Girard JP. 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:e3331. doi: 10.1371/journal.pone.0003331
19. Kuchler AM, Pollheimer J, Balogh J, Sponheim J, Manley L, Sorensen DR, et al. Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation. *Am J Pathol.* (2008) 173:1229–42. doi: 10.2353/ajpath.2008.080014
20. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N. 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) 188:3488–95. doi: 10.4049/jimmunol.1101977
21. Wei J, Zhao J, Schrott V, Zhang Y, Gladwin M, Bullock G. et al. Red blood cells store and release interleukin-33. *J Investig Med.* (2015) 63:806–10. doi: 10.1097/JIM.0000000000000213
22. Luzina IG, Kopach P, Lockatell V, Kang PH, Nagarsekar A, Burke AP, et al. Interleukin-33 potentiates bleomycin-induced lung injury. *Am J Respir Cell Mol Biol.* (2013) 49:999–1008. doi: 10.1165/rcmb.2013-0093OC
23. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, et al. Sleeman and T. mustelin: oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun.* (2015) 6:8327. doi: 10.1038/ncomms9327
24. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA.* (2009) 106:9021–6. doi: 10.1073/pnas.0812690106
25. Ohto-Ozaki H, Kuroiwa K, Mato N, Matsuyama Y, Hayakawa M, Tamemoto H. et al. Characterization of ST2 transgenic mice with resistance to IL-33. *Eur J Immunol.* (2010) 40:2632–42. doi: 10.1002/eji.200940291
26. Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S, et al. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med.* (2001) 164:277–81. doi: 10.1164/ajrccm.164.2.2008120
27. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol.* (2010) 10:103–10. doi: 10.1038/nri2692
28. Kageyama Y, Torikai E, Tsujimura K, Kobayashi M. Involvement of IL-33 in the pathogenesis of rheumatoid arthritis: the effect of etanercept on the serum levels of IL-33. *Mod Rheumatol.* (2012) 22:89–93. doi: 10.1007/s10165-011-0480-1
29. Hong YS, Moon SJ, Joo YB, Jeon CH, Cho ML, Ju JH, et al. Measurement of interleukin-33 (IL-33) and IL-33 receptors (sST2 and ST2L) in patients with rheumatoid arthritis. *J Korean Med Sci.* (2011) 26:1132–9. doi: 10.3346/jkms.2011.26.9.1132
30. Kuroiwa K, Arai T, Okazaki H, Minota S, Tominaga S. Identification of human ST2 protein in the sera of patients with autoimmune diseases. *Biochem Biophys Res Commun.* (2001) 284:1104–8. doi: 10.1006/bbrc.2001.5090
31. Wang S, Ding L, Liu SS, Wang C, Leng RX, Chen GM, et al. IL-33: a potential therapeutic target in autoimmune diseases. *J Investig Med.* (2012) 60:1151–6. doi: 10.2310/JIM.0b013e31826d8fcb
32. Hladinova Z, Hruskova Z, Svobodova B, Malickova K, Lanska V, Konopasek P, et al. Increased levels of soluble ST2 in patients with active newly diagnosed ANCA-associated vasculitis. *Mediators Inflamm.* (2015) 2015:603750. doi: 10.1155/2015/603750
33. Furukawa S, Moriyama M, Miyake K, Nakashima H, Tanaka A, Maehara T, et al. Interleukin-33 produced by M2 macrophages and other immune cells contributes to Th2 immune reaction of IgG4-related disease. *Sci Rep.* (2017) 7:42413. doi: 10.1038/srep42413
34. Byrne SN, Beaugie C, O'Sullivan C, Leighton S, Halliday GM. The immune-modulating cytokine and endogenous Alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation. *Am J Pathol.* (2011) 179:211–22. doi: 10.1016/j.ajpath.2011.03.010
35. Gautier V, Cayrol C, Farache D, Roga S, Monsarrat B, Burlet-Schiltz O, et al. Extracellular IL-33 cytokine, but not endogenous nuclear IL-33, regulates protein expression in endothelial cells. *Sci Rep.* (2016) 6:34255. doi: 10.1038/srep34255
36. Pei C, Barbour M, Fairlie-Clarke KJ, Allan D, Mu R, Jiang, HR. Emerging role of interleukin-33 in autoimmune diseases. *Immunology* (2014) 141:9–17. doi: 10.1111/imm.12174
37. Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. *Nat Rev Rheumatol.* (2011) 8:42–54. doi: 10.1038/nrrheum.2011.149
38. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest.* (2007) 117:557–67. doi: 10.1172/JCI31139
39. Zhang YJ, Zhang Q, Yang GJ, Tao JH, Wu GC, Huang XL, et al. Elevated serum levels of interleukin-1beta and interleukin-33 in patients with systemic sclerosis in Chinese population. *Z Rheumatol.* (2016) 77:151–9. doi: 10.1007/s00393-016-0202-3
40. Terras S, Opitz E, Moritz R K, Hoxtermann S, Gambichler T, Kreuter A. Increased serum IL-33 levels may indicate vascular involvement in systemic sclerosis. *Ann Rheum Dis.* (2013) 72:144–5. doi: 10.1136/annrheumdis-2012-201553
41. Yanaba K, Yoshizaki A, Asano Y, Kadono T, Sato S. Serum IL-33 levels are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *Clin Rheumatol.* (2011) 30:825–30. doi: 10.1007/s10067-011-1686-5
42. Manetti M, Guiducci S, Ceccarelli C, Romano E, Bellando-Randone S, Conforti ML, et al. Increased circulating levels of interleukin 33 in systemic sclerosis correlate with early disease stage and microvascular involvement. *Ann Rheum Dis.* (2011) 70:1876–8. doi: 10.1136/ard.2010.148247
43. Wagner A, Kohm M, Nordin A, Svenungsson E, Pfeilschifter JM, Radeke HH. Increased serum levels of the IL-33 neutralizing sST2 in limited cutaneous systemic sclerosis. *Scand J Immunol.* (2015) 82:269–74. doi: 10.1111/sji.12317
44. Mostmans Y, Cutolo M, Giddelo C, Decuman S, Melsens K, Declercq H, et al. The role of endothelial cells in the vasculopathy of systemic sclerosis: a systematic review. *Autoimmun Rev.* (2017) 16:774–86. doi: 10.1016/j.autrev.2017.05.024
45. Vettori S, Cuomo G, Iudici M, D'Abrosca V, Giacco V, Barra G, et al. Early systemic sclerosis: serum profiling of factors involved in endothelial, T-cell, and fibroblast interplay is marked by elevated interleukin-33 levels. *J Clin Immunol.* (2014) 34:663–8. doi: 10.1007/s10875-014-0037-0
46. Manetti M, Ibba-Manneschi L, Liakouli V, Guiducci S, Milia AF, Benelli G, 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) 69:598–605. doi: 10.1136/ard.2009.119321
47. Kakkar R, Hei H, Dobner S, Lee RT. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J Biol Chem.* (2012) 287:6941–8. doi: 10.1074/jbc.M111.298703

48. Lee JU, Chang HS, Lee HJ, Jung CA, Bae DJ, Song HJ, et al. Upregulation of interleukin-33 and thymic stromal lymphopoietin levels in the lungs of idiopathic pulmonary fibrosis. *BMC Pulm Med.* (2017) 17:39. doi: 10.1186/s12890-017-0380-z
49. Luzina IG, Pickering EM, Kopach P, Kang PH, Lockatell V, Todd NW, et al. Full-length IL-33 promotes inflammation but not Th2 response *in vivo* in an ST2-independent fashion. *J Immunol.* (2012) 189:403–10. doi: 10.4049/jimmunol.1200259
50. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, et al. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J Allergy Clin Immunol.* (2014) 134:1422–32. e11doi: 10.1016/j.jaci.2014.05.011
51. Greenblatt MB, Aliprantis AO. The immune pathogenesis of scleroderma: context is everything. *Curr Rheumatol Rep.* (2013) 15:297. doi: 10.1007/s11926-012-0297-8
52. Zuo X, Zhang L, Luo H, Li Y, Zhu H. Systematic approach to understanding the pathogenesis of systemic sclerosis. *Clin Genet.* (2017) 92:365–71. doi: 10.1111/cge.12946
53. Koca SS, Pehlivan Y, Kara M, Alibaz-Oner F, Oztuzcu S, Yilmaz N, et al. The IL-33 gene is related to increased susceptibility to systemic sclerosis. *Rheumatol Int.* (2016) 36:579–84. doi: 10.1007/s00296-015-3417-8
54. Varga J, Pasche B. Transforming growth factor beta as a therapeutic target in systemic sclerosis. *Nat Rev Rheumatol.* (2009) 5:200–6. doi: 10.1038/nrrheum.2009.26
55. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med.* (2007) 13:1324–32. doi: 10.1038/nm1663
56. Csak T, Velayudham A, Hritz I, Petrasek J, Levin I, Lippai D, et al. Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice. *Am J Physiol Gastrointest Liver Physiol.* (2011)300:G433–41. doi: 10.1152/ajpgi.00163.2009
57. Kim D, Peck A, Santer D, Patole P, Schwartz SM, Molitor JA, et al. Induction of interferon-alpha by scleroderma sera containing autoantibodies to topoisomerase I: association of higher interferon-alpha activity with lung fibrosis. *Arthritis Rheum.* (2008) 58:2163–73. doi: 10.1002/art.23486
58. Torok KS, Kurzinski K, Kelsey C, Yabes J, Magee K, Vallejo AN, et al. Peripheral blood cytokine and chemokine profiles in juvenile localized scleroderma: T-helper cell-associated cytokine profiles. *Semin Arthritis Rheum.* (2015) 45:284–93. doi: 10.1016/j.semarthrit.2015.06.006
59. Rankin AL, Mumm JB, Murphy E, Turner S, Yu N, McClanahan TK, et al. IL-33 induces IL-13-dependent cutaneous fibrosis. *J Immunol.* (2010) 184:1526–35. doi: 10.4049/jimmunol.0903306
60. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol.* (2009) 183:6469–77. doi: 10.4049/jimmunol.0901575
61. Hayakawa H, Hayakawa M, Tominaga SI. Soluble ST2 suppresses the effect of interleukin-33 on lung type 2 innate lymphoid cells. *Biochem Biophys Rep.* (2016) 5:401–07. doi: 10.1016/j.bbrep.2016.02.002
62. Molofsky AB, Van Gool F, Liang HE, Van Dyken SJ, Nussbaum JC, Lee J, et al. Interleukin-33 and interferon-gamma counter-regulate group 2 innate lymphoid cell activation during immune perturbation. *Immunity* (2015) 43:161–74. doi: 10.1016/j.immuni.2015.05.019
63. Kopach P, Lockatell V, Pickering EM, Haskell RE, Anderson RD, Hasday JD, et al. IFN-gamma directly controls IL-33 protein level through a STAT1- and LMP2-dependent mechanism. *J Biol Chem.* (2014) 289:11829–43. doi: 10.1074/jbc.M113.534396
64. Sun L, Zhu Z, Cheng N, Yan Q, Ye RD. Serum amyloid A induces interleukin-33 expression through an IRF7-dependent pathway. *Eur J Immunol.* (2014) 44:2153–64. doi: 10.1002/eji.201344310
65. Su Z, Lin J, Lu F, Zhang X, Zhang L, Gandhi NB, et al. Potential autocrine regulation of interleukin-33/ST2 signaling of dendritic cells in allergic inflammation. *Mucosal Immunol.* (2013) 6:921–30. doi: 10.1038/mi.2012.130
66. Heger K, Fierens K, Vahl J C, Aszodi A, Peschke K, Schenten D, et al. A20-deficient mast cells exacerbate inflammatory responses *in vivo*. *PLoS Biol.* (2014) 12:e1001762. doi: 10.1371/journal.pbio.1001762
67. Tjota MY, Hrusch CL, Blaine KM, Williams JW, Barrett NA, Sperling AI. Signaling through FcRgamma-associated receptors on dendritic cells drives IL-33-dependent TH2-type responses. *J Allergy Clin Immunol.* (2014) 134:706–13. e8doi: 10.1016/j.jaci.2014.06.013
68. Molofsky AB, Savage AK, Locksley nRM. Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation. *Immunity* (2015) 42:1005–19. doi: 10.1016/j.immuni.2015.06.006
69. Zhao W, Hu Z. The enigmatic processing and secretion of interleukin-33. *Cell Mol Immunol* (2010) 7:260–2. doi: 10.1038/cmi.2010.3
70. Rak GD, Osborne LC, Siracusa MC, Kim BS, Wang K, Bayat A, et al. IL-33-dependent group 2 innate lymphoid cells promote cutaneous wound healing. *J Invest Dermatol.* (2016) 136:487–96. doi: 10.1038/JID.2015.406
71. Castiglioni A, Corna G, Rigamonti E, Basso V, Vezzoli M, Monno A, et al. FOXP3+ T cells recruited to sites of sterile skeletal muscle injury regulate the fate of satellite cells and guide effective tissue regeneration. *PLoS ONE* (2015) 10:e0128094. doi: 10.1371/journal.pone.0128094
72. Kuswanto W, Burzyn D, Panduro M, Wang KK, Jang YC, Wagers AJ, et al. Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells. *Immunity* (2016) 44:355–67. doi: 10.1016/j.immuni.2016.01.009
73. Natarajan C, Yao SY, Sriram S. TLR3 agonist poly-IC induces IL-33 and promotes myelin repair. *PLoS ONE* (2016) 11:e0152163. doi: 10.1371/journal.pone.0152163
74. Millar NL, Gilchrist DS, Akbar M, Reilly JH, Kerr SC, Campbell AL, et al. MicroRNA29a regulates IL-33-mediated tissue remodelling in tendon disease. *Nat Commun.* (2015) 6:6774. doi: 10.1038/ncomms7774
75. Huang XL, Wu GC, Wang YJ, Yang XK, Yang GJ, Tao JH, et al. Association of interleukin-1 family cytokines single nucleotide polymorphisms with susceptibility to systemic sclerosis: an independent case-control study and a meta-analysis. *Immunol Res.* (2016) 64:1041–52. doi: 10.1007/s12026-016-8797-7
76. Li C, Mu R, Guo J, Wu X, Tu X, Liu X, et al. Genetic variant in IL33 is associated with susceptibility to rheumatoid arthritis. *Arthritis Res Ther.* (2014) 16:R105. doi: 10.1186/ar4554
77. Angeles-Martinez J, Posadas-Sanchez R, Llorente L, Alvarez-Leon E, Ramirez-Bello J, Villarreal-Molina T, et al. The rs7044343 polymorphism of the interleukin 33 gene is associated with decreased risk of developing premature coronary artery disease and central obesity, and could be involved in regulating the production of IL-33. *PLoS ONE* (2017) 12:e0168828. doi: 10.1371/journal.pone.0168828
78. Stri I. Cytokines of the IL-1 family: recognized targets in chronic inflammation underrated in organ transplantations. *Clin Sci.* (2017) 131:2241–56. doi: 10.1042/CS20170098
79. Mueller T, Zimmermann M, Dieplinger B, Ankersmit HJ, Haltmayer M. Comparison of plasma concentrations of soluble ST2 measured by three different commercially available assays: the MBL ST2 assay, the Presage ST2 Assay, and the R&D ST2 assay. *Clin. Chim.* (2012) 413:1493–94. doi: 10.1016/j.cca.2012.06.021
80. Mueller T, Dieplinger B. The Presage ® ST2 Assay: analytical considerations and clinical applications for a high-sensitivity assay for measurement of soluble ST2. *Expert Rev Mol Diagn.* (2013) 13:13–30. doi: 10.1586/erm.12.128

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Li, Zhu and Zuo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 15 November 2018
doi: 10.3389/fimmu.2018.02646

IL-33 Prevents MLD-STZ Induction of Diabetes and Attenuate Insulitis in Prediabetic NOD Mice

Sladjana Pavlovic[1*†], *Ivica Petrovic*[1,2†], *Nemanja Jovicic*[1,3†], *Biljana Ljujic*[1,4], *Marina Miletic Kovacevic*[3], *Nebojsa Arsenijevic*[1] *and Miodrag L. Lukic*[1*]

[1] *Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research, University of Kragujevac, Kragujevac, Serbia,* [2] *Department of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia,* [3] *Department of Histology and Embryology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia,* [4] *Department of Genetics, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*

Type 1 diabetes is an autoimmune disease caused by the immune-mediated destruction of pancreatic β-cells. Prevention of type 1 diabetes requires early intervention in the autoimmune process against beta-cells of the pancreatic islets of Langerhans, which is believed to result from disordered immunoregulation. $CD4^+Foxp3^+$ regulatory T cells (Tregs) participate as one of the most important cell types in limiting the autoimmune process. The aim of this study was to investigate the effect of exogenous IL-33 in multiple low dose streptozotocin (MLD-STZ) induced diabetes and to delineate its role in the induction of protective Tregs in an autoimmune attack. C57BL/6 mice were treated i. p. with five doses of 40 mg/kg STZ and 0.4 μg rIL-33 four times, starting from day 0, 6, or 12 every second day from the day of disease induction. 16 weeks old NOD mice were treated with 6 injections of 0.4 μg/mouse IL-33 (every second day). Glycemia and glycosuria were measured and histological parameters in pancreatic islets were evaluated at the end of experiments. Cellular make up of the pancreatic lymph nodes and islets were evaluated by flow cytometry. IL-33 given simultaneously with the application of STZ completely prevented the development of hyperglycemia, glycosuria and profoundly attenuated mononuclear cell infiltration. IL-33 treatment was accompanied by higher number of IL-13 and IL-5 producing $CD4^+$ T cells and increased presence of $ST2^+Foxp3^+$ regulatory T cells in pancreatic lymph nodes and islets. Elimination of Tregs abrogated protective effect of IL-33. We provide evidence that exogenous IL-33 completely prevents the development of T cell mediated inflammation in pancreatic islets and consecutive development of diabetes in C57BL/6 mice by facilitating the induction Treg cells. To extend this finding for possible relevance in spontaneous diabetes, we showed that IL-33 attenuate insulitis in prediabetic NOD mice.

Keywords: IL-33, diabetes, C57BL/6 mice, NOD mice, streptozotocin

OPEN ACCESS

Edited by:
Teizo Yoshimura,
Okayama University, Japan

Reviewed by:
Girdhari Lal,
National Centre for Cell Science (NCCS), India
Toshihiro Ito,
Nara Medical University, Japan

***Correspondence:**
Sladjana Pavlovic
sladjadile@ugmail.com
Miodrag L. Lukic
miodrag.lukic@medf.kg.ac.rs

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *28 May 2018*
Accepted: *26 October 2018*
Published: *15 November 2018*

Citation:
Pavlovic S, Petrovic I, Jovicic N, Ljujic B, Miletic Kovacevic M, Arsenijevic N and Lukic ML (2018) IL-33 Prevents MLD-STZ Induction of Diabetes and Attenuate Insulitis in Prediabetic NOD Mice. Front. Immunol. 9:2646. doi: 10.3389/fimmu.2018.02646

INTRODUCTION

Diabetes mellitus type 1 is a chronic inflammatory disease characterized by the progressive destruction of pancreatic β-cells of Langerhans islets caused by autoimmune processes (1–3). The development of these autoimmune processes is thought to be the result of disorders of immunoregulation (3). This failure allows Th1/Th17 lymphocytes to trigger a cascade of immune/inflammatory processes in the pancretic islets causing β cell destruction. Both the

numerical and functional equilibrium between effector and regulatory T lymphocytes in pancreatic infiltrates determine the extent of destruction of β cells (4–6).

IL-33 is a member of the IL-1 cytokine family (7). Receptor for IL-33 is ST2 (IL-33R) molecule that is constitutively expressed on various cells including subpopulation of Foxp3 regulatory cells Th2 lymphocytes, innate lymphoid cells (ILC2), mast cells, basophiles, and eosinophils (8–10). IL-33/ST2 axis is important in several inflammatory diseases (11, 12).

The idea behind this work stems from our previous data on the role of IL-33/ST2 axis in the models of the two Th1 mediated diseases: MLD-STZ and experimental allergic encephalomyelitis (EAE) (5, 13–15). Deletion of ST2 abrogates resistance to EAE in BALB/C mice by enhancing polarization of antigen presenting cells to inflammatory phenotypes and enhancing IL-17 and IFN-γ production. In MLD-STZ diabetes model we showed that resistance to disease in BALB/C mice depends partially on $CD4^+CD25^+Foxp3^+$ cells (5, 15).

Further, Yuan et al. (16) recently showed that in prediabetic NOD mice a combination of CD122 and IL-33 promotes Tregs abundance and function in pancreatic islets. Finally Ryba-Stanislawowska et al. (17) reported that *in vitro* IL-33 treatment of Tregs derived from patients with type 1 diabetes resulted in quantitative and qualitative enhancement of their suppressive activity.

Siede et al. (18) have reported that IL-33 receptor expressing Treg cells acquire capacity to produce IL-5 and IL-13 and suppress T effectors cells by producing IL-10. Taken together these data suggested that *in vivo* treatment of IL-33 may have beneficial effects in MLD-STZ diabetes by promoting Tregs and in particular $ST2^+$ Tregs producing IL-10 and possibly IL-5 and/or IL-13.

MLD-STZ induced diabetes appears to be an experimental model for studying T cell-dependent inflammatory pathology in the islets (19). We used this model to investigate the immunomodulatory capacity of IL-33 and to delineate the mechanisms influencing effectors immune cell functions. Our study has shown that IL-33 prevents MLD-STZ diabetes induction if given at the time of disease induction. If given 6 and 12 days after the disease induction IL-33 can still significantly attenuate development of hyperglycemia. Finally, in order to show relevance of our findings for the development of "spontaneous" diabetes, we looked at the possibility that exogenous IL-33 alter the onset of insulitis in prediabetic NOD mice. IL-33 treated NOD mice showed significantly lower mononuclear cells infiltration but higher percentage and number of $CD4^+IL\text{-}5^+$, $CD4^+IL\text{-}13^+$, and $CD4^+Foxp3^+$ cells expression in the islets.

This beneficial effect appears to be mainly due to the ability of IL-33 to enhance induction of regulatory $CD4^+Foxp3^+$ $ST2^+$ T cells.

MATERIALS AND METHODS

Experimental Animals

C57BL/6 mice male 8–10 week old, housed under conventional conditions and allowed laboratory chow and water *ad libitum*, were used in the experiments. Within each experiment, animals were matched by age and weight (18–24 g) and randomly divided into groups of 7–8 to receive different treatments. Breeding pairs of NOD mice were purchased from Charles River Laboratories SRL (Calco, Italy) and maintained in specific pathogen free facilities. Female NOD mice at the 16 week of age were used in the experiment. In our animal facilities only 25–35% of NOD mice develop diabetes typically at the 24th week of age. In attempt to standardize the evaluation of IL-33 in these mice we used group of 20 female mice at the age of 16 weeks and 10 of them were treated with IL-33.

All animal procedures were approved by the Ethics Committee for Animal studies of the Faculty of Medical Science, University in Kragujevac.

Diabetes Induction

Diabetes was induced by MLD-STZ, as described earlier (20). Briefly, animals received intraperitoneally (i. p.) 40 mg/kg (b.w.) STZ (Sigma-Aldrich, St Louis, MO) dissolved in citrate buffer on pH 4.5 for 5 consecutive days. The daily dose of STZ is determined by the daily weight measurement of each mouse immediately prior to the injection of the substance.

Diabetes Evaluation

Clinical diabetes was defined by hyperglycaemia (blood glucose levels > 10.3 mmol/l) and glycosuria (urine glucose levels > 7.1 mmol/l or 128 mg/100 ml) in fasted animals. Blood glucose levels and urine glucose were measured three times per week. Samples were taken from the tail tip after starvation for 4 h. Blood glucose levels (mmol/l) were determined using the Accu-Chek Performa glucometer (Roshe Diagnostics, Mannheim, Germany) and urine glucose was analyzed by Uriscan 2 test strip (YD diagnostics, Korea).

Glucose Tolerance Test (GTT)

GTT analyses were performed both in C57BL/6 and NOD mice at the end of experiment. To this end food was withheld 16 h before testing. Animals were weighed and injected with 2 g/kg of glucose (i.p.). Glucose concentrations were measured before and at 0, 15, 30, 60, and 120 min after glucose injection.

IL-33 Application

C57BL/6 mice received exogenous mouse IL-33 (0.4 μg/injection; eBioscience) intraperitoneally, according to the following scheme: I group: days 0, 2, 4, and 6; II group: days 6, 8, 10, and 12; III group: days 12, 14, 16, and 18. Control animals were treated with intraperitoneally PBS + citrate buffer (CB) solution or IL-33 + citrate buffer solution at the same time interval.

The effects of IL-33 on development of periinsulitis and insulitis in prediabetic NOD mice were evaluated. Sixteen weeks old animals that were all normoglycemic, free of glycosuria and with normal GTT test were treated with 6 injections of 0.4 μg/mouse IL-33 (every second day) and sacrificed for histological analysis at 18th week of age.

Cyclophosphamide (CY) Administration

CY was administered at a dose 200 mg/kg of body weight twice on 5 and 7th day of the treatment. The first CY injection was given ∼8 h after the last administration of STZ as a precaution against a possible interaction of CY with STZ (21).

Histological Examination of Pancreata

Pancreata of all groups were excised and placed in 10% buffered formaldehyde fixative solution overnight at room temperature. Pancreatic tissue paraffin sections were stained with hematoxylin-eosin were used for the analysis of lymphocytic infiltrates in the Langerhans pancreatic islets by light microscope (BX51; Olympus, Japan) using a magnification lens of 40 × (14). Histological analysis of the distribution of inflammatory cell infiltrate in pancreatic islets was performed in blinded fashion by two independent observers (MM and SP). Insulitis was graded and a mean insulitis score was calculated as described previously (22).

Isolation of Lymphoid Cells for Phenotypic Assessment

Pancreatic draining lymph nodes were removed, and single-cell suspensions were obtained by mechanical disruption. After removing pancreatic lymph nodes, pancreas was processed through three steps: *in situ* perfusion with collagenase, pancreatic digestion, and isolation of the islet. The cells were separated according to the protocol as describe elsewhere (23) and analyzed by flow cytofluorimetry. Data was shown as percentage of mononuclear cells and absolute number of cells per islets from one pancreas.

Flow Cytometric Analysis

Cells suspensions were prepared from lymph nodes and pancreatic islets. Single-cell suspensions were labeled with fluorochrome-conjugated monoclonal antibodies: anti-mouse CD3, CD4, CD8, ST2, and CXCR3 (BD Biosciences), CD11c and CD11b antibodies (BioLegend, San Diego, CA) or with isotype-matched control and analyzed on a FACSCalibur (BD) using CELLQUEST software (BD). The intracellular staining was performed with lymph node cells incubated for 6 h in the presence of Phorbol 12-myristate13-acetate (50 ng/ml) (Sigma, USA), Ionomycin (Sigma, USA) (500 ng/ml), and GolgyStop (BD Pharmingen) at 37°C, 5% CO2, stained with anti-CD4 monoclonal antibodies or appropriate isotype controls, fixed and permeabilized with a Cytofix/Cytoperm solution. Intracellular staining was performed using monoclonal antibodies: IFN-γ, IL-17, IL-10, IL-5, IL-13, IL-2, and Foxp3 (BD Biosciences) or appropriate negative controls. Cells were analyzed with the FACSCalibur Flow Cytometer (BD Biosciences), and analysis was conducted with FlowJo (Tree Star).

Statistical Analysis

All variables were continuous and values were described by the means ± SEM. In order to determine differences in the mean values of continuous variables with a normal distribution of values, parametric Student's *t*-test was used, and its non-parametric alternative Mann-Whitney test if data did not follow a normal distribution. All data were analyzed using the statistical program SPSS version 20 (SPSS Inc., Chicago, IL) where p-value < 0.05 was considered statistically significant.

RESULTS

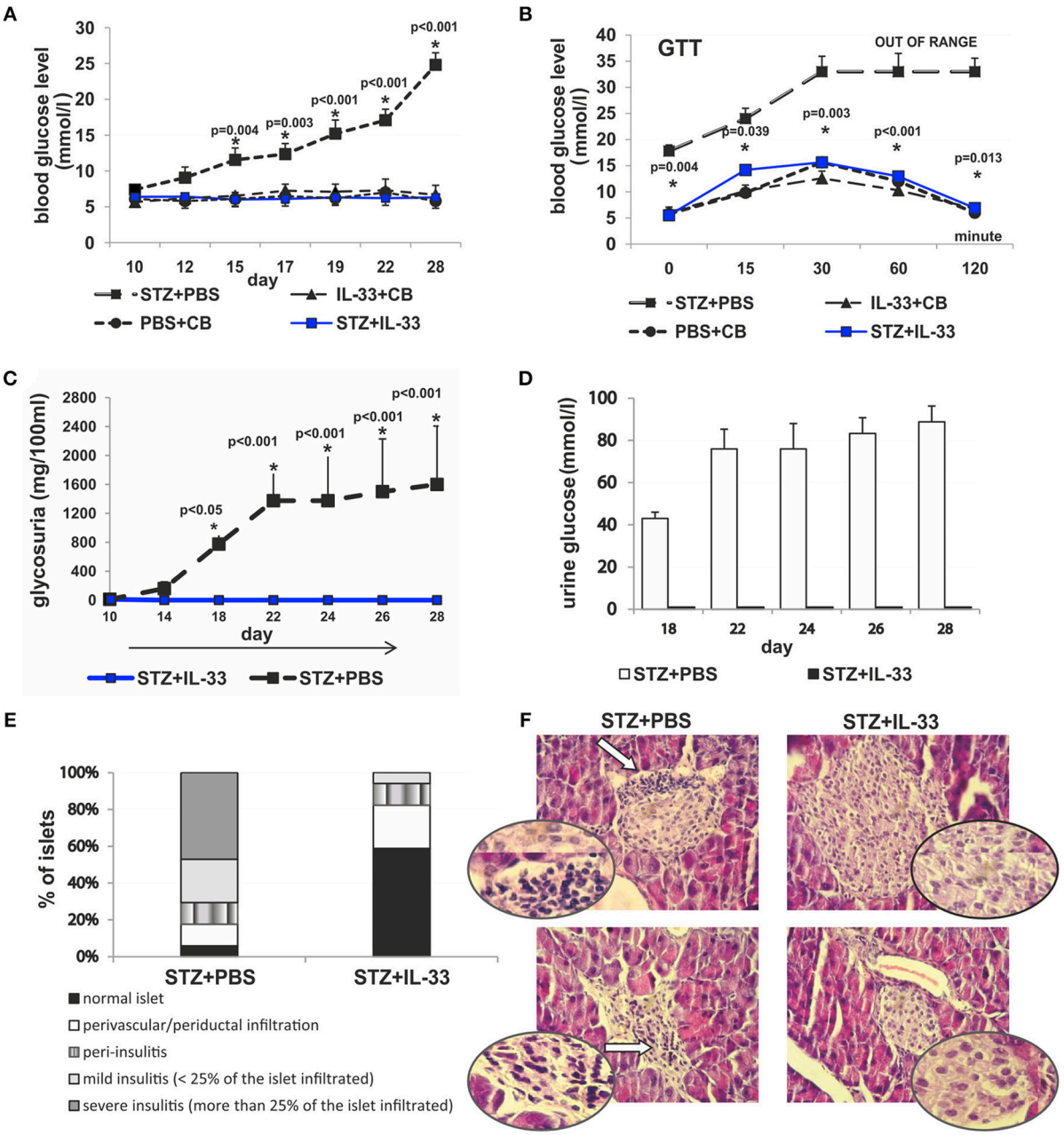
IL-33 Treatment Prevents Diabetes Induction in C57BL/6 Mice as Evaluated by Glycemia, Glucose Tolerance Test, Glycosuria, and Islet Infiltration

Present study was undertaken to analyze the effect of exogenous IL-33 on the onset and the development of type 1 diabetes as evaluated by glycemia, glucose tolerance test, glycosuria, and islet infiltration. C57BL/6 mice were injected with four injections of IL-33 or PBS starting from the day 0 as described in material and methods section (I group). As shown in **Figure 1**, exogenous IL-33 showed strong suppressive effects on diabetes induction and no biochemical parameters of the disease onset were noticed. Significant difference in values of glycemia was observed from the day 15 and remained until the end of the experiment (**Figure 1A**).

We examined blood sugar values of the experimental mice after glucose loading using a glucose tolerance test. All measurements were conducted within 120 min in line with the schedule: 0, 15, 30, 60, and 120. Assessing the differences between the group that received IL-33 from the day 0 and the control group, we found significant differences in all five time points. Significances (p-values) according to the schedule of measurements were as follows: 0.004, 0.039, 0.003, < 0.001, and 0.013, respectively (**Figure 1B**). Control animals did not develop hyperglycemia by the end of the experiment (**Figures 1A**,**B**).

We also evaluated the onset and the differences in levels of glycosuria between the observed groups of animals. Measurements were performed on days 10, 14, 18, 22, 24, 26, and 28. First two measurements (days 10 and 14) did not detect glucose in animal's urine in either group. Glycosuria occurred on the day 18 in mice treated with STZ only. Complete prevention of glycosuria was achieved by administration IL-33 (**Figures 1C**,**D**).

The degree of insulitis was graded according to Hall et al. (22) and Pejnovic et al. (24): normal islet, score 1; perivascular/periductal infiltration, score 2; peri-insulitis, score 3; mild insulitis (< 25% of the islet infiltrated), score 4; and severe insulitis (more than 25% of the islet infiltrated), score 5. The histological examination of the pancreata revealed differences in the intensity of mononuclear cells infiltration in the islets. No islets with severe insulitis were detected in the group of mice treated with IL-33, while the control group of mice had 47.06% of islets with severe insulitis ($p < 0.001$). The percentage of total intact islets was 58.82% in IL-33 treated mice compared to control group of mice with 5.8% of healthy islets ($p < 0.001$) (**Figures 1E**,**F**).



	normal islets	perivascular/ periductal infiltration	peri-insulitis	mild insulitis	severe insulitis
STZ+PBS vs. STZ+IL-33	p<0,001	/	/	p<0,001	p<0,001

FIGURE 1 | Concomitant treatment with recombinant IL-33 completely abrogates induction of diabetes by MLD-STZ. Effect of four injections of 0.4 μg/mouse IL-33 on glycemia **(A)**, GTT test **(B),** and glycosuria **(C,D)**. Histology of the islets showed highly significant ($p < 0.001$) decrease of mononuclear cells influx in IL-33 treated group compared to control group **(E,F).** An analysis of lymphocytic infiltrates in Langerhans's pancreatic islands was performed by light microscope using a magnifying lens of 40 X.

Exogenous IL-33 Particularly Enhances the Number of $CD^{4+}Foxp^{3+}$ Cells in Pancreatic Lymph Nodes in C57BL/6 Mice

IL-33 did not cause significant differences in the total number and percentage of $CD4^+$ (**Figures 2A**,**B**) and $CD8^+$ lymphocytes (**Figures 2C**,**D**) in pancreatic lymph nodes of examined animals. There were no significant differences in percentage and total number of IFN-γ producing $CD4^+$ T lymphocytes between the group that were treated with IL-33 and the group that were treated with STZ only (**Figures 2E**,**F**). IL-33 treatment significantly reduced the percentage of CD8 lymphocytes producing IFN-γ ($p = 0.011$) (**Figure 2G**). Likewise, the absolute number of $CD8^+$IFN-γ^+ cells was also significantly lower in the group of mice treated with IL-33, compared to the control group ($p = 0.029$) (**Figure 2H**). There was no significant difference in the percentage and number of IL-17 producing $CD4^+$cells (**Figures 2I**,**J**). IL-33 treatment significantly increased the percentage ($p = 0.005$) and the total number ($p = 0.038$) of $CD4^+$IL-5^+ cells (**Figures 2K**,**L**) and the percentage and total number of $CD4^+$IL-13^+ cells ($p = 0.02$, $p = 0.028$, respectively) (**Figures 2M**,**N**).

We noticed a significant increase in the percentage ($p = 0.038$) and number ($p = 0.037$) of regulatory $CD4^+Foxp3^+$ cells in the group treated with IL-33 (**Figures 3A**,**B**). Further, the IL-33 treatment led to highly significant increase of the percentage and the number of Treg cells expressing ST2 molecule ($p < 0.001$) (**Figures 3C**,**D**). Exogenous IL-33 significantly increased percentage and number of $CD4^+Foxp3^+ST2^+$IL-10^+ ($p = 0.037$, $p < 0.001$, respectively) (**Figures 3E**,**F**), $CD4^+Foxp3^+ST2^+$IL-13^+ ($p < 0.001$, $p < 0.001$, respectively) (**Figures 3G**,**H**) and percentage of $CD4^+Foxp3^+ST2^+$IL-5^+ ($p < 0.001$) (**Figure 3J**) cells in pancreatic lymph nodes. However, IL-33 treatment did not increase the percentage and number of $CD11c^+$ cells (**Figures 3K**,**L**), but significantly increased percentage of $CD11c^+$ population that produces IL-2 which is important for the survival of Treg cells ($p = 0.023$; **Figure 3M**). Dot plots relevant for **Figures 2**, **3** are given in **Supplementary Figure S1**.

IL-33 Treatment Leads to Significant Decrease in $CXCR^{3+}$ Diabetogenic Cells in the Islets

We also investigated mononuclear infiltrate in pancreatic islets at the end of the experiments (**Figure 4**). There was no difference in the percentage and number of $CD4^+$ cells (**Figures 4A,B**) and $CD8^+$ cells (**Figures 4C,D**). Results showed significantly decreased percentage ($p = 0.029$) and number ($p < 0.001$) of effectors $CD4^+CXCR3^+$ cells in the group of mice treated with IL-33 (**Figures 4G,H**). Similarly, we noticed lower percentage of $CD8^+CXCR3^+$ cells (**Figures 4I,J**) and higher percentage of $CD4^+$IL-10^+ cells (**Figures 4K,L**) but differences did not reach statistical significance. The treatment with IL-33 induced higher percentage ($p = 0.042$) and total number ($p = 0.038$) of $CD4^+Foxp3^+ST2^+$ (**Figures 4O,P**) in mononuclear cell population in the islets. Total number of $CD4^+Foxp3^+ST2^+$IL-10^+ cell was also increased ($p = 0.038$) (**Figures 4Q,R**). Furthermore, we have shown that exogenous IL-33 increased the percentage and the total number of tolerogenic dendritic $CD11b^+CD11c^+$ cells ($p = 0.013$, $p < 0.001$, respectively) (**Figures 4S,T**) in mononuclear cell population in the islets. Dot plots relevant for **Figure 4** are given in **Supplementary Figure S2**.

Low Dose of Cyclophosphamide (CY) Affects Regulatory Cells and Attenuates Protective Effect of IL-33 in Autoimmunity Including Type 1 Diabetes

We and others have shown previously that in mice (15) and rats (25) low dose of CY enhances diabetes induction by affecting regulatory cells. Therefore, we tested by day 18 whether protective effect of IL-33 will be affected by pretreatment with CY. There was significant difference in the streptozotocin induced level of glycemia between the groups treated with IL-33 + CY and IL-33 only ($p < 0.001$). Animals treated with STZ + PBS showed high glycemia (23 ± 2.16) vs. animals treated with IL-33+CB or PBS+CB (glycemia 6.8 ± 0.36; 7.0 ± 0.28) which did not develop disease (**Figure 5A**).

Phenotyping of the cells in pancreatic lymph nodes at the end point of the experiment confirmed the low total number of $CD4^+Foxp3^+$ positive cells ($p = 0.013$) (**Figure 5C**) as well as $CD4^+Foxp3^+ST2^+$ cells ($p = 0.008$) (**Figure 5D**) after CY treatment that correlated with higher glycemia. The percentage of $CD4^+Foxp3^+$ positive cells was also increased in pancreatic islets ($p = 0.004$) (**Figure 5E**). These data suggest that $CD4^+Foxp3^+$ ($ST2^+$) cells are major downregulatory cells induced by IL-33 in MLD-STZ diabetes.

IL-33 Downregulates Diabetes If Given After the Onset of Disease

Exogenous IL-33 treatment, applied after MLD-STZ, had also antidiabetogenic effects in mice received IL-33 from the day 6 and 12, respectively (**Figure 6**). In the groups of mice that received IL-33 from day 6, two out of 7 animals and from day 12 three out of 7 developed hyperglycemia presenting the partial effect of IL-33 if given after the onset of disease. There was significant difference in glycemia level between IL-33 treated and control animals (**Figures 6A**,**B**).

GTT results were significantly different when comparing the group that received IL-33 from the day 6 to control with the exception of GTT measurement in minute 15 showing similar results within the groups. Quantifications of GTT in remaining four-time intervals were significantly different with *p*-values in minute 0, 0.008; min 30, 0.005; min 60, 0.002; min 120, 0.023 (**Figure 6C**). Comparing group that received IL-33 from the day 12 with control, the only result with similar GTT levels was evaluated in min 30. Remaining four of scheduled measurements revealed significant differences with *p*-values of 0.004, 0.034, 0.001, and 0.004, respectively (**Figure 6D**).

Initiated on the day 6 and 12, IL-33 caused a development of a low level of glycosuria. Those low levels were significantly different ($p = 0.011$ from day 18) when IL-33 was initiated on the day 6 and remained different until the end of the experiment on

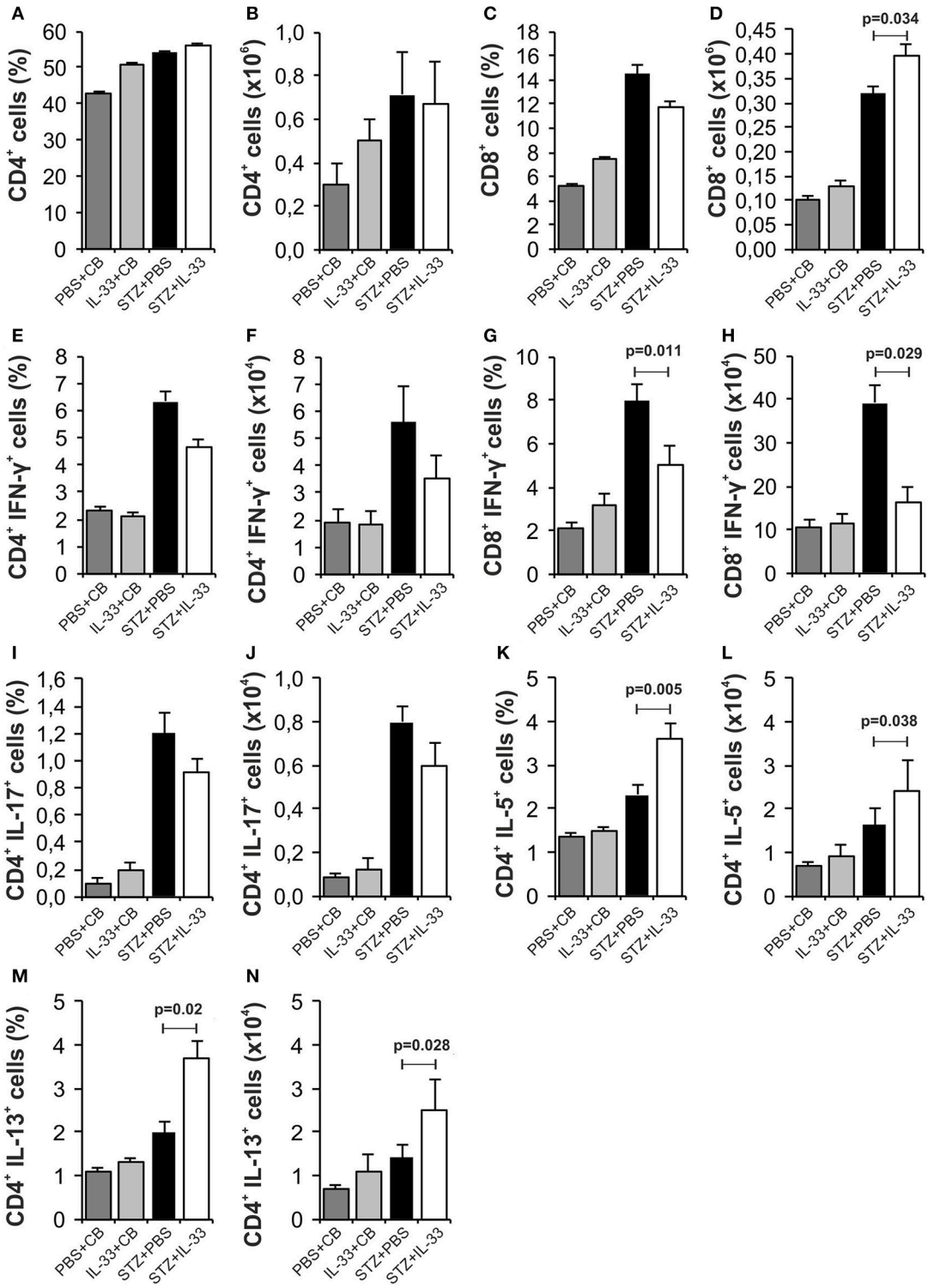


FIGURE 2 | IL-33 attenuates inflammatory cells and increases IL-5 and IL-13 producing $CD4^+$ cells. The percentage and total number of $CD4^+$ **(A,B)**, $CD8^+$ **(C,D),** $CD4^+IFN\text{-}\gamma^+$ **(E,F)**, $CD8^+IFN\text{-}\gamma^+$ **(G,H),** $CD4^+IL\text{-}17^+$ **(I,J)**, $CD4^+IL\text{-}5^+$ **(K,L)**, $CD4^+IL\text{-}13^+$ **(M,N)** were examined. The animals were treated with 0.4 μg/injection IL-33 together with MLD-STZ (i.p. 40 mg/kg for 5 consecutive days) or of an equimolar dose of PBS or 0.4 μg/injection IL-33 together with citrate buffer. Cells were obtained from pancreatic lymph nodes on day 28 after diabetes induction. Data from two individual experiments with at least 8 mice per group are shown as mean ± SEM; by paired *t*-test when compared with values obtained with phosphate-buffered saline.

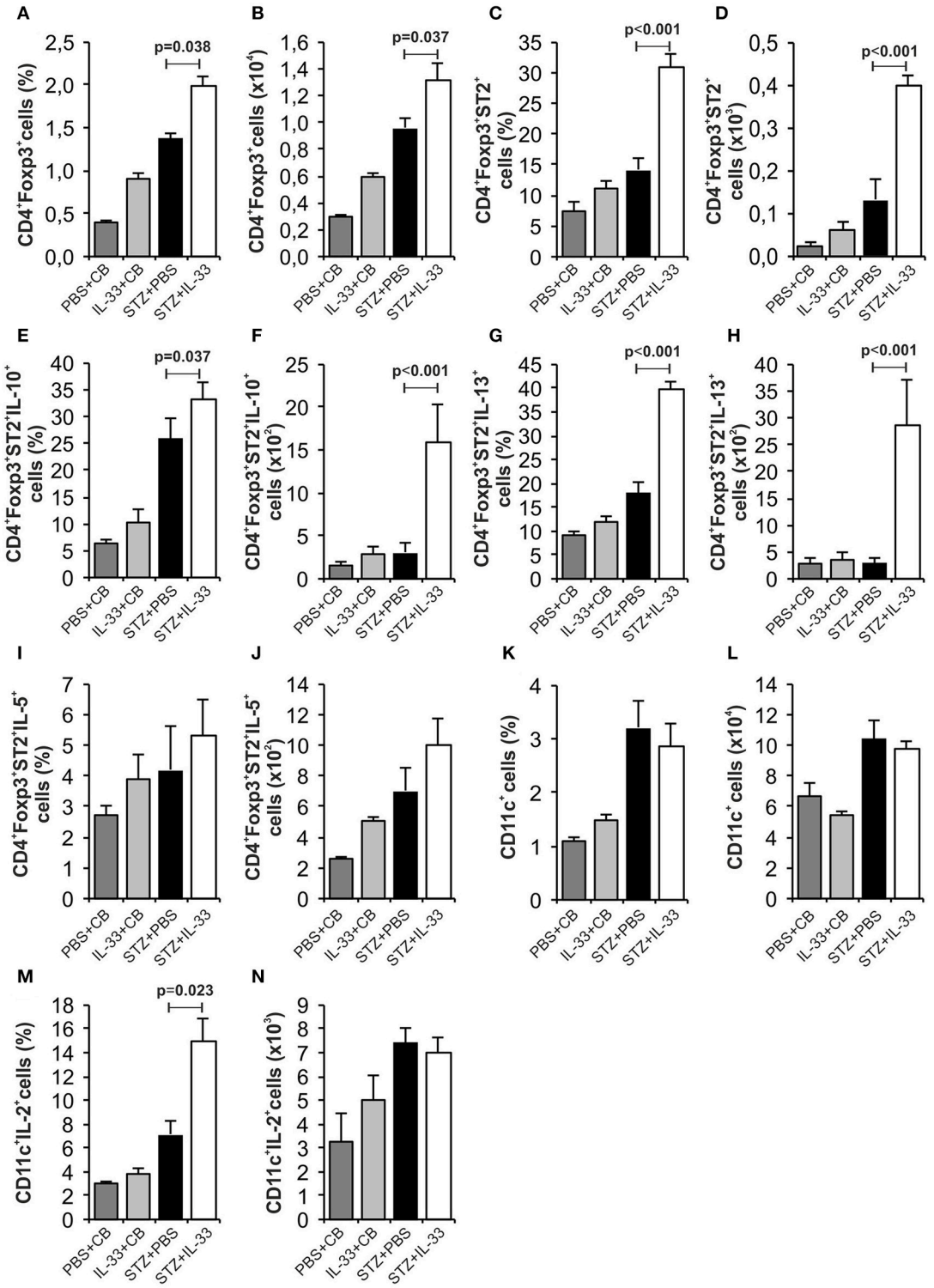


FIGURE 3 | Exogenous IL-33 significantly increases the percentage and total number of regulatory T cells ($Foxp3^+ST2^+IL\text{-}10^+$) in the pancreatic lymph node. The percentage and total number of $CD4^+Foxp3^+$ **(A,B)**, $CD4^+Foxp3^+ST2^+$ **(C,D),** $CD4^+Foxp3^+ST2^+IL\text{-}10^+$ **(E,F)**, $CD4^+Foxp3^+ST2^+IL\text{-}13^+$ **(G,H)**, $CD4^+Foxp3^+ST2^+IL\text{-}5^+$ **(I,J)**, $CD11c^+$ **(K,L)**, $CD11c^+IL\text{-}2^+$ **(M,N)** were examined. The animals were treated with 0.4 μg/injection IL-33 together with MLD-STZ (i.p. 40 mg/kg for 5 consecutive days) or of an equimolar dose of PBS or with 0.4 μg/injection IL-33 together with citrate buffer. Cells were obtained from pancreatic lymph nodes on day 28 after diabetes induction. Data from two individual experiments with at least 8 mice per group are shown as mean ± SEM; by paired *t*-test when compared with values obtained with phosphate-buffered saline.

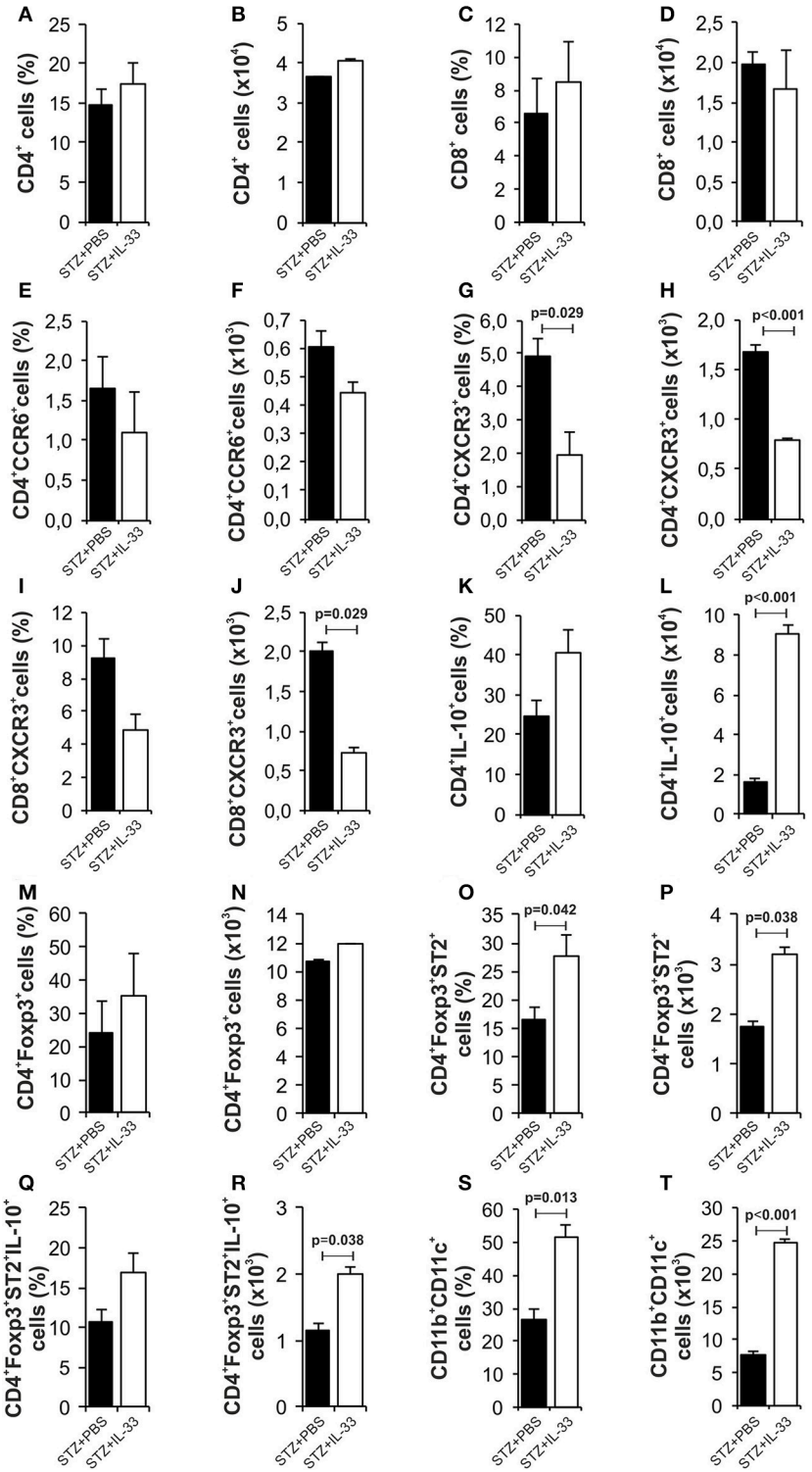


FIGURE 4 | IL-33 attenuates influx of inflammatory cells and promotes regulatory cells infiltration in the pancreatic islets. The percentage and total number of $CD4^+$ **(A,B), $CD4^+CCR6^+$ (C,D),** $CD4^+CXCR3^+$ **(E,F),** $CD4^+IL\text{-}10^+$ **(G,H),** $CD8^+$ **(I,J)**, $CD8^+CXCR3^+$ **(K,L)**, $CD4^+Foxp3^+$ **(M,N)**, $CD4^+Foxp3^+ST2^+$ **(O,P)**, $CD4^+Foxp3^+ST2^+IL\text{-}10^+$ **(Q,R),** $CD11b^+CD11c^+$ **(S,T)** were examined. IL-33 treatment significant decreases effector $CD4^+CXCR3^+$ and $CD8^+CXCR3^+$ cells and increases percentage of regulatory cells, myeloid dendritic cells. The animals were treated with 0.4 μg/injection IL-33 together with MLD-STZ (i.p. 40 mg/kg for 5 consecutive days) or of an equimolar dose of PBS or with 0.4 μg/injection IL-33 together with citrate buffer. Cells were obtained from pancreatic islets on day 28 after diabetes induction. Data from two individual experiments with at least 8 mice per group are shown as mean ± SEM; by paired *t*-test when compared with values obtained with phosphate-buffered saline.

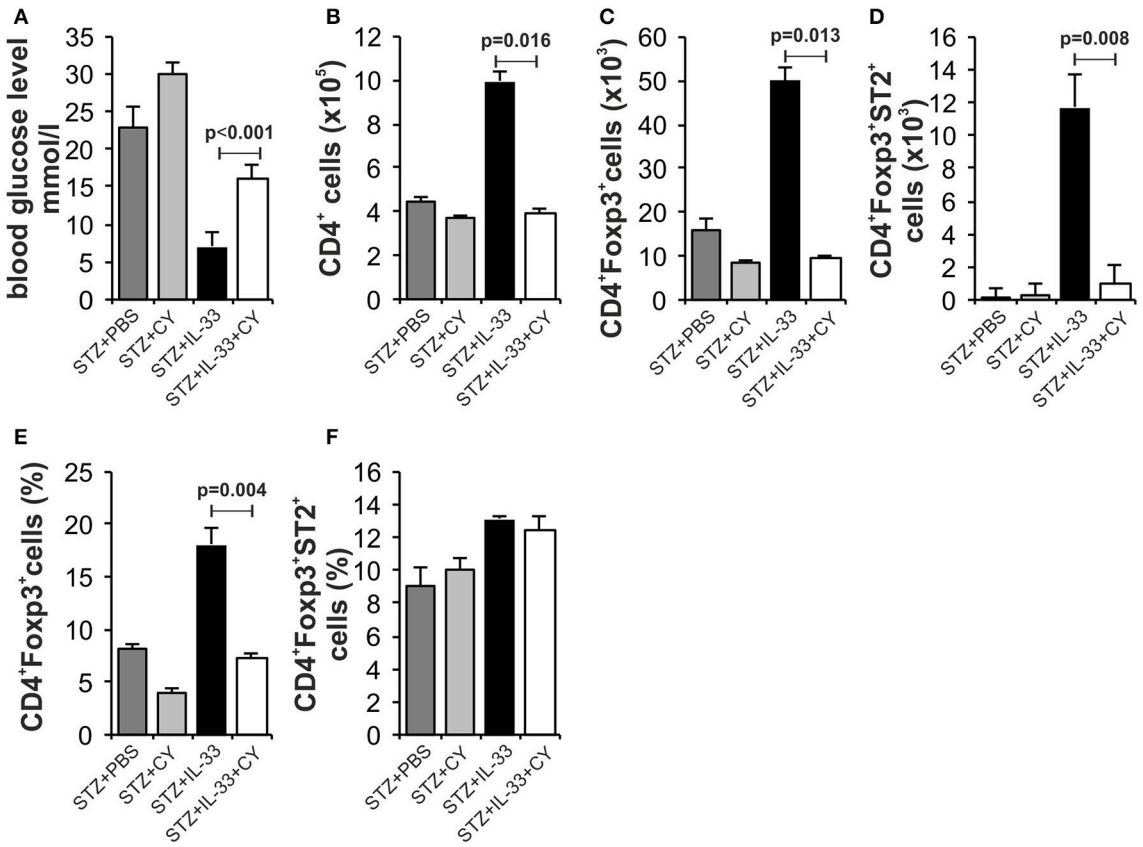


FIGURE 5 | Low dose of cyclophosphamide (CY) affects regulatory cells and attenuates protective effect of IL-33. In order to confirm our hypothesis that the effect of Il-33 administration leads to activation and an increase in the number of regulatory T cells, we added a group that, in addition to MLD STZ and IL-33, also received a low dose of CY (2 × 100 mg/kg) that eliminates regulatory T cells (21). Effect of four injections of 0.4 μg/mouse IL-33, MLD STZ and CY on glycemia **(A)**. The number of $CD4^+$ **(B)**, $CD4^+Foxp3^+$ **(C),** $CD4^+Foxp3^+ST2^+$ **(D)** in pancreatic lymph nodes and percentage of $CD4^+Foxp3^+$ **(E),** $CD4^+Foxp3^+ST2^+$ **(F)** in pancreatic islets. The animals were treated with MLD-STZ (i.p. 40 mg/kg for 5 consecutive days) with PBS or 0.4 μg/injection of IL-33 or CY or both. Cells were obtained from pancreatic islets and pancreatic lymph nodes on day 28 after diabetes induction. Data from one experiment with at least 8 mice per group are shown as mean ± SEM; by paired *t*-test.

the day 28 ($p = 0.044$) when compared with MLD-STZ treated mice (**Figures 6E**,**F**).

IL-33 Treatment Attenuates Insulitis in Prediabetic NOD Mice

In our further analysis, we wanted to examine the effect of exogenous IL-33 on the development of mononuclear infiltrates in spontaneous diabetes NOD mice. Our results clearly showed a significant difference in the infiltration of pancreatic islets among the mice received IL-33 and untreated NOD mice. We evaluated 70 islets and found 60% intact islets in the IL-33 treated group of mice and only 27.5% in the untreated group. Furthermore, in untreated group of mice, 45% of the islets were affected by severe or mild insulitis (**Figure 7A**). Exogenous IL-33 significantly decreased percentage ($p < 0.001$) and number ($p = 0.038$) of $CD4^+$ (**Figures 7B,C**) cells in NOD mice. Significantly decreased percentage ($p = 0.042$) and number of $CD8^+$ T cells ($p < 0.001$) (**Figures 7D,E**) was also noticed after IL-33 treatment in NOD mice. The treatment with IL-33 induced higher percentage and number of of $CD4^+IL\text{-}5^+$ ($p = 0.042$, $p < 0.001$, respectively) (**Figures 7H**,**I**) and $CD4^+IL\text{-}13^+$ ($p = 0.003$; $p < 0.001$, respectively) (**Figures 7J**,**K**). Percentage of $CD4^+Foxp3^+$ T cells was significantly increased after IL-33 treatment ($p = 0.046$) (**Figure 7L**) in pancreatic lymph nodes. There was no significant difference between groups in percentage and number of $CD4^+IL\text{-}17^+$ cells (**Figures 7F,G**). Dot plots relevant for **Figure 7** are give in **Supplementary Figure S3**.

DISCUSSION

In this paper we describe the experiments showing that exogenous IL-33 may prevent development of MLD-STZ diabetes in C57BL/6 mice and significantly attenuate development of insulitis in prediabetic NOD mice. These effects were accompanied by alteration of inflammatory cellular make up in the draining pancreatic lymph nodes as well as in the islets of the pancreas.

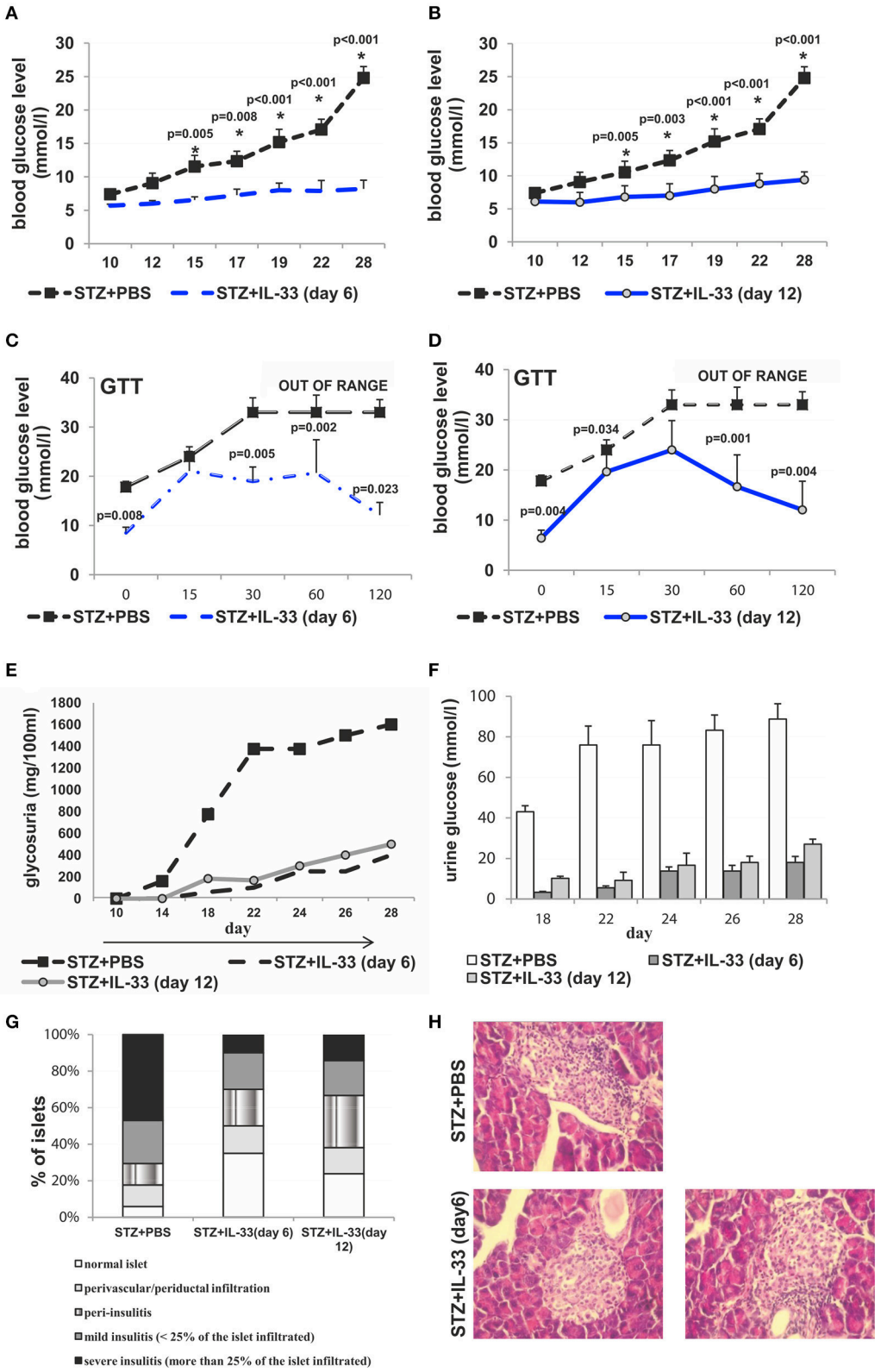


FIGURE 6 | IL-33 given 6 and 12 days after diabetes induction partially attenuates clinical signs and influx of inflammatory cells in the islets. Effect of four injections of 0.4 μg/mouse IL-33 on glycemia **(A,B)**, GTT test **(C,D)** and glycosuria **(E,F)** Histology of the islet showed significantly ($p < 0.001$) decreased influx of mononuclear cells in IL-33 treated group in comparison with control group **(G,H)**. An analysis of lymphocytic infiltrates in Langerhans's pancreatic islands was performed by light microscope using a magnifying lens of 40 X.

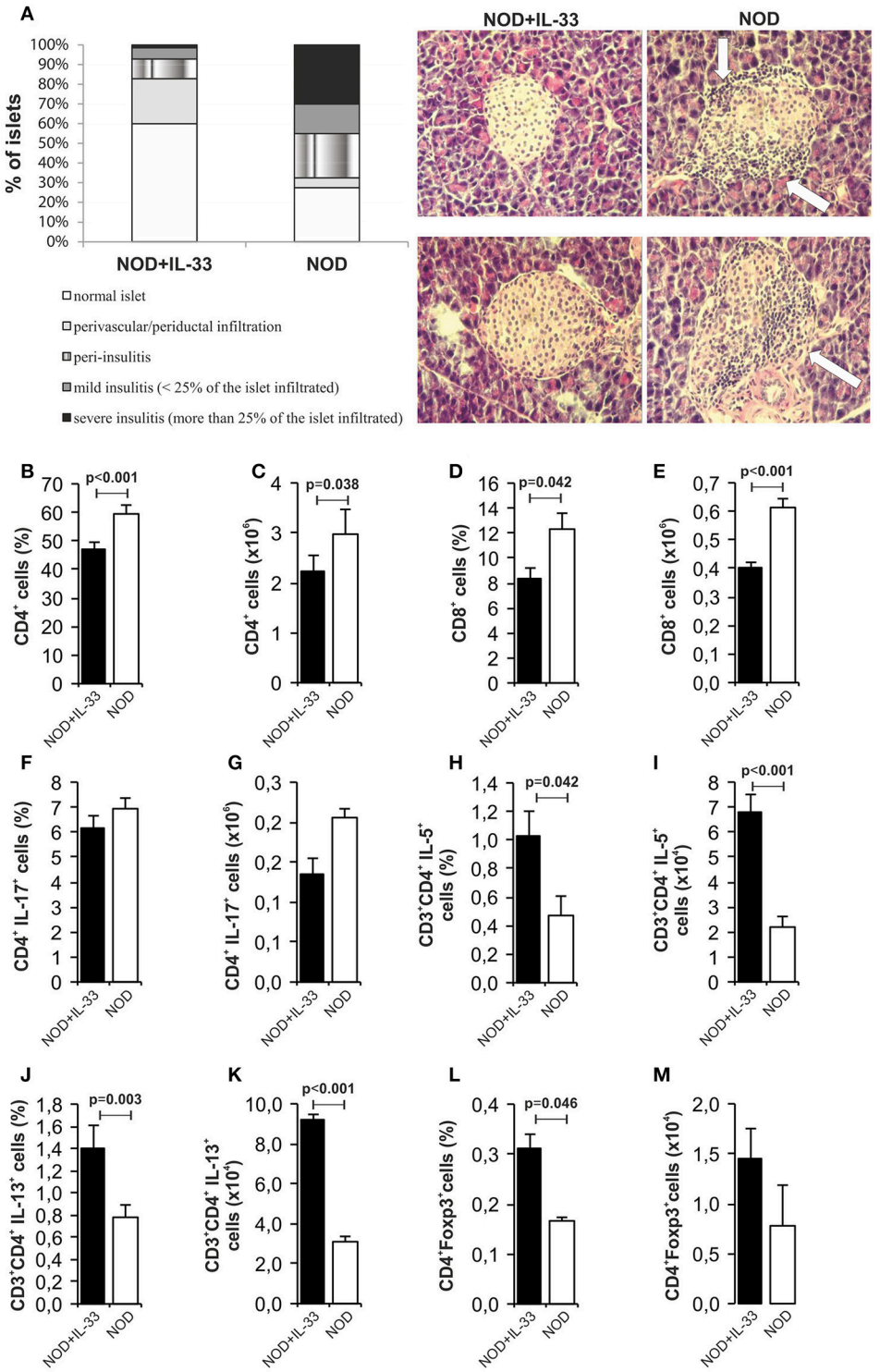


FIGURE 7 | IL-33 decrease insulitis and change composition of mononuclear islet infiltration in prediabetic NOD mice. 16 weeks old animals that were all normoglycemic, free of glycosuria and with normal GTT test were treated with 6 injections of 0,4 μg/mouse IL-33 (every second day) and sacrificed for histological analysis at 18th week of age **(A)**. The percentage and number of $CD4^+$ **(B,C)**, $CD8^+$ **(D,E)**, $CD4^+IL\text{-}17^+$ **(F,G)**, $CD3^+CD4^+IL\text{-}5^+$ **(H,I)**, $CD3^+CD4^+IL\text{-}13^+$ **(J,K)**, $CD4^+Foxp3^+$ **(L,M)** was examined by flow cytometric analysis. IL-33 treatment significant increase percentage of $CD3^+CD4^+IL\text{-}5^+$ **(H,I)**, $CD3^+CD4^+IL\text{-}13^+$ **(J,K)** and regulatory $CD4^+Foxp3^+$ **(L,M)** cells. Data from one experiment with 10 mice per group are shown as mean ± SEM; by paired *t*-test when compared with values obtained with phosphate-buffered saline.

There is growing evidence suggesting that IL-33/ST2 axis plays an important role in chronic in?ammatory and autoimmune diseases (26); type 2 diabetes (27), in?ammatory bowel disease (28), cardiac disease (29), graft-vs.-host disease (30) and small bowel transplant rejection (31). Lack of IL-33/ST2 signaling enhances acute hepatitis (32) and EAE (14).

In this paper we add the evidence that not only genetic deletion of IL-33 receptor (ST2) enhance T cell mediated autoimmune inflammatory diseases but also the exogenous IL-33 has shown powerful preventive effect in a model of type 1 diabetes (**Figure 1**). Moreover, an attempt to therapeutically apply IL-33, 6 and 12 days after diabetes induction had significant effects on the development of clinical and laboratory signs of the disease (**Figure 6**). This protective effect was also confirmed by the GTT test. Furthermore, IL-33 significantly reduced mononuclear infiltration in the pancreatic islets and we did not notice infiltration in vast majority of IL-33 treated mice. Same animals developed only mild periductal infiltrations (**Figure 1**).

Interestingly Oboki et al. (33) did not find the differences in susceptibility to MLD-STZ diabetes between IL-33$^{-/-}$ and "wild type mice." Although the reason for the discrepancy with our results is not clear, it may be assumed that the lack of endogenous IL-33 does not alter other regulatory mechanisms such as ST2 negative T regulatory cells observed in our previous experiments (15).

It was shown that IL-33-activated dendritic cells (DCs) do not produce IL-12, while LPS-activated DCs produce abundant IL-12. It is believed that the pathway IL-33/ST2 may counteract the LPS/TLR4 pathway and thus may control the production of ?h1 cytokines (34). Therefore, it was important to analyse cellular events in the draining pancreatic lymph node and the islets (**Figures 2**, **4**). Application of IL-33 modulates the Th1-Th2 balance and promotes Th2 cells only when given in early phase of the immune response during parasitic infection (35). Similarly, in our investigation the treatment with exogenous IL-33 led to an increase of total number and percentage CD4$^+$IL-5$^+$ and CD4$^+$IL-13$^+$ cells in groups of C57BL/6 mice treated with IL-33 at the day 0 and day 6 (also treated with MLD-STZ) as shown in **Figures 2**, **6**. These findings were also observed in NOD mice treated with IL-33 (**Figure 7**).

As Th2 immune response has a protective role in pathogenesis of diabetes (36), it is possible that one of potential mechanisms of attenuation of the disease mediated by IL-33 is directing immune response toward Th2. However, low dose CY sensitivity of IL-33 antidiabetogenic effect suggests that CD4$^+$Foxp3$^+$ positive cells are the most important regulators in MLD-STZ model in particular ST2$^+$ cells of this subset (**Figure 5**). The activity of Th1 immune response is controlled by the regulatory T lymphocytes (Tregs), which play a key role in maintaining immune homeostasis. Their quantitative and/or qualitative deficiency is often observed in autoimmune and/or inflammatory diseases. IL-33 is an essential cytokine for induction of ILC2. IL-33 promotes and maintains Tregs directly (37) by affecting maturation of antigen presenting cells (13, 14) and by intrinsic activation of ILC2 which affect Tregs via ICOSL-ICOS interaction (37). In this paper we did not evaluate possible contribution of ILC2 which is shown to play a role in metabolic disorders such as obesity and type 2 diabetes but not in T cells mediated autoimmune diseases such as diabetes or EAE which have similar pathogenesis (38, 39). Alteration of antigen presenting cells as previously shown in EAE and MLD-STZ as well as direct effect of IL-33 on maintenance of Foxp3$^+$ST2$^+$Treg$^+$ appear to be explanations of observed preventive and therapeutic effect of IL-33 in our model. However, it remains to be elucidated whether ILC2 may also have importance in Th1 mediated autoimmunity as shown in lymphoid cell activation during immune perturbation metabolic disorders (37, 40, 41) and allergic inflammation (42).

It was shown that ST2$^+$ Tregs are expanded through IL-33-driven production of IL-2 by CD11c$^+$ DC. IL-33/IL-2 axis in CD11c$^+$ DC has the ability to preferentially expand an activated subset of suppressive Foxp3$^+$ Treg expressing ST2. The development of Tregs induces the consumption of IL-2 and therefore the inability to develop Th1 subpopulation.

Several studies have shown the immunoregulatory effect of IL-33 on regulatory cells in the mouse model of inflammatory bowel disease (28) and in mice following cardiac transplantation (43). In both of these studies, IL-33 causes upregulation of CD4$^+$CD25^{high}Foxp3$^+$T cells followed by increasing their number, promoting suppressive activity, as well as ST2 surface expression (28, 43). In addition, two recently published papers have confirmed that IL-33 has the ability to induce regulatory phenotype promoting the expansion of ST2$^+$Tregs (43, 44). *In vitro* treatment of mononuclear cells with IL-33 increases the number of CD4$^+$CD25^{high}Foxp3$^+$ cells expressing the ST2 molecule (45).

Our study also showed that the exogenous IL-33 leads to the expansion of CD11c$^+$IL-2$^+$ cells (**Figures 3M,N**) and increased number of CD4$^+$Foxp3$^+$ST2$^+$ (**Figures 3C,D**) immunoregulatory cells that suppress the development of diabetes in mice treated with IL-33 during the induction of the disease. The importance of CD11c$^+$CD11b$^+$ dendritic cells was indicated previously. It was shown that this DC subset has tolerogenic characteristics and that could be used for immunotherapy of autoimmune diseases (46, 47).

Thus, we showed that exogenous IL-33 attenuates MLD-STZ diabetes induction. The cellular basis of this prevention appears to be mainly due to increased number of Foxp3$^+$ cells in particular those expressing ST2 molecule.

The treatment of IL-33 of 16 weeks old NOD mice in our experiment significantly suppressed the development of mononuclear infiltrates in the pancreatic islets. We showed increased percentage of downregulatory cells in IL-33 treated NOD mice (**Figure 7**). Whether the mechanism is the same as in MLD-STZ diabetes remains to be elucidated. As it has been shown recently that Type 1 interferon inhibits IL-10 signaling and development of diabetes in NOD mice which is promoted by IL-33, the mechanism may be similar (48).

AUTHOR CONTRIBUTIONS

SP, BL, IP, NJ, and MM performed experiments. SP, BL, IP, NA, and MM analyzed data. ML, NA, and SP conceived and designed experiments. SP and ML wrote the article.

FUNDING

This work was supported by a grant from the Serbian Ministry of Science and Education (projects no.175069 and 175071), research grant from University of Kragujevac, Faculty of Medical Sciences, Serbia (project no. JP 12/17) to SP and grant from Swiss National Science Foundation (project no.IZ73Z0_152407) to ML.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.02646/full#supplementary-material

Figure S1 | Gating strategy and representative dot and contour plots for the data presented in **Figure 2** and **Figure 3.** The main findings are indicated in corresponding quadrant.

Figure S2 | Gating strategy and representative dot and contour plots for the data presented in **Figure 4**. The main findings are indicated in corresponding quadrant.

Figure S3 | Representative dot and contour plots for the data presented in **Figure 7.** The main findings are indicated in corresponding quadrant.

REFERENCES

1. Diana J, Simoni Y, Furio L, Beaudoin L, Agerberth B, Barrat F, et al. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. *Nat Med.* (2013) 19:65–73. doi: 10.1038/nm.3042
2. Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. *Immunity* (2010) 32:468–78. doi: 10.1016/j.immuni
3. You S, Chatenoud L. Autoimmune diabetes: an overview of experimental models and novel therapeutics. *Methods Mol Biol.* (2016) 1371:117–42. doi: 10.1007/978-1-4939-3139-2_8
4. Mensah-Brown EP, Shahin A, Al-Shamisi M, Wei X, Lukic ML. IL-23 leads to diabetes induction after subdiabetogenic treatment with multiple low doses of streptozotocin. *Eur J Immunol.* (2006) 36:216–23. doi: 10.1002/eji.200535325
5. Zdravkovic N, Shahin A, Arsenijevic N, Lukic ML, Mensah-Brown EP. Regulatory T cells and ST2 signaling control diabetes induction with multiple low doses of streptozotocin. *Mol Immunol.* (2009) 47:28–36. doi: 10.1016/j.molimm.2008.12.023
6. Dwyer CJ, Ward NC, Pugliese A, Malek TR. Promoting immune regulation in type 1 diabetes using low-dose interleukin-2. *Curr Diab Rep.* (2016) 16:46. doi: 10.1007/s11892-016-0739-1
7. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-elated protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
8. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
9. Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC, et al. IL-33 induces antigen-specific IL-5^+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol.* (2008) 181:4780–90. doi: 10.4049/jimmunol.181.11.8170
10. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* (2009) 113:1526–34. doi: 10.1182/blood-2008-05-157818
11. Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol.* (2011) 41:1675–86. doi: 10.1002/eji.201041033
12. Popovic B, Golemac M, Podlech J, Zeleznjak J, Bilic-Zulle L, Lukic ML, et al. IL-33/ST2 pathway drives regulatory T cell dependent suppression of liver damage upon cytomegalovirus infection. *PLoS Pathog.* (2017) 13:e1006345. doi: 10.1371/journal.ppat.1006345
13. Jiang HR, Milovanović M, Allan D, Niedbala W, Besnard AG, Fukada SY, et al. IL-33 attenuates EAE by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages. *Eur J Immunol.* (2012) 42:1804–14. doi: 10.1002/eji.201141947
14. Milovanovic M, Volarevic V, Ljujic B, Radosavljevic G, Jovanovic I, Arsenijevic N, et al. Deletion of IL-33R (ST2) abrogates resistance to EAE in BALB/C mice by enhancing polarization of APC to inflammatory phenotype. *PLoS ONE* (2012) 7:e45225. doi: 10.1371/journal.pone.0045225
15. Zdravkovic N, Pavlovic S, Zdravkovic V, Pejnovic N, Arsenijevic N, Lukic ML. ST2 Gene-Deletion reveals a role of Foxp3+ regulatory t cells in diabetes modulation in BALB/c mice. *Transl Res.* (2013) 161:118–29. doi: 10.1016/j.trsl.2012.10.005
16. Yuan X, Dong Y, Tsurushita N, Tso JY, Fu W. CD122 blockade restores immunological tolerance in autoimmune type 1 diabetes via multiple mechanisms. *JCI Insight* (2018) 3:96600. doi: 10.1172/jci.insight.96600
17. Ryba-Stanisławowska M, Buksa L, Brandt A, Juhas U, Myśliwiec M. IL-33 improves the suppressive potential of regulatory T cells in patients with type 1 diabetes. *Diabetes Res Clin Pract.* (2017) 128:67–73. doi: 10.1016/j.diabres.2017.04.011
18. Siede J, Fröhlich A, Datsi, Hegazy AN, Varga DV, Holecska V, et al. IL-33 Receptor-expressing regulatory T cells are highly activated, Th2 biased and suppress CD4 T cell proliferation through IL-10 and TGFβ release. *PLoS ONE* (2016) 11:e0161507. doi: 10.1371/journal.pone.0161507
19. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis in mice. Morphologic and physiologic studies. *Science* (1976) 193:415–7. doi: 10.1126/science.180605
20. Pavlovic S, Zdravkovic N, Dimitrov JD, Djukic A, Arsenijevic N, Vassilev TL, et al. Intravenous immunoglobulins exposed to heme (heme IVIG) are more efficient than IVIG in attenuating autoimmune diabetes. *Clin Immunol.* (2011) 138:162–71. doi: 10.1016/j.clim.2010.10.010
21. Ablamunits V, Quintana F, Reshef T, Elias D, Cohen IR. Acceleration of autoimmune diabetes by cyclophosphamide is associated with an enhanced IFN-gamma secretion pathway. *J Autoimmun.* (1999) 3:383–92. doi: 10.1006/jaut.1999.0331
22. Hall TR, Bogdani M, Leboeuf RC, Kirk EA, Maziarz M, Banga JP, et al. Modulation of diabetes in NOD mice by GAD65-specific monoclonal antibodies is epitope specific and accompanied by anti-idiotypic antibodies. *Immunology* (2008) 123:547–54. doi: 10.1111/j.1365-2567.2007.02724.x
23. Li DS, Yuan YH, Tu HJ, Liang QL, Dai LJ. A protocol for islet isolation from mouse pancreas. *Nat Protoc.* (2009) 4:1649–52. doi: 10.1038/nprot.2009.150
24. Pejnovic NN, Pantic JM, Jovanovic IP, Radosavljevic GD, Milovanovic MZ, Nikolic IG, et al. Galectin-3 deficiency accelerates high-fat diet-induced obesity and amplifies inflammation in adipose tissue and pancreatic islets. *Diabetes* (2013) 62:1932–44. doi: 10.2337/db12-0222
25. Mostarica-Stojković M, Petrović M, Lukić ML. Resistance to the induction of EAE in AO rats: its prevention by the pre-treatment with cyclophosphamide or low dose of irradiation. *Clin Exp Immunol.* (1982) 50:311–7.
26. Zhao Q, Chen G. Role of IL-33 and its receptor in T cell-mediated autoimmune diseases. *Biomed Res Int.* (2014) 2014:587376. doi: 10.1155/2014/587376
27. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circ Res.* (2010) 107:650–8. doi: 10.1161/CIRCRESAHA.110.218867

28. Zhu J, Yang F, Sang L, Zhai J, Zhang X, Yue D, et al. IL-33 Aggravates DSS-induced acute colitis in mouse colon lamina propria by enhancing Th2 cell responses. *Mediators Inflamm.* (2015) 2015:913041. doi: 10.1155/2015/913041
29. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, et al. IL 33 reduces the development of atherosclerosis. *J Exp Med.* (2008) 205:339–46. doi: 10.1084/jem.20071868
30. Vander Lugt MT, Braun TM, Hanash S, Ritz J, Ho VT, Antin JH, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. *N Engl J Med.* (2013) 369:529–39. doi: 10.1056/NEJMoa1213299
31. Mathews LR, Lott JM, Isse K, Lesniak A, Landsittel D, Demetris AJ, et al. Elevated ST2 distinguishes incidences of pediatric heart and small bowel transplant rejection. *Am J Transplant* (2016) 16:938–50. doi: 10.1111/ajt.13542
32. Volarevic V, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, et al. Protective role of IL-33/ST2 axis in Con A-induced hepatitis. *J Hepatol.* (2012) 56:26–33. doi: 10.1016/j.jhep.2011.03.022
33. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci USA.* (2010) 107:18581–6. doi: 10.1073/pnas.1003059107
34. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol.* (2009) 123:1047–54. doi: 10.1016/j.jaci.2009.02.026
35. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol.* (2008) 180:2443–9. doi: 10.4049/jimmunol.180.4.2443
36. Hill NJ, Van Gunst K, Sarvetnick N. Th1 and Th2 pancreatic inflammation differentially affects homing of islet-reactive CD4 cells in nonobese diabetic mice. *J Immunol.* (2003) 170:1649–58. doi: 10.4049/jimmunol.170.4.1649
37. Molofsky AB, Van Gool F, Liang HE, Van Dyken SJ, Nussbaum JC, Lee J, et al. Interleukin-33 and interferon-γ counter-regulate group 2 innate lymphoid cell activation during immune perturbation. *Immunity* (2015) 43:161–74. doi: 10.1016/j.immuni.2015.05.019
38. Milovanovic M, Volarevic V, Radosavljevic G, Jovanovic I, Pejnovic N, Arsenijevic N, et al. IL-33/ST2 axis in inflammation and immunopathology. *Immunol Res.* (2012) 52:89–99. doi: 10.1007/s12026-012-8283-9
39. Ankathatti Munegowda M, Deng Y, Chibbar R, Xu Q, Freywald A, Mulligan SJ, et al. A distinct role of CD^{4+}Th17- and Th17-stimulated CD^{8+} CTL in the pathogenesis of type 1 diabetes and experimental autoimmune encephalomyelitis. *J Clin Immunol.* (2011) 31:811–26. doi: 10.1007/s10875-011-9549-z
40. McHedlidze T, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M et al. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunit*y (2013) 39:357–71. doi: 10.1016/j.immuni.2013.07.018
41. Jeftic I, Jovicic N, Pantic J, Arsenijevic N, Lukic ML, Pejnovic N. Galectin-3 ablation enhances liver steatosis, but attenuates inflammation and IL-33-dependent fibrosis in obesogenic mouse model of nonalcoholic steatohepatitis. *Mol Med.* (2015) 21:453–65. doi: 10.2119/molmed.2014.00178
42. Takatori H, Makita S, Ito T, Matsuki A, Nakajima H. Regulatory mechanisms of IL-33-ST2-mediated allergic inflammation. *Front Immunol.* (2018) 9:2004. doi: 10.3389/fimmu.2018.02004
43. Matta BM, Lott JM, Mathews LR, Liu Q, Rosborough BR, Blazar BR, 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) 193:4010–20. doi: 10.4049/jimmunol.1400481
44. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* (2014) 513:564–8. doi: 10.1038/nature13577
45. Ryba-Stanisławowska M, Werner P, Skrzypkowska M, Brandt A, Myśliwska J. IL-33 Effect on quantitative changes of $CD4^{+}CD25^{high}FOXP3^{+}$ regulatory T cells in children with type 1 diabetes. *Mediators Inflamm.* (2016) 2016:9429760. doi: 10.1155/2016/9429760
46. Li H, Zhang GX, Chen Y, Xu H, Fitzgerald DC, Zhao Z, et al. $CD11c^{+}CD11b^{+}$ dendritic cells play an important role in intravenous tolerance and the suppression of experimental autoimmune encephalomyelitis. *J Immunol.* (2008) 181:2483–93. doi: 10.4049/jimmunol.181.4.2483
47. Kriegel MA, Rathinam C, Flavell RA. Pancreatic islet expression of chemokine CCL2 suppresses autoimmune diabetes via tolerogenic $CD11c^{+}CD11b^{+}$ dendritic cells. *Proc Natl Acad Sci USA.* (2012) 109:3457–62. doi: 10.1073/pnas.1115308109
48. Iglesias M, Arun A, Chicco M, Lam B, Talbot CC Jr, Ivanova V, et al. Type-I Interferons inhibit interleukin-10 signaling and favor type 1 diabetes development in nonobese diabetic mice. *Front Immunol.* (2018) 9:1565. doi: 10.3389/fimmu.2018.01565

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Pavlovic, Petrovic, Jovicic, Ljujic, Miletic Kovacevic, Arsenijevic and Lukic. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 20 November 2018
doi: 10.3389/fimmu.2018.02596

Expression and Function of IL-33/ST2 Axis in the Central Nervous System Under Normal and Diseased Conditions

*Karen Fairlie-Clarke, Mark Barbour, Chelsey Wilson, Shehla U. Hridi, Debbie Allan and Hui-Rong Jiang**

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

Interleukin-33 (IL-33) is a well-recognized immunomodulatory cytokine which plays critical roles in tissue function and immune-mediated diseases. The abundant expression of IL-33 in brain and spinal cord prompted many scientists to explore its unique role in the central nervous system (CNS) under physiological and pathological conditions. Indeed emerging evidence from over a decade's research suggests that IL-33 acts as one of the key molecular signaling cues coordinating the network between the immune and CNS systems, particularly during the development of neurological diseases. Here, we highlight the recent advances in our knowledge regarding the distribution and cellular localization of IL-33 and its receptor ST2 in specific CNS regions, and more importantly the key roles IL-33/ST2 signaling pathway play in CNS function under normal and diseased conditions.

Keywords: IL-33, ST2, central nervous system, expression, neurological diseases

OPEN ACCESS

Edited by:
Detlef Neumann,
Hannover Medical School, Germany

Reviewed by:
Jorg Hermann Fritz,
McGill University, Canada
Fouad Zouein,
American University of Beirut, Lebanon
Christopher John Nile,
University of Glasgow, United Kingdom

***Correspondence:**
Hui-Rong Jiang
huirong.jiang@strath.ac.uk

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *03 August 2018*
Accepted: *22 October 2018*
Published: *20 November 2018*

Citation:
Fairlie-Clarke K, Barbour M, Wilson C, Hridi SU, Allan D and Jiang H-R (2018) Expression and Function of IL-33/ST2 Axis in the Central Nervous System Under Normal and Diseased Conditions. Front. Immunol. 9:2596. doi: 10.3389/fimmu.2018.02596

INTRODUCTION

The active and extensive communication between the immune and central nervous system (CNS) is essential in maintaining homeostasis of the CNS and mediating the pathogenesis of neurological diseases. Over recent decades, rapid development in the area suggests many immune cytokines play pivotal roles facilitating this complex neuroimmune crosstalk, and interleukin-33 (IL-33) is one such key cytokine.

IL-33, a member of IL-1 cytokine family, was first identified in 2005 (1) as the ligand for ST2 (also known as IL-1RL1) (2). Extensive research in the following years has shown that full length IL-33 is bioactive and can be released by living cells (3), or by necrotic cells following tissue damage acting as an endogenous danger signal or alarmin [reviewed previously (4, 5)]. In apoptotic cells, IL-33 is inactivated by released caspases (6). IL-33 binds to ST2 and then recruits IL-1 receptor accessory protein (IL-1RAcP), which leads to the activation of MyD88 and NF-κB signaling pathway (7). Soluble ST2 (sST2), a decoy receptor for IL-33, binds to IL-33, and blocks its function. While IL-33 is expressed by many types of cells, in particular stromal and tissue barrier cells and some innate immune cells, ST2 is expressed by various immune cells including T cells, B cells, macrophages, dendritic cells, and innate lymphoid cells (7). The IL-33/ST2 signaling pathway has pleitropic functions in a range of infectious and inflammatory diseases often with dual roles, mediating both pathological immune responses and tissue repair (4, 8–11).

Remarkably IL-33 is expressed constitutively within the brain and spinal cord tissues. Around 33% of isolated brain cells of naïve mouse are IL-33 positive (12) and its mRNA expression level is higher than any other tissues and organs tested (1). These findings together with the recent evidence of ST2 expression by CNS resident cells (13, 14) indicate a unique role for the IL-33/ST2 signaling pathway within the CNS. Indeed accumulating evidence in recent years suggests that IL-33 mediates the interaction between immune, endothelial and CNS resident cells and plays a key role in the development and homeostasis of the CNS. IL-33 is also prominently involved in the neuroinflammation of many neurological diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS) through action mechanisms beyond immunomodulation (15, 16).

This paper will summarize the current findings of the expression of IL-33 and ST2 in different regions and cells of the CNS. This is an area which remains poorly characterized and sometimes controversial, however it constitutes a key step toward our understanding of the function of IL-33/ST2 axis in CNS. We will then discuss the functional implications of the IL-33/ST2 signaling pathway in the CNS compartment under normal and diseased conditions.

DIVERSE EXPRESSION OF IL-33 AND ITS RECEPTOR ST2 IN CNS REGIONS AND CELLS

IL-33 Expression

Despite suggestions from Wicher et al. that IL-33 is only expressed in CNS during late embryogenesis and becoming absent in adult brain (17), others show that its expression is increased during postnatal development (16, 18). IL-33 is also constitutively highly expressed in adult CNS tissues in both human and mouse (13, 16, 19, 20). Interestingly the levels of IL-33 expression across brain and spinal cord regions are not uniform and constant, which potentially reflects specific functions of the IL-33/ST2 signaling pathway in region-associated neuronal activities and disorders. IL-33 expression is particularly abundant in white matter areas such as: corpus callosum (CC) (12, 21) and the anterior forceps of the CC (fmi) (**Figure 1A**), anterior commissure (aco) (**Figure 1B**), hippocampal fringe (fi) and the stria terminalus (st) (**Figure 1C**) (21, 22). IL-33 expression is also distributed throughout the olfactory cortex as well as scattered expression in the somatosensory (S1) and motor cortex (M1/M2; **Figure 1D**). While the hippocampus is another region which is often affected during CNS inflammatory diseases, in particular AD, a disease accompanied by cognition and memory impairments (23), hippocampal expression of IL-33 is surprisingly low (21). Within the cerebellum, IL-33 expressing cells are scattered through the granular, and white matter layers with no expression detected in the molecular layer (**Figure 1E**).

Co-localization of IL-33 with various CNS resident cells has been reported across brain regions (**Table 1**). Within the CC, IL-33 is predominantly expressed by astrocytes (not all though) in murine tissues using GFAP (**Figure 2A**) or S-100 antibodies (27). High level of co-expression with Oligo2$^+$ oligodendrocytes is also shown within this region (21), which is enhanced by Poly-IC treatment after gliotoxic injury and therefore promotes oligodendrocyte progenitor cell (OPC) maturation and myelin production (27). Whether IL-33 is expressed by microglia and neuron cells is less clear and remains controversial (**Table 1**). IL-33 colocalisation with Iba-1$^+$ microglia and neuronal somas (NeuN$^+$) (**Figure 2A**) in CC region is evident however at much lower levels. In the hippocampus, the low level of IL-33 expression appears to co-localize with GFAP$^+$ astrocytes predominantly within the stratum oriens and stratum radiatum layers of the CA1, CA2, and CA3 region as well as molecular layer of the dentate gyrus (DG; **Figure 2B**). Consistent with previous findings (21), low level neuronal expression of IL-33 is observed in the granular layer (**Figure 2B**) within the DG, while low number of IL-33-expressing microglia was also observed in the polymorph layer of the DG (**Figure 2B**) (21).

The spinal cord is made up of bundles of nerve fibers and forms an important part of the CNS. Currently it is not clear whether IL-33/ST2 exhibit different roles in the spinal cord compared to brain. Data of IL-33 expression in the spinal cord has been primarily documented in animal studies (**Figure 2C**) (16, 29, 30, 34). In contrast to its abundant expression in the white matter region in brain (12), IL-33 immune reactivity is prominent in spinal cord gray matter and significantly lower in the white matter (29). This suggests potentially different functions of IL-33 in brain and spinal cord. Similar to findings in the brain, IL-33 is shown to be expressed by oligodendrocytes (34) and astrocytes (29, 30) (**Figure 2C**). In a recent study, Vainchtein et al. identified astrocytes as the primary source of local IL-33 in both brain and spinal cord using two IL-33 reporter mouse models (16). Further evidence also suggests that IL-33 is expressed by a limited number of NeuN$^+$ neurons and Iba1$^+$ microglia cells (**Figure 2C**), similar to its expression in brain.

ST2 Expression

Extracellular IL-33 exerts its effect through binding to its receptor which consists of a heterodimer between ST2 and IL-1RAcP (4). Although the expression pattern and cellular distribution of ST2 in CNS remains understudied and somewhat disputed, there has been some exciting progress in recent years identifying IL-33 target cells. This has provided new insights into the functional mechanisms of the signaling pathway in CNS.

Expression of ST2 in the brain appears to mirror IL-33 expression in regard to distribution pattern. This includes regions such as the olfactory bulb, hippocampus, and hippocampal fringe; anterior commissure; CC as well as a number of other regions throughout the cortex, in particular within the somatosensory regions (S1FL/HL; **Figure 3A**). ST2 expression within the cerebellum is distinct with low levels in the granular and white matter layer, and likely on Purkinje cells (personal observation), a class of GABAergic neurons located in the cerebellum and cerebellar nuclei which play a fundamental role in controlling motor movement. Whether IL-33/ST2 in cerebellum region relates to motor movement activities is not clear. In contrast to the low level of IL-33 expression observed

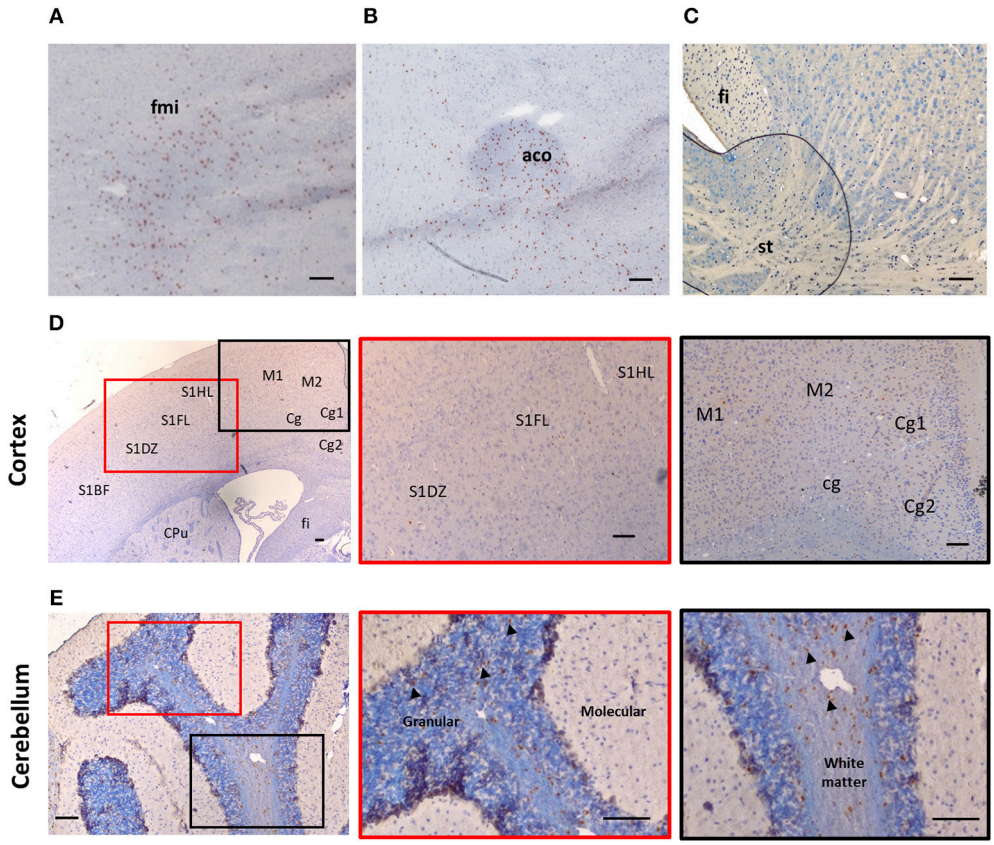


FIGURE 1 | Expression of IL-33 in several brain regions. Brains from C57BL/6 mice were sectioned coronally and immunohistochemically stained for the expression of IL-33 (red/brown) and counterstained with haematoxylin. **(A–C)** IL-33 is predominantly expressed in several white matter rich regions including **(A)** anterior forceps of corpus collosum (fmi); **(B)** anterior commissure (aco) and **(C)** the hippocampal fringe (fi) and stria terminalus (st). **(D)** Within the cortex, IL-33 is expressed at low levels in the somatosensory (S1) and motor cortex regions (M1/M2). **(E)** In the cerebellum, IL-33 is expressed through the granular, and white matter regions with minimal expression within the molecular layer. Arrowheads indicate IL-33$^+$ cells. CPu, striatum; Cg, cingulate cortex. Scale bars represent 100 μm.

in the hippocampus (**Figure 2B**), ST2 is highly expressed throughout all regions which include CA1-CA3 and DG (**Figure 3B**).

Different CNS cells have been proposed as IL-33 target cells (**Table 2**). *St2* mRNA was initially identified in cultured murine astrocytes and microglia (14). However only microglia, not astrocytes, neurons or any other CNS cells, flow-sorted from thalamus region tissues are confirmed to express *St2* (16). Extensive staining in naïve mouse brain tissue demonstrates that within the CC region, ST2 is surprisingly expressed by some astrocytes, with lower expression level observed in neuron cell bodies and microglia (**Figure 4A**). Prominent but not complete neuronal co-localization is also seen in the cortex (**Figure 4B**), indicating the presence of ST2 on other CNS cell types such as GFAP$^+$ astrocytes (**Figure 4B**). The expression of ST2 on astrocytes close to the endothelial layer of the cortex is interesting, as these cells could be involved in the astrocytic interactions with endothelial cells and pericytes making up the blood brain barrier (BBB) (38). IL-33 may interact with the BBB via these ST2$^+$ astrocytes as reported in an experimental cerebral malaria (ECM) model (39). Furthermore, ST2 expression in various brain regions changes under different conditions, e.g., the level is upregulated in the lesion of ischemic brain (28).

The expression of ST2 in the spinal cord was confirmed years ago (29) however it is yet to be agreed which CNS cell population is the target for IL-33. Findings from several groups have indicated that neurons (29, 33, 35), astrocytes (33, 35) and oligodendrocytes (35, 37) can all express ST2 in the spinal cord as also shown in **Figure 4C**. Neuronal expression of ST2 within the gray matter is particularly evident (**Figure 4C**) and its staining pattern is consistent with the morphology of neurons, which was confirmed by dual staining with NeuN antibody. Less is known about ST2 expression by spinal cord microglia cells (33, 35). Immunohistochemical staining of naïve mouse tissue confirms the expression of ST2 in some astrocytes and microglia (**Figure 4C**), albeit at far lower levels. In the inflammatory spinal cord of experimental autoimmune encephalomyelitis (EAE) mice, mRNA of *St2* is shown to be increased (29) and the upregulation is predominantly in the lesion area in the white matter (29, 37).

TABLE 1 | Expression of IL-33 in central nervous system cells.

CNS Tissue	Neuron (NeuN Ab or stated)	Astrocyte (GFAP Ab or stated)	Microglia (Iba-1 Ab or stated)	Oligodendrocytes (Olig 2 Ab or stated)	References
Mouse mixed glia culture: *Il33* mRNA	ND	√	ND	NT	(14)
Mouse mixed glial culture	NT	√	NT	NT	(24)
Mouse mixed glial culture	NT	√	NT	√	(17)
Mouse mixed glial culture	NT	√	NT	√	(25)
Mouse P9 brain	ND in most MAP-2+ cells	√	NT	√	(17)
Rat brain	√	√	ND	NT	(26)
Rat brain	NT	√√ (S-100)	Infrequent (CD68)	√	(27)
MS patient brain	NT	√	NT	NT	(19)
Healthy and MS brain	√ (SMI-31)	√	√	√ (CA-II)	(13)
Mouse brain	ND (MAP-2 and DCX)	√	ND	√	(25)
Mouse brain	√ (ventral dentate gyrus)	√ (S-100β)	ND	√	(21)
Mouse brain	ND	√	ND	√ (CC-1, not NG2)	(28)
Mouse brain	NT	√	NT	NT	(18)
Mouse thalamus and spinal cord	NT	√ (ALDH1L1)	NT	√ (CC-1)	(16)
Mouse spinal cord	√	√	NT	NT	(29)
Mouse spinal dorsal horn	ND	√	ND (CD11b)	NT	(30)
Mouse spinal cord	ND	√	ND	NT	(31)
Mouse spinal cord	√	√	ND (CD11b)	NT	(32)
Mouse spinal cord	ND	√	ND (CD11b)	NT	(33)
Mouse spinal cord	Minimal to none	√ (ALDH1L1)	Minimal to none	√ (Olig2+CC-1)	(12)
Mouse spinal cord	√	√	√	√	(34)
Rat spinal cord	√	√	ND (OX-42)	√	(35)

Expression of Il33 mRNA was performed using PCR while protein expression was studied using immunohistochemical staining and other methods. √, positive expression of IL-33; ND, not detectable; NT in gray square, not tested. Ab, antibody; MS, multiple sclerosis.

Implications of IL-33 and ST2 Expression in CNS

Thus, the current research evidence illustrates a complex pattern of IL-33 and ST2 expression in CNS tissue and cells. The variation of the expression levels in different regions of the CNS may indicate that the IL-33/ST2 signaling pathway has specific roles in different regions. For example, CC region is the largest commissure in the brain connecting both hemispheres homologous regions and providing interhemispheric communication (40). It is also involved in a number of CNS inflammatory diseases including MS (41, 42) and cerebral malaria (25). High levels of both IL-33 and ST2 positive cells in the CC may imply a role of the signaling pathway in these diseases. While it is surprising that IL-33 expression in hippocampus is low, high levels of ST2 expression in the region may indicate an important hippocampal response to IL-33 released from nearby tissues following injury/inflammation.

Numerous studies have substantiated that astrocytes and oligodendrocytes are the predominant source of IL-33 production, while its expression on neurons and microglia (**Table 1**) is less consistent and requires further investigation. Once produced, IL-33 acts through binding to ST2 receptor expressed by various cell populations including microglia, oligodendrocytes, astrocytes and neurons. The variation and disagreement about IL-33$^+$ and ST2$^+$ cells in CNS tissues between reports (**Tables 1**, **2**) may be explained by the heterogeneity of each type of CNS cell. For example astrocytes differ in morphology, receptor expression, and function across different CNS regions, this has been reported in both mice and human (43–45). Furthermore, without doubt, the dynamic neurological and immunological changes within the tissue under various physiopathological conditions also contribute to the complex heterogeneity of CNS resident cells. This inevitably influences the expression of IL-33 and ST2 on these cells. IL-33 expression is increased in the CNS of MS patients (13) but the level is decreased in AD (46). ST2 expression is significantly increased in the spinal cord lesions composed of infiltrating immune cells in EAE mice (37), and in microglia which are essential in mitigating the severity of ischemic lesions in mice (28). Gadani et al. even discovered a flip of the expression levels of *St2* gene between CD11b$^+$ microglia and CD11b$^-$ astrocyte cell populations before and after injury (12). It is also important to note the different methods used to determine the molecule expression in tissues, e.g., *Il-33* reporter mice (16, 25) or immunohistochemical staining. Although there is variation in the quality of the IL-33 and ST2 antibodies used in different experiments, IL-33$^{-/-}$ mice (21) or specific blocking peptides (36) are used in some but not all studies to verify the specificity

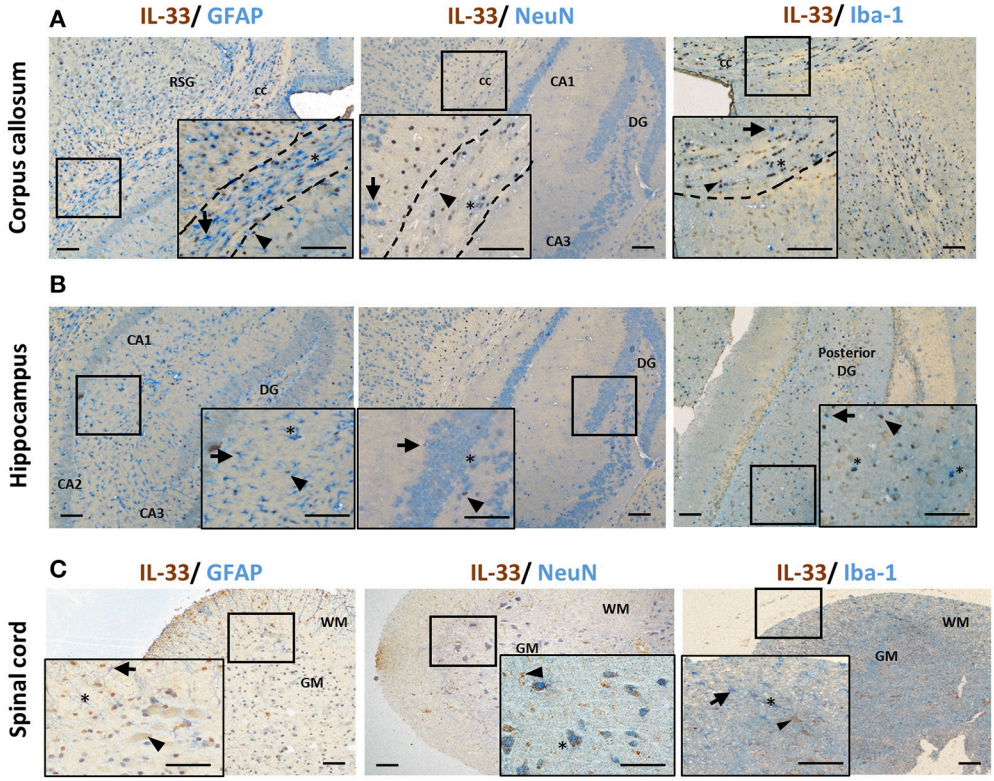


FIGURE 2 | IL-33 expression by CNS resident cells in mouse brain and spinal cord. Tissues from C57BL/6 mice were sectioned and immunohistochemically stained for the expression of IL-33 (brown), and astrocytes (GFAP), neurons (NeuN) and microglia (Iba-1) (all blue) within the **(A)** corpus callosum and **(B)** hippocampus and IL-33 co-localization assessed. RSG, retrosplenial granular cortex; CC, corpus callosum; DG, dentate gyrus; CA1-3, hippocampus. **(C)** In spinal cord, the majority of IL-33 expressing cells are localized to the GM, with lower levels present in the WM. IL-33 is expressed by GFAP$^+$ astrocytes in both GM and WM regions with lower level co-localization with neurons and microglia in the GM and, GM/WM, respectively. Arrowheads indicate IL-33$^+$ cells, arrows indicate NeuN, GFAP or Iba-1$^+$ cells and asterisks indicate double positive cells. GM, gray matter; WM, white matter. Scale bars represent 100 μm.

of the antibodies. Improvement in the standards for antibody validation is therefore essential in the future.

Taken together, the dynamic expression of IL-33 and its receptor ST2 within the CNS regions and cells indicates a complex network of communication between the immune and CNS resident cells. This underscores the fundamental role endogenous IL-33/ST2 signaling pathway plays in the CNS under physiological and pathological conditions.

ROLE OF IL-33/ST2 AXIS IN CNS DEVELOPMENT AND FUNCTION

Despite recent effort, whether IL-33 has detrimental or beneficial effects on the growth and function of neurons is still undetermined. 10 ng/ml of recombinant IL-33 (rIL-33) in a mouse mixed glia cell culture or pure neuronal culture decreases neuronal number together with the loss of neuritis-like appearance when compared with untreated control neurons (47). However 25 or 100 ng/ml of rIL-33 has no significant impact on the growth or viability of neurons and axonal densities in a rat myelinating culture system (13). Similarly, there is contradictory evidence regarding the role of IL-33/ST2 axis in CNS myelination. Treatment with rIL-33 promotes the differentiation and maturation of rat OPCs cultured *in vitro* (27), but fails to improve axonal myelination in a rat myelinating culture as the treatment significantly reduces the proportion of myelinated axons (13). It is worth noting that this inhibitory effect on myelination is not observed in a mouse myelinating culture system (13). This may indicate an important species-specific difference or indeed culture condition difference resulting in the discrepancy reported by different research groups. Nevertheless, the above findings, together with the recent identification of ST2 expression by oligodendrocytes (**Table 2**) indicate an important role for IL-33/ST2 signaling pathway in the myelination process during the CNS development, and also likely the repair phase in demyelinating diseases such as MS. Additionally, ST2 is shown to be expressed in small to medium-sized dorsal root ganglion (DRG) neurons and IL-33 induces Ca^{2+} influx into a subsets of neurons dissociated from the cervical DRG, suggesting IL-33/ST2 signaling excites sensory neurons (36).

The unique role of IL-33 in CNS physiological function *in vivo* has been reported using *Il33* gene knockout mice (21). Deficiency of *Il33* alters the expression of c-Fos proteins, an indicator of neuronal activities, in brain regions implicated in

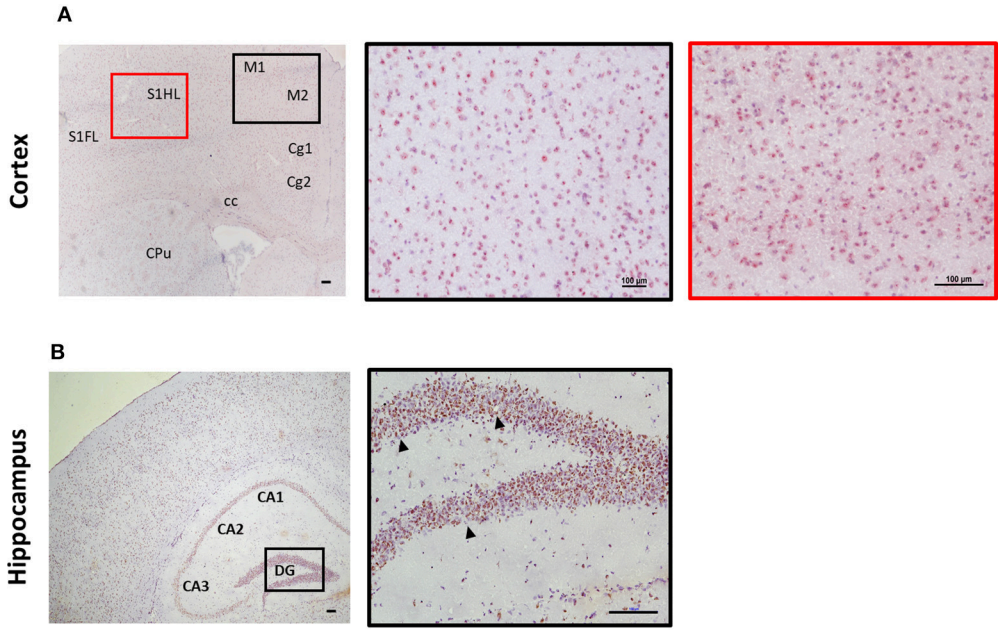


FIGURE 3 | Expression of ST2 in mouse brain regions. Brains from C57BL/6 mice were sectioned coronally and stained for the expression of ST2 (red/brown) and counterstained with haematoxylin. **(A)** Within the cortex, ST2 was expressed at high levels in the somatosensory (S1) and motor cortex regions (M1/M2). **(B)** ST2 was also highly expressed throughout the hippocampus (CA1–CA3) including the dentate gyrus (DG). Arrowheads indicate $ST2^+$ cells. CPu, striatum; Cg, cingulate cortex. Scale bars represent 100 μm.

anxiety-related behaviors. These IL-33 deficient mice exhibit reduced anxiety-like behaviors, as well as deficits in social novelty recognition, despite intact sociability (21). The authors suggest that IL-33 may regulate the development and/or maturation of neuronal circuits, rather than control neuronal activities in adult mice. Indeed IL-33 mediated signaling is required in brain development (16). IL-33 deficient mice contain an excess number of excitatory synapse and show deficits in acoustic startle response, a sensorimotor reflex mediated by motor neurons in the brain stem and spinal cord. Furthermore, IL-33 produced by synapse-associated astrocytes is required for the development of normal synapse numbers and circuit function in the thalamus and spinal cord, signaling primarily through microglia cells under physiologic conditions to promote increased synaptic engulfment (16).

While the above findings reveal a key role for IL-33 during CNS development and activities in some brain regions, it is important to remember that the mechanisms of action of the IL-33/ST2 axis in the CNS are likely to be complicated and engage the multi-cell network consisting of neurons, oligodendrocytes, astrocytes, and microglia cells. The findings of IL-33 involvement in exciting sensory neurons, axonal myelination and synapse homeostasis also imply that dysregulated IL-33/ST2 signaling may lead to CNS neurological and behavioral disorders.

ROLE OF IL-33/ST2 AXIS IN CNS DISEASE

Alzheimer's Disease

AD is the most common form of dementia and its pathology can be characterized by extracellular amyloid deposits- made of Aβ peptides- and intracellular tau-based neurofibrillary tangles (NFTs) (48). Genetic studies have identified single nucleotide polymorphisms (SNPs) within *Il-33* to be associated with a decreased risk of developing AD in Caucasian (46) and Han Chinese populations (49, 50). It is not yet clear how the expression levels of IL-33 and ST2 in tissues correlate with AD. Chapuis observed a reduced level of IL-33 expression in the brains of AD patients (46). However, increased IL-33 and ST2 levels are found in proximity to amyloid plaques and NFTs in AD patients in comparison to healthy controls (51). Patients with mild cognitive impairment, who have an increased risk of AD development, also exhibit a significantly higher serum level of sST2 (52).

It remains debatable whether and how IL-33 affects AD. The induction of IL-33 production by β-amyloid peptide in astrocytes at the vicinity of plaques indicates IL-33 may contribute to AD pathogenesis as one of the inflammatory molecules (51). Indeed an effective treatment with a nanomaterial in *APP/PS1* transgenic mice, a transgenic animal model of amyloid deposition, shows improved learning and memory capability associated with decreased levels of several pro-inflammatory cytokines including IL-33 (53). Other investigators disagree and have presented research evidence to substantiate a potentially therapeutic role for IL-33 in AD (18, 46, 52). Systemic injection of rIL-33 in *APP/PS1* mice reverses synaptic plasticity impairment and memory deficits with reduced soluble Aβ levels and amyloid plaque deposition (52). This is likely mediated by promoting the recruitment of microglia and enhancing their Aβ phagocytic activity. Their subsequent experiments revealed that IL-33 polarizes microglia and macrophages toward an anti-inflammatory M2 phenotype, a

TABLE 2 | Expression of IL-33 receptor ST2 in central nervous system cells.

Tissue or cells	Neuron (NeuN Ab or stated)	Astrocyte (GFAP Ab or stated)	Microglia (Ab tested)	Oligodendrocytes (Olig 2 Ab or stated)	References
Mouse mixed glia culture: *St2* and *IL-1RAcP* mRNA	√ (*IL-1RAcP* only)	√	√	NT	(14)
Mouse mixed glia culture: *St2* mRNA	NT	√	√	NT	(12)
Rat myelinating co-culture	√	ND	NT	√ (O4)	(13)
MS patient brain	√ (SMI-31)	NT	NT	√ (CA-II)	(13)
Mouse brain	ND (by FC)	√ (GLAST) (by FC)	√ (Iba-1)	ND (by FC)	(28)
Mouse thalamus and spinal cord flow sorted cells (*St2* mRNA)	ND	ND	√	NT	(16)
Mouse spinal cord	√	ND	NT	NT	(29)
Mouse spinal cord	√	√	ND (CD11b)	NT	(36)
Mouse dorsal root ganglia	√ (Nissi)	NT	NT	NT	(36)
Human DRG: *St2 and IL-1RAcP* mRNA	√	NT	NT	NT	(36)
Mouse spinal cord (EAE)	NT	√	NT	√ (O1)	(37)
Rat spinal cord	√	√	ND (OX-42)	√	(35)

Expression of St2 mRNA was performed using PCR while protein expression was studied using immunohistochemical staining or other methods. √, positive expression of ST2; ND, not detectable; NT in gray square, not tested. Ab, antibody; EAE, experimental autoimmune encephalomyelitis; FC, flow cytometry; MS, multiple sclerosis; DRG, dorsal root ganglia.

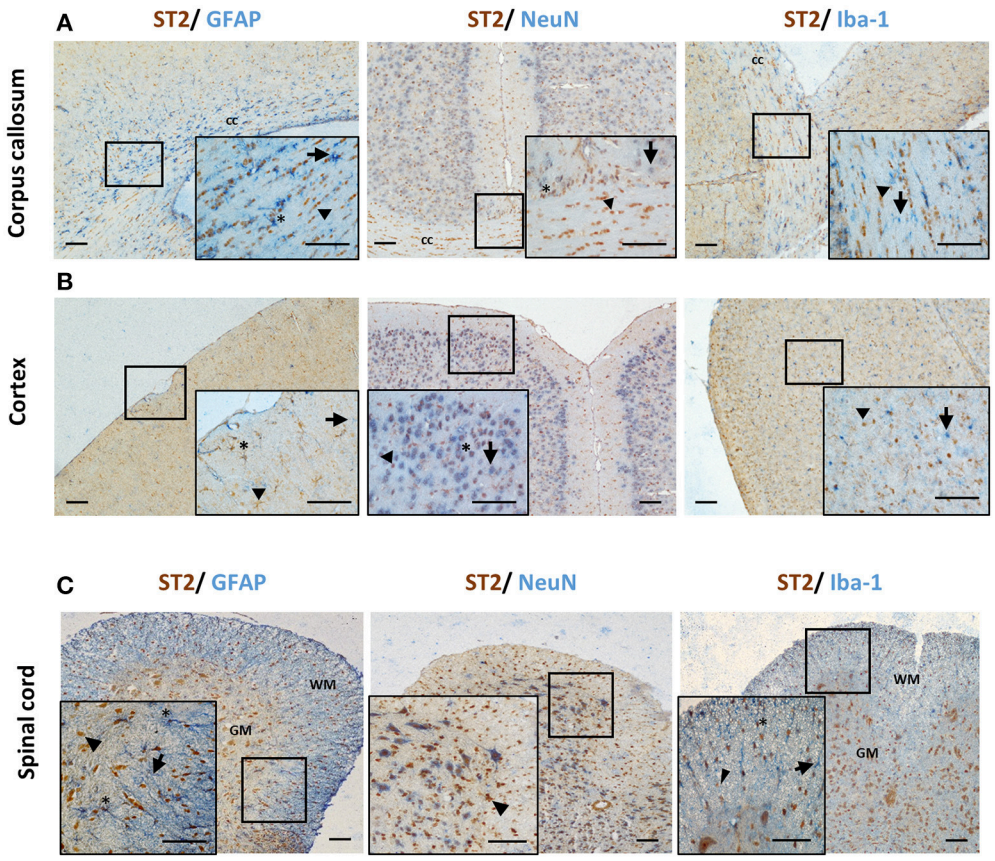


FIGURE 4 | ST2 expression by CNS resident cells in mouse brain and spinal cord. Tissues from C57BL/6 mice were sectioned and stained for the expression of ST2 (brown) and astrocytes (GFAP), neurons (NeuN) and microglia (Iba-1) (all blue) within the **(A)** corpus callosum and **(B)** hippocampus and ST2 co-localization assessed. The majority of ST2 expressing cells co-localized with GFAP$^+$ astrocytes while low level co-localization with neurons and microglia was observed in both regions. **(C)** In spinal cord, the majority of ST2 expressing cells were localized to the GM, with lower levels present in the WM. ST2 was expressed by NeuN$^+$ neurons in both GM and WM regions with lower level co-localization with astroyctes and microglia. Arrowheads indicate ST2$^+$ cells, arrows indicate NeuN, GFAP or Iba-1$^+$ cells and asterisks indicate double positive cells. cc, corpus callosum; GM, gray matter; WM, white matter. Scale bars represent 100 μm.

well-recognized mechanism of IL-33 action in several immune-mediated diseases including spinal cord injury (12), asthma (54), and EAE (29). Such a beneficial role of IL-33 in AD is further supported in a study using aging mice. IL-33 expression in astrocytes is dramatically increased by up to 74% in aged mice, which appears to be critical for the repair of aged neurons (18). Conversely IL-33 deficient mice have an uncontrolled surge of neuronal aging due to failed repair at middle age and ultimately develop neurodegeneration and late-onset AD-like symptoms in old age (18). This is characterized by Tau deposition and heavy neuronal loss in both the cerebral cortex and the hippocampus and accompanied with cognition and memory impairment. These findings indicate a critical role for IL-33 in the maintenance and repair of aging and stressed neurons.

In summary, further investigation is required to determine whether and how the changed levels of IL-33, ST2 and/or sST2 in CNS tissues imply a specific role of IL-33/ST2 signaling pathway in AD. Evidences from *in vivo* research using animal models support a neuroprotective role by modulating neuroinflammation and promoting neuronal repair process, indicating a potential IL-33 based novel therapeutic strategy for AD patients.

Multiple Sclerosis

The involvement of IL-33 in the development of MS, a CNS inflammatory demyelinating autoimmune disease, has been supported by the increased expression of IL-33 and/or ST2 in the CNS lesions of MS patients (13, 19) and EAE mice (29, 55). In addition, effective treatment in EAE rats downregulates IL-33/ST2 expression (55). However, its precise function in disease development remains controversial (29, 32, 56). While Li et al. reported a detrimental effect of IL-33 treatment on EAE severity (56), others argue a protective role for the cytokine in attenuating EAE. The studies suggest IL-33 inhibits EAE development through mechanisms such as promoting type 2 T cell- and macrophage-mediated immune responses and inhibiting production of IL-17 and IFN-γ (11, 29). A more recent investigation suggests that a dramatic decrease of intracellular IL-33 is accompanied by increased extracellular IL-33 in the spinal cord of EAE mice (32). This subsequently promotes the expansion and function of $ST2^+$ Treg cells in inhibiting CNS inflammation. Interestingly, IL-33 has been reported to regulate sex-dimorphic susceptibility of MS (57). Male-specific expression of IL-33 by mast cells expands ST2 positive (58) type 2 innate lymphoid cells (ILC2s) and drives a Th2 immune response in attenuating EAE clinical symptoms in mice. To date no data are available indicating a gender-based differential expression/production of IL-33 and/or ST2 in tissues of other sex-biased diseases. The involvement of mast cells and ILC2s as the mediating cells in the above study indicates that this special function of IL-33 is unlikely to be limited to CNS diseases. Thus, future studies in immune disorders with pronounced gender differences such as systemic lupus erythematosus and ankylosing spondylitis may provide improved knowledge of the molecular basis of sex-dimorphic disease.

Another emerging issue of recent debate is whether IL-33/ST2 plays an important role in remyelination, an essential CNS repair process in MS. This is of particular interest after the confirmation of ST2 expression on oligodendrocytes (12, 13). Despite the findings that rIL-33 reduces the proportion of myelinated axons in a rat myelinating culture (13), higher levels of IL-33 expression are observed in tissues with higher myelin content *in vivo* (12). rIL-33 also promotes the differentiation and maturation of rat oligodentrocytes in culture as shown with increased transcription of myelin genes and phosphorylation of p38MAPK, a signaling molecule involved in myelination (27). Furthermore, *in vivo* subcutaneous administration of Poly-IC results in greater recruitment of OPCs and enhances remyelination following gliotoxic injury with lysolecithin to the brain CC region (27). This is associated with increased expression of IL-33 in astrocytes and an upregulation of Arg and CD206 in macrophages (indicating they are type 2 like macrophages) in the local region.

Therefore, it is clear that current research findings support the involvement of IL-33/ST2 axis in MS development through modulating both the immune response and the CNS repair process. However, its precise function remains to be determined before any potential strategies of therapies can be developed for patients.

Schizophrenia

Schizophrenia is a complex neuropsychiatric disorder which is characterized by a heterogeneous combination of symptoms such as hallucinations and delusions, social withdrawal as well as cognitive impairment. It affects approximately 1% of the worldwide population, with its pathogenesis yet to be fully characterized. Recent studies have shown that specific cytokines might be the neurobiological mediators underlying the pathology of the disease (59). Elevated levels of inflammatory cytokines such as IL-1β, IL-12, IFN-γ, and TNF-α are detected both in the brain and blood of schizophrenia patients (60). Furthermore, increased levels of serum IL-18 is positively associated with cognitive deficits in schizophrenia patients (61). To date very little is known about IL-33 in schizophrenia. However, the expression of IL-33 and ST2 in schizophrenia associated brain regions such as prefrontal cortex, basal ganglia, hippocampus, and amygdala (62–65) may suggest its involvement in disease development. Indeed polymorphism of IL-33 is associated with decreased susceptibility to schizophrenia within an Iranian population (66). Additionally, increased levels of serum IL-33 and sST2 correlate with improved cognitive performance in schizophrenia patients, although no difference of either molecules is observed between patients with schizophrenia and healthy controls (67).

Schizophrenia patients frequently exhibit behavioral abnormalities such as enhanced persistence of resting and active periods of locomotor activities (68). IL-33 deficient mice display multiple behavioral deficits such as reduced anxiety and impaired social recognition (21). However, these mice show no obvious changes in locomotor activities, with the exception of older mice (60 weeks old) at which point the activities are increased (18). Naïve adult age (8–10 weeks) mice receiving intraperitoneal injection of rIL-33 do not show any change in locomotor activity when analyzing parameters such as distance moved, velocity, rotations and meander in an open-field test (personal observation). Interestingly in a mouse model of

schizophrenia induced by phencyclidine (PCP) (69–71), a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor (72, 73), rIL-33 dramatically increases the PCP-induced locomotor activity. This is characterized by increased animal moving distance and velocity in open-field tests relative to PCP mice (**Figures 5A–C**; $P < 0.0001$).

The effect of IL-33/ST2 signaling pathway on locomotor activity in both aging (18) and PCP treated mice, but not normal age naïve mice, is very interesting and important. Future studies should reveal whether the IL-33/ST2 signaling pathway only influences locomotor activity in individuals with specific CNS conditions. The underlying mechanism of altered locomotor activities by IL-33 remains unclear, however as an antagonist of NMDA receptors, IL-33 may act on glutamate synapses in a synergistic fashion similarly to IL-1β (74) and TNF-α (75). It is also possible that IL-33 may influence the secretion of glutamine synthetase by astrocytes. This would potentially have an impact on the ability of astrocytes to protect neurons from excitotoxicity which has been shown before with IL-1β and TNF-α (76).

CNS Injury

CNS traumatic or ischemic injury is one of the leading causes of death and permanent disability worldwide. Neuroinflammation, often characterized by type 1 or type 2 immune responses (although an oversimplified description, but helpful in our understanding of the immune responses in diseases), is a prominent feature of CNS injury and highly influences CNS repair and thus the injury recovery process (77–80). As a nuclear alarmin molecule released by damaged dying cells (6) and as an important immunomodulatory cytokine, IL-33 plays an important role in the pathophysiology of CNS injury.

An association between IL-33/ST2 signaling pathway and CNS ischaemic injury has been highlighted by the findings of elevated level of sST2 in the plasma of stroke patients and its correlation with a worsened clinical outcome (81). Significantly increased level of serum IL-33 is also detected in patients with acute ischemic stroke (AIS) compared with healthy controls (82), and is shown to be a novel diagnostic and prognostic biomarker for AIS. Patients with higher IL-33 demonstrate a favorable outcome 3 months after the stroke. In a mouse model of stroke, the expression of IL-33 by oligodendrocytes and astrocytes is rapidly increased together with an upregulation of ST2 on microglia after inducing ischemic brain injury (28). Increased ischemic lesion size and long term behavioral deficits are observed in ST2 deficient mice in comparison to the wild type controls indicating a possible protective role of IL-33 in stroke (28). Indeed administration of rIL-33 locally (28, 83) or peripherally (81) reduces stroke-induced CNS damage and ameliorates neurological deficits via inducing anti-inflammatory responses systemically and M2 type macrophages and microglia in the CNS. This occurs at least partially in an IL-4-dependent manner (81). Meanwhile infusion of rIL-33 intracerebroventricularly protects mice from ischemic injury by inducing a switch from Th1 to Th2 and suppressing Th17 responses (83), or by potentiating the expression of IL-10 and other M2 genes in microglia (28). Indeed mice deficient in ST2 gene have impaired expression of M2 polarizing markers (28).

The IL-33/ST2 signaling pathway is also closely involved in the neuroinflammatory response following CNS traumatic injury. Significantly increased levels of IL-33, likely released by oligodendrocytes (12) and astrocytes (31), are detected in injured spinal cord segments (12, 31) and in the cerebrospinal fluid (CSF) (12). Gadani et al. further discovered that the baseline expression level of *St2* transcript by astrocytes is low but significantly elevated after injury. At the same time its expression level on microglia is shifted from high at baseline to low after injury. From these data the authors concluded that astrocytes but not microglia are likely to be the primary target cells of IL-33 mediating the neuroinflammation after injury. It also confirms the dynamic change of IL-33 expression in CNS tissues under diseased conditions (12). Mice with spinal cord injury exhibit significantly reduced tissue damage, demyelination and astrogliosis after treatment with rIL-33 (31). This is associated with suppressed pro-inflammatory immune responses and biased anti-inflammatory M2 microglia/macrophages and Treg cells both locally in the spinal cord and systemically (31). On the contrary, mice deficient of IL-33 gene develop increased lesion size together with significantly decreased number of M2 microglia and macrophages (12). The investigators further demonstrated that IL-33 released from damaged oligodendrocytes upon injury orchestrates the production of various chemokines such as CCL2 and CXCL2 by astrocytes, resulting in the recruitment of monocytes into the CNS. As a potent activator of ST2 expressing ILC2s (39, 58, 84), IL-33 released into the CSF immediately after injury also mediates CNS inflammation through the newly discovered meningeal ILC2s (85). IL-33 therefore is released as an alarmin after injury to orchestrate the activations of both CNS cells and immune cells with the aim to promote recovery (12).

Taken together, in both ischemic and traumatic CNS injury, IL-33 signaling appears to be beneficial. IL-33 released by damaged oligodendrocytes, astrocytes and possibly other cells at the injury site coordinates the immune responses mediated by CNS resident cells, meningeal ILC2s and infiltrating immune cells. This ultimately promotes a type 2-like neuroinflammation and confers protection from tissue damage and neurological deficit.

Pain

Given the prevalence of chronic pain and the fact that current treatment only provides moderate degrees of relief, it is both challenging and important to understand the molecular mechanisms of pain. Over the years astrocytes and microglia cells have emerged as key contributors to the pathological development of pain (86) through releasing a number of important molecules such as IL-33.

IL-33 was first identified as a key mediator of inflammatory hypernociception in an animal model of arthritis (87), and in carrageenan-induced inflammatory pain likely through triggering the production of inflammatory mediators such as TNF-α (88). In a more recent study, Zarpelon et al. show that intrathecal injection of IL-33 induces hyperalgesia

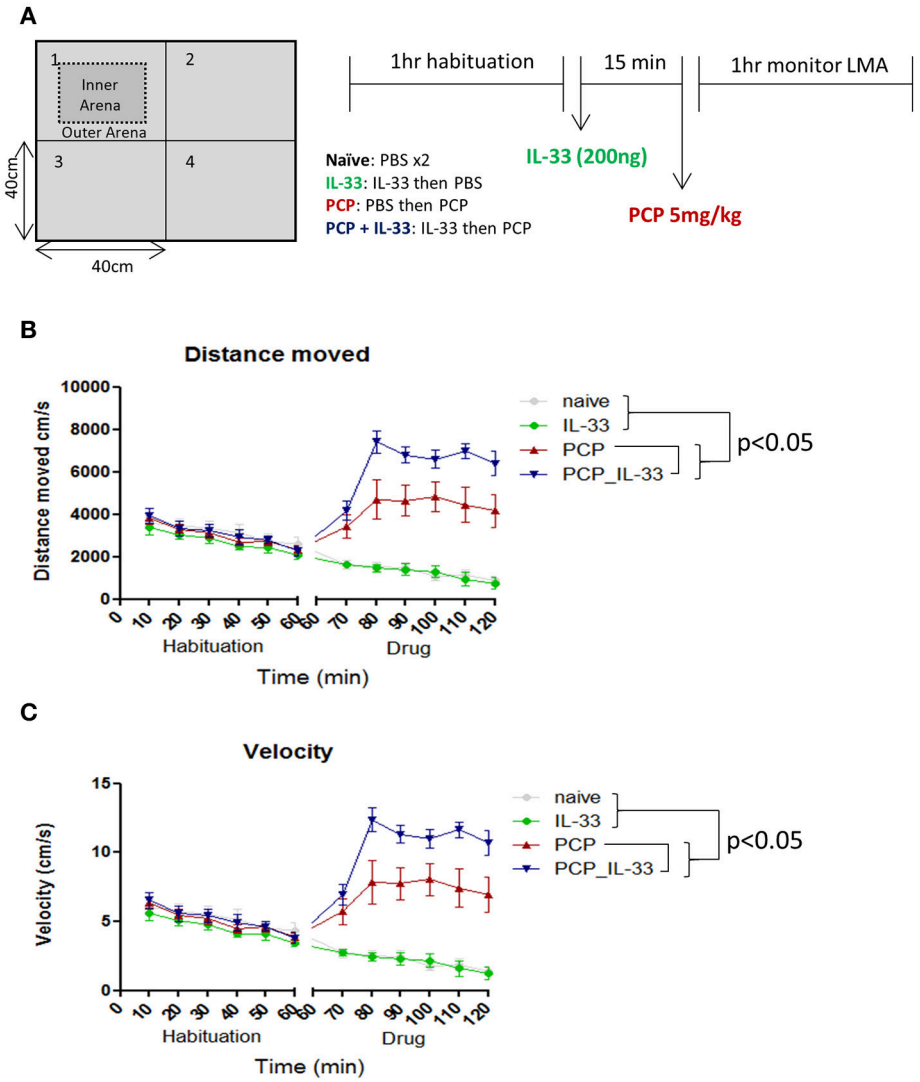


FIGURE 5 | IL-33 enhances PCP-induced changes in locomotor activity. **(A)** C57BL/6 mice were first habituated inside a 40 × 40cm open field maze for 1 h before being taken out of the maze and injected intraperitoneally with vehicle control (PBS) or 200 ng IL-33. After 15 min mice received an i.p. injection of either PBS or PCP (5 mg/kg), and were then placed back into the same open field maze for 1 h. Their **(B)** distance moved and **(C)** velocity were tracked via overhead cameras and analyzed. Significant ($p < 0.05$) pairwise differences according to Tukey's *post hoc* test show PCP_IL-33 > PCP > naïve and IL-33 for both distance moved and velocity. LMA: locomotor activity.

in naïve mice and enhances hyperalgesia caused by chronic constriction injury (CCI) (34). Furthermore, hyperalgesia is reduced in mice deficient of *St2* gene or treated with IL-33 decoy receptor sST2 suggesting IL-33 has an important role in neuropathic pain. The group further demonstrated IL-33 is mainly produced by spinal cord oligodendrocytes after CCI which subsequently acts on astrocytes and microglia. Furthermore, IL-33-mediated hyperalgesia is dependent on a reciprocal relationship with TNF-α and IL-1β. This mechanism of action of IL-33 is supported by findings from a rat model of radicular pain (35). Non-compressive lumbar disc herniation induces expression of IL-33 and ST2 in the spinal cord which subsequently mediates the development and progression of radicular pain through modulating TNF-α, IL-1β, and COX-2. The pathogenic contribution of IL-33 toward pain is further confirmed in a spared nerve injury induced neuropathic pain model (33). Blocking of IL-33/ST2 signaling with ST2 neutralizing antibody or *St2* gene depletion significantly attenuates mechanical and cold allodynia. Liu et al. reported that IL-33 induced nociceptive behavior involves the spinal NMDAR and is mediated through activation of the astroglial JAK2–STAT3 cascade and the neuronal CaMKII–CREB cascade (33).

Confirming the pathological contribution of IL-33/ST2 signaling pathway in pain development, effective reduction of CCI induced pain by different analgesics is shown to

TABLE 3 | Role of IL-33/ST2 axis in neurological disease.

Disease	Role of IL-33	References
Alzheimer disease (AD)	• Reduced IL-33 expression in AD brain; *Il33* genetic variants associate with a decrease risk in AD	(46)
	• Increased IL-33 and ST2 in AD brains, Aβ induces IL-33 production by astrocytes to mediate AD pathogenesis	(51)
	• Increased sST2 in serum of AD patients; IL-33 reduces Aβ levels and amyloid plagues and reverses synaptic plasticity impairment and memory deficit in a mouse model	(52)
	• Effective treatment of *APP/PS1* mice with improved learning and memory capability is associated with reduced level of IL-33	(53)
	• IL-33 expression in astrocytes increases with age and IL-33$^{-/-}$ aged mice display AD-like symptoms with tau abnormality and neurodegeneration	(18)
Multiple sclerosis (MS)	• Elevated IL-33 protein, mRNA levels in NAWM and plaque areas, and in plasma and PBMCs	(19, 106)
	• Enhanced expression of IL-33 and ST2 in MS CNS lesion	(13)
	• mRNA of IL-33 and ST2 increased in spinal cord of EAE rats	(55)
	• IL-33 blockade suppresses EAE development in mice	(56)
	• IL-33 attenuates EAE by inducing type 2 immune response	(29)
	• IL-33 released by astrocytes and neurons suppresses EAE	(32)
	• Male-specific IL-33 attenuates EAE through ILC2s and mast cells	(57)
Pain	• IL-33 is a key mediator of inflammatory hyper nociception	(87)
	• IL-33 mediates carrageenan-induced inflammatory pain via trigging TNF-α	(88)
	• IL-33 induces hyperalgesia in naïve mice and enhanced hyperalgesia in a mouse model of chronic constriction injury via reciprocal relationship with TNF-α and IL-1β	(34)
	• IL-33 inhibits electroacupuncture analgesia in formalin mice	(92)
	• Spinal IL-33 and ST2 contribute to bone cancer induced pain in mice	(30)
	• IL-33 contributes to neuropathic pain through activating astroglial and neuronal cascades, and up-regulate NMDA	(33)
	• IL-33 induced by non-compressive lumber disk herniation in rat mediates pain through TNF-α, IL-1β and COX-2	(35)
Psychiatric disorders	• IL-33 polymorphism associates with decreased susceptibility to schizophrenia	(66)
	• Serum levels of IL-33 and sST2 positively correlate with cognitive performance in schizophrenia patients, but with no difference between patients and controls	(67)
	• IL-33$^{-/-}$ mice display multiple behavioral deficits, e.g., reduced anxiety and impaired social recognition	(21)
	• IL-33$^{-/-}$ aged mice exhibit increased locomotor activities	(18)
	• IL-33, sST2 and IL-1β plasma levels are not changed in ASD patients	(107)
	• Reduced plasma IL-33 in ASD patients	(108)
Stroke	• sST2 is increased in plasma of stroke patients, IL-33 protects mice against stroke through IL-4	(81)
	• IL-33 ameliorates brain injury in stroke model through promoting Th2 and suppressing Th17 responses	(83)
	• Increased serum IL-33 in acute ischemic stroke patients	(82)
	• IL-33/ST2 dependent microglial response limits acute ischemic brain injury	(28)
Traumatic CNS injury	• Oligodendrocyte-derived IL-33 induces M2 genes and aids the recovery of optic nerve injury in mice	(12)
	• IL-33 reduces secondary injury and improves recovery in a mouse contusion injury model through inducing Th2 and Treg responses	(31)
	• IL-33 improves spinal cord injury via activating meningeal ILC2s to produce type 2 cytokines	(85)
Cerebral malaria	• IL-33 treatment reduces parasitaemia at early phase of infection through inducing type 2 immune responses	(39)
	• ST2 deficient mice survived longer than WT after infection	(97)
CNS encephalitis	• Levels of IL-33 protein and mRNA increased in the serum and CSF of neuro-Behcet's disease patients	(109)
	• IL-33 attenuates viral induced encephalitis by downregulating IFN-γ and NO production	(110)
CNS Toxoplasmosis	• ST2 Levels increased in brain of infected mice, ST2 deficient mice had increased susceptibility, parasite burden and encephalitis to cerebral infection	(95)
CNS hemorrhage	• IL-33 encoding gene *Dvs 27* is active after experimental subarachnoid hemorrhage	(111)
	• *Il33* mRNA elevated in cerebral cortex in subarachnoid hemorrhage	(26)
	• IL-33 has neuroprotective effects in intracerebral hemorrhage in mouse	(112)

be mediated through inhibition of the IL-33/ST2 signaling pathway (89–91). This also agrees with a report by Han et al. suggesting electro acupuncture analgesia alleviates formalin-induced inflammatory pain in mice, at least partially, through inhibition of spinal IL-33/ST2 signaling and the downstream ERK and JNK pathways (92).

Based on the above findings, it is likely that IL-33/ST2 induces and/or augments pain in the nervous system through

potentiating TNF-α and IL-1β mediated inflammation. Thus, future therapeutic strategies for patients with pain may be developed by targeting the IL-33/ST2 signaling pathway along with current treatments.

CNS Parasitic Infection

IL-33 is important in limiting protozoan parasite infections systemically (93, 94), with several studies particularly focused on its role in neuropathology induced by parasite infections. Toxoplasma gondii (*T. gondii*) is an obligate, intracellular parasite which is able to persist, often asymptomatically, for a lifetime within the CNS of immunocompetent hosts. *St2* mRNA is upregulated in the brain of mice infected with *T. gondii* (95). ST2 deficient mice ($T1/St2^{-/-}$) demonstrate an increased susceptibility to cerebral infection with an increased parasite burden and more severe encephalitis which is associated with greater cerebral expression of iNOS, TNF-α and IFN-γ (95). This indicates a possible protective role for IL-33/ST2 signaling in *T. gondii* infection.

A specific role of IL-33 has been investigated in cerebral malaria which is the most severe form of neurological complications associated with *Plasmodium falciparum* infection. It results in long term cognitive deficits, behavioral difficulties, epilepsy, coma and, in many cases, death (96). Increased IL-33 expression is shown in the brains of experimental cerebral malaria (ECM) mice induced by murine *Plasmodium berghei* ANKA (*Pb*A) (25, 97), which is likely expressed by astrocytes and oligodendrocytes (25). Meanwhile, ST2 deficient mice do not exhibit ECM associated neurological signs or associated cognitive deficits unlike their wild type counterparts despite similar levels of parasitaemia and parasite load (25, 97). There appears to be reduced neuroinflammation together with a reduction in activated $CD4^+$ and $CD8^+$ T cells and pro-inflammatory cytokines and chemokine levels within the brain parenchyma of ST2 deficient mice. A potential feedback loop has been further suggested between microglia and oligodendrocytes in exacerbating neuroinflammation by the initial infection. IL-33 stimulates IL-1β production by microglia, which in turn induces IL-33 expression by oligodendrocytes (25). These results suggest IL-33/ST2 signaling plays a role in promoting the effector and cytotoxic T cell responses and enhancing the CNS resident immune response, which combined contributes to the neuropathology and cognitive deficits associated with ECM. Paradoxically, administration of IL-33 in the early stages of ECM improves animal survival time with reduced weight loss and clinical score, but not from malaria-induced hyperparasitemia and death compared to control mice (39). The study provided further evidence of a protective immune response via IL-33-induced ILC2s, M2 macrophages, and T regs. It is not known what has caused the discrepancy between the above findings, the data may highlight the difference between research approaches using gene modified animals and exogenous administration of reagents.

Thus, while the study by Jones et al. illustrates a protective role for IL-33 in *T. gondii* infection (95), the precise pathophysiological function of IL-33/ST2 in ECM remains to be determined.

Glioma

The role of IL-33 in tumor progression has been illustrated elsewhere, with overexpression of IL-33 identified as a diagnostic and prognostic marker for several types of cancer (98–101). Within the CNS, IL-33 is abundantly expressed by rat glioma cells together with its receptor ST2 (102). In glioma patients, increased expression of IL-33 (103, 104) and ST2 (104) is detected in tumor tissues albeit heterogeneously, compared with normal brain tissue (103). Zhang et al. also reported higher expression of *Il33* mRNA in glioma tissues than in normal brain tissue, and that the level correlates with a shorter progression-free survival and overall survival than those with low expression (105). Furthermore, while there is no differential IL-33 protein expression by tumor grade, elevated levels of IL-33 protein, and mRNA are associated with inferior survival in patients with recurrent glioblastomas. All of these data suggest a pivotal role for IL-33 in the disease pathogenesis and as a potential biomarker for prognosis of human gliomas.

It has become clear that the IL-33/ST2 signaling pathway facilitates glioma cell proliferation and migration, as treatment of the cells with IL-33 shRNA or ST2 shRNA reduces cell growth and colony formation in culture and reduces tumor volume *in vivo* (102). Further evidence indicates that the involvement of IL-33 in glioma cell invasion and migration is through upregulation of MMP2 and MMP9 via the ST2-NF-κB signaling pathway (104).

Thus, IL-33 may become a useful biomarker in predicting prognosis in glioma patients, and a novel therapeutic target for glioma treatment.

Other CNS Diseases

Emerging evidence suggests that the role of IL-33 is not limited to the diseases highlighted in this paper (**Table 3**) and is expanding to other CNS diseases. Expression level of IL-33 is significantly reduced while sST2 increased in the serum samples of amyotropic lateral sclerosis patients (113). In a rat model of subarachnoid hemorrhage (SAH), expression of IL-33 in the cerebral cortex after injury is markedly elevated in the SAH together with IL-1β and TNF-a (26). IL-33 signaling pathway is also shown to be essential in attenuating viral-induced encephalitis development by downregulating iNOS expression in the CNS (110). Although it is not changed in autism spectrum disorder patients (107), increased plasma level of IL-33 is also observed in bipolar disorder patients (114). While it will take time to appreciate the specific and important function of IL-33/ST2 in various CNS disorders, future research progress will provide new knowledge and lead to improved diagnosis and therapies for patients.

CONCLUSIONS

The broad expression of IL-33 and ST2 in different CNS regions and cells, in addition to many well-documented immune cells, suggests the involvement of IL-33/ST2 signaling pathway in the complex network of multi-cell interaction between the immune and nervous systems in CNS health and disease. Scientific advancements during the last decade have provided some initial insights into the diverse and pleiotropic role IL-33

plays in certain aspects of CNS function and disease through interactions between glia, neuron, and immune cells. However, as the CNS is the most complex organ, and human disease often displays multiple layers of pathophysiology, the challenge now is to determine in more detail the intensive and dynamic communications among the CNS resident and infiltrating cells. This would enhance our understanding of the important roles the IL-33/ST2 signaling pathway plays in CNS homoeostasis and neurological disorders. These findings will advance our diagnosis of CNS diseases, and develop novel effective therapeutics for patients.

ETHICS STATEMENT

Tissues in **Figures 1–4** were collected from naïve C57BL/6 mice at 8–10 weeks of age. All animal care and experimental procedures were conducted in accordance with relevant guidelines and regulations with the approval of the University of Strathclyde Animal Welfare and Ethical Review Body (AWERB), under UK Home Office regulations [Animals (Scientific Procedures) Act 1986, UK]. Animals are housed according to the Home Office Code of Practice for the housing and care of animals bred, supplied or used for scientific purposes.

AUTHOR CONTRIBUTIONS

KF-C, MB, SH, and DA performed the experiments and analyzed the data. MB, CW, SH, DA, H-RJ and KF-C made contribution to data interpretation, figure and manuscript preparation. MB and H-RJ revised and finalized the files for submission.

ACKNOWLEDGMENTS

The authors are grateful to Professor Judith A Pratt and Dr David M Thomson for their expert advice in PCP induced disease model and locomotor activity data analysis. The study received financial support from Medical Research Scotland (Grant no. 440FRG).

REFERENCES

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
2. Tominaga S, A putative protein of a growth specific cDNA from BALB/C-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS Lett.* (1989) 258:301–4. doi: 10.1016/0014-5793(89)81679-5
3. Kakkar R, Hei H, Dobner S, Lee RT. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J Biol Chem.* (2012) 287:6941–8. doi: 10.1074/jbc.M111.298703
4. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol.* (2010) 10:103–10. doi: 10.1038/nri2692
5. Pei C, Barbour M, Fairlie-Clarke KJ, Allan D, Mu R, Jiang HR. Emerging role of interleukin-33 in autoimmune diseases. *Immunology* (2014) 141:9–17. doi: 10.1111/imm.12174.
6. Lüthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* (2009) 31:84–98. doi: 10.1016/j.immuni.2009.05.007
7. Mirchandani AS, Salmond RJ, Liew FY. Interleukin-33 and the function of innate lymphoid cells. *Trends Immunol.* (2012) 33:389–96. doi: 10.1016/j.it.2012.04.005
8. De la Fuente M, MacDonald TT, Hermoso MA. The IL-33/ST2 axis: Role in health and disease. *Cytokine Growth Factor Rev.* (2015) 26:615–23. doi: 10.1016/j.cytogfr.2015.07.017.
9. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Immunol.* (2008) 7:827–40. doi: 10.1111/j.1743-6109.2008.01122.x.Endothelial
10. Liew FY, Girard J-P, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
11. Milovanovic M, Volarevic V, Ljujic B, Radosavljevic G, Jovanovic I, Arsenijevic N, et al. Deletion of IL-33R (ST2) abrogates resistance to EAE in BALB/C mice by enhancing polarization of APC to inflammatory phenotype. *PLoS ONE* (2012) 7:1–13. doi: 10.1371/journal.pone.0045225.
12. Gadani SP, Walsh JT, Smirnov I, Zheng J, Kipnis J. The glia-derived alarmin IL-33 orchestrates the immune response and promotes recovery following CNS injury. *Neuron* (2015) 85:703–9. doi: 10.1016/j.neuron.2015.01.013
13. Allan D, Fairlie-Clarke KJ, Elliott C, Schuh C, Barnett SC, Lassmann H, et al. Role of IL-33 and ST2 signaling pathway in multiple sclerosis: expression by oligodendrocytes and inhibition of myelination in central nervous system. *Acta Neuropathol Commun.* (2016) 4:75. doi: 10.1186/s40478-016-0344-1
14. Yasuoka S, Kawanokuchi J, Parajuli B, Jin S, Doi Y, Noda M, et al. Production and functions of IL-33 in the central nervous system. *Brain Res.* (2011) 1385:8–17. doi: 10.1016/j.brainres.2011.02.045
15. Cao K, Liao X, Lu J, Yao S, Wu F, Zhu X, et al. IL-33 / ST2 plays a critical role in endothelial cell activation and microglia-mediated neuroinflammation modulation. *J Neuroinflammation* (2018) 15:1–12. doi: 10.1186/s12974-018-1169-6
16. Vainchtein ID, Chin G, Cho FS, Kelley KW, Miller JG, Chien EC, et al. Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. *Science* (2018) 359:1269–73. doi: 10.1126/science.aal3589
17. Wicher G, Husic E, Nilsson G, Forsberg-Nilsson K. Developmental expression of IL-33 in the mouse brain. *Neurosci Lett.* (2013) 555:171–6. doi: 10.1016/j.neulet.2013.09.046
18. Carlock C, Wu J, Shim J, Moreno-Gonzalez I, Pitcher MR, Hicks J, et al. Interleukin33 deficiency causes tau abnormality and neurodegeneration with Alzheimer-like symptoms in aged mice. *Transl Psychiatry* (2017) 7:e1164. doi: 10.1038/tp.2017.142
19. Christophi GP, Gruber RC, Panos M, Christophi RL, Jubelt B, Massa PT. Interleukin-33 upregulation in peripheral leukocytes and CNS of multiple sclerosis patients. *Clin Immunol.* (2012) 142:308–19. doi: 10.1016/j.clim.2011.11.007.Interleukin-33
20. Zandee SEJ, O'Connor RA, Mair I, Leech MD, Williams A, Anderton SM. IL-10-producing, ST2-expressing Foxp3 + T cells in multiple sclerosis brain lesions. *Immunol Cell Biol.* (2017) 95:484–90. doi: 10.1038/icb.2017.3
21. Dohi E, Choi EY, Rose IVL, Murata AS, Chow S, Niwa M, et al. Behavioral changes in mice lacking interleukin-33. *J Neurosci.* (2017) 4:ENEURO.0147-17.2017. doi: 10.1523/ENEURO.0147-17.2017
22. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, 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) 188:3488–95. doi: 10.4049/jimmunol.1101977
23. Feng X, Valdearcos M, Uchida Y, Lutrin D, Maze M, Koliwad SK. Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. *JCI Insight* (2017) 2:e91229. doi: 10.1172/jci.insight.91229
24. Hudson CA, Christophi GP, Gruber RC, Wilmore JR, Lawrence DA, Massa PT. Induction of IL-33 expression and activity in central nervous system glia. *J. Leukoc. Biol.* (2008) 84:631–43. doi: 10.1189/jlb.1207830

25. Reverchon F, Mortaud S, Sivoyon M, Maillet I, Laugeray A, Palomo J, et al. IL-33 receptor ST2 regulates the cognitive impairments associated with experimental cerebral malaria. *PLoS Pathog.* (2017) 13:e1006322. doi: 10.1371/journal.ppat.1006322
26. Huang L-T, Li H, Sun Q, Liu M, Li W-D, Li S, et al. IL-33 expression in the cerebral cortex following experimental subarachnoid hemorrhage in rats. *Cell Mol Neurobiol.* (2015) 35:493–501. doi: 10.1007/s10571-014-0143-9
27. Natarajan C, Yao SY, Sriram S. TLR3 agonist poly-IC induces IL-33 and promotes myelin repair. *PLoS ONE* (2016) 11:e0152163. doi: 10.1371/journal.pone.0152163
28. Yang Y, Liu H, Zhang H, Ye Q, Wang J, Yang B, et al. ST2/IL-33-dependent microglial response limits acute ischemic brain injury. *J Neurosci.* (2017) 37:4692–704. doi: 10.1523/JNEUROSCI.3233-16.2017
29. Jiang HR, Milovanović M, Allan D, Niedbala W, Besnard AG., Fukada SY, et al. IL-33 attenuates EAE by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages. *Eur. J. Immunol.* (2012) 42:1804–14. doi: 10.1002/eji.201141947
30. Zhao J, Zhang H, Han P, Hu S, Li Q. Spinal interleukin-33 and its receptor ST2 contribute to bone cancer-induced pain in mice. *Neuroscience* (2013) 253:172–82. doi: 10.1016/j.neuroscience.2013.08.026
31. Pomeshchik Y, Kidin I, Korhonen P, Savchenko E, Jaronen M, Lehtonen S, et al. Interleukin-33 treatment reduces secondary injury and improves functional recovery after contusion spinal cord injury. *Brain Behav Immun.* (2015) 44:68–81. doi: 10.1016/j.bbi.2014.08.002
32. Chen H, Sun Y, Lai L, Wu H, Xiao Y, Ming B, et al. Interleukin-33 is released in spinal cord and suppresses experimental autoimmune encephalomyelitis in mice. *Neuroscience* (2015) 308:157–68. doi: 10.1016/j.neuroscience.2015.09.019
33. Shenbin L, Wen-Li M, Quan L, Meng-Ting Z, Ping H, Shan H, et al. Spinal IL-33/ST2 Signaling Contributes to Neuropathic Pain. *Anesthesiology* (2015) 123:1154–69. doi: 10.1097/ALN.0000000000000850
34. Zarpelon AC, Rodrigues FC, Lopes AH, Souza GR, Carvalho TT, Pinto LG, et al. Spinal cord oligodendrocyte-derived alarmin IL-33 mediates neuropathic pain. *FASEB J.* (2016) 30:54–65. doi: 10.1096/fj.14-267146
35. Huang SJ, Yan JQ, Luo H, Zhou LY, Luo JG. IL-33/ST2 signaling contributes to radicular pain by modulating MAPK and NF-κB activation and inflammatory mediator expression in the spinal cord in rat models of noncompressive lumber disk herniation. *J Neuroinflammation* (2018) 15:1–12. doi: 10.1186/s12974-017-1021-4
36. Liu B, Tai Y, Achanta S, Kaelberer MM, Caceres AI, Shao X, et al. IL-33/ST2 signaling excites sensory neurons and mediates itch response in a mouse model of poison ivy contact allergy. *Proc Natl Acad Sci.* (2016) 113:7572–9. doi: 10.1073/pnas.1606608113
37. Barbour M, Wood R, Hridi SU, Wilson C, Mckay G, Bushell TJ. The therapeutic effect of anti-CD52 treatment in murine experimental autoimmune encephalomyelitis is associated with altered IL-33 and ST2 expression levels. *J Neuroimmunol.* (2018) 318:87–96. doi: 10.1016/j.jneuroim.2018.02.012
38. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci.* (2006) 7:41–53. doi: 10.1038/nrn1824
39. Besnard AG, Guabiraba R, Niedbala W, Palomo J, Reverchon F, Shaw TN, et al. IL-33-mediated protection against experimental cerebral malaria is linked to induction of type 2 innate lymphoid cells, M2 macrophages and regulatory T cells. *PLoS Pathog.* (2015) 11:e1004607. doi: 10.1371/journal.ppat.1004607
40. Bloom JS, Hynd GW. The role of the corpus callosum in interhemispheric transfer of information: Excitation or inhibition? *Neuropsychol Rev.* (2005) 15:59–71. doi: 10.1007/s11065-005-6252-y
41. Ozturk A, Smith S, Gordon-Lipkin E, Harrison D, Shiee N, Pham D, et al. MRI of the corpus callosum in multiple sclerosis: association with disability. *Mult Scler.* (2010) 16:166–77. doi: 10.1177/1352458509353649.MRI
42. Rimkus CDM, Junqueira TDF, Lyra KP, Jackowski MP, Machado MAR, Miotto EC, et al. Corpus callosum microstructural changes correlate with cognitive dysfunction in early stages of relapsing-remitting multiple sclerosis: axial and radial diffusivities approach. *Mult Scler Int.* (2011) 2011:1–31. doi: 10.1155/2011/304875
43. Kimelberg HK, Nedergaard M. Functions of astrocytes and their potential as therapeutic targets. *Neurotherapeutics* (2010) 7:338–53. doi: 10.1016/j.nurt.2010.07.006
44. Lanjakornsiripan D, Pior BJ, Kawaguchi D, Furutachi S, Tahara T, Katsuyama Y, et al. Layer-specific morphological and molecular differences in neocortical astrocytes and their dependence on neuronal layers. *Nat Commun.* (2018) 9:1–13. doi: 10.1038/s41467-018-03940-3
45. Verkhratsky A, Nedergaard M. The homeostatic astroglia emerges from evolutionary specialization of neural cells. *Philos Trans R Soc B Biol Sci.* (2016) 371:1–29. doi: 10.1098/rstb.2015.0428
46. Chapuis J, Hot D, Hansmannel F, Kerdraon O, Ferreira S, Hubans C, et al. Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. *Mol Psychiatry* (2009) 14:1004–16. doi: 10.1038/mp.2009.10
47. Kempuraj D, Khan MM, Thangavel R, Xiong Z, Yang E, Zaheer A. Glia maturation factor induces interleukin-33 release from astrocytes: implications for neurodegenerative diseases. *J Neuroimmune Pharmacol.* (2013) 8:643–50. doi: 10.1007/s11481-013-9439-7
48. Glenner GG. Reprint of "Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein." *Biochem Biophys Res Commun.* (2012) 425:534–9. doi: 10.1016/j.bbrc.2012.08.020
49. Tian M, Deng YY, Hou DR, Li W, Feng XL, Yu ZL. Association of IL-1, IL-18, and IL-33 gene polymorphisms with late-onset Alzheimer's disease in a Hunan Han Chinese population. *Brain Res.* (2015) 1596:136–45. doi: 10.1016/j.brainres.2014.11.019
50. Yu JT, Song JH, Wang ND, Wu ZC, Zhang Q, Zhang N, et al. Implication of IL-33 gene polymorphism in Chinese patients with Alzheimer's disease. *Neurobiol. Aging* (2012) 33:1014.e11–1014.e14. doi: 10.1016/j.neurobiolaging.2010.07.003
51. Xiong Z, Thangavel R, Kempuraj D, Yang E, Zaheer S, Zaheer A. Alzheimer's disease: Evidence for the expression of interleukin-33 and its receptor ST2 in the brain. *J Alzheimer's Dis.* (2014) 40:297–308. doi: 10.3233/JAD-132081
52. Fu AKY, Hung K-W, Yuen MYF, Zhou X, Mak DSY, Chan ICW, et al. IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline. *Proc Natl Acad Sci.USA*. (2016) 113:2705–13. doi: 10.1073/pnas.1604032113
53. Xiao S, Zhou D, Luan P, Gu B, Feng L, Fan S, et al. Graphene quantum dots conjugated neuroprotective peptide improve learning and memory capability. *Biomaterials* (2016) 106:98–110. doi: 10.1016/j.biomaterials.2016.08.021
54. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol.* (2009) 183:6469–77. doi: 10.4049/jimmunol.0901575
55. Zhao X, Zhang X, Lv Y, Xu Y, Li M, Pan Q, et al. Matrine downregulates IL-33/ST2 expression in the central nervous system of rats with experimental autoimmune encephalomyelitis. *Immunol Lett.* (2016) 178:97–104. doi: 10.1016/j.imlet.2016.08.007
56. Li M, Li Y, Liu X, Gao X, Wang Y. IL-33 blockade suppresses the development of experimental autoimmune encephalomyelitis in C57BL/6 mice. *J Neuroimmunol.* (2012) 247:25–31. doi: 10.1016/j.jneuroim.2012.03.016
57. Russi AE, Ebel ME, Yang Y, Brown MA. Male-specific IL-33 expression regulates sex-dimorphic EAE susceptibility. *Proc Natl Acad Sci USA*. (2018) 115:1520–9. doi: 10.1073/pnas.1710401115
58. Lefrancais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc Natl Acad Sci USA*. (2014) 111:15502–7. doi: 10.1073/pnas.1410700111
59. Watanabe Y, Someya T, Nawa H. Cytokine hypothesis of schizophrenia pathogenesis: Evidence from human studies and animal models. *Psychiatry Clin Neurosci.* (2010) 64:217–30. doi: 10.1111/j.1440-1819.2010.02094.x
60. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol Psychiatry* (2011) 70:663–71. doi: 10.1016/j.biopsych.2011.04.013
61. Zhang XY, Tang W, Xiu MH, Chen DC, Yang FD, Tan YL, et al. Interleukin 18 and cognitive impairment in first episode and drug naïve schizophrenia versus healthy controls. *Brain Behav Immun.* (2013) 32:105–111. doi: 10.1016/j.bbi.2013.03.001

62. Duan M, Chen X, He H, Jiang Y, Jiang S, Xie Q, et al. Altered basal ganglia network integration in schizophrenia. *Front Hum Neurosci.* (2015) 9:561. doi: 10.3389/fnhum.2015.00561
63. Heckers S, Konradi C. GABAergic mechanisms of hippocampal hyperactivity in schizophrenia. *Schizophr Res.* (2016) 167:4–11. doi: 10.1016/j.schres.2014.09.041.GABAergic
64. Lawrie S, Whalley H, Job D, Johnstone E. Structural and functional abnormalities of the amygdala in schizophrenia. *Ann N Y Acad Sci.* (2003) 985:445–60. doi: 10.1196/annals.1340.018
65. Wible CG, Anderson J, Shenton ME, Kricun A, Hirayasu Y, Tanaka S, et al. Prefrontal cortex, negative symptoms, and schizophrenia: an MRI study. *Psychiatry Res.* (2001) 108:65–78. doi: 10.1016/S0925-4927(01)00109-3
66. Kordi-tamandani DM, Bahrami AR, Sabbaghi,- R, Soleimani H, and Baranzehi T. Analysis of IL-33 gene polymorphism (rs11792633 C/T) and risk of schizophrenia. *Mol Biol Res Commun.* (2016) 5:45–48. doi: 10.22099/MBRC.2016.3485
67. de Campos-Carli SM, Miranda AS, Dias ICS, de Oliveira A, Cruz BF, Vieira ÉLM, et al. Serum levels of interleukin-33 and its soluble form receptor (sST2) are associated with cognitive performance in patients with schizophrenia. *Compr Psychiatry* (2017) 74:96–101. doi: 10.1016/j.comppsych.2017.01.008
68. Sano W, Nakamura T, Yoshiuchi K, Kitajima T, Tsuchiya A, Esaki Y, et al. Enhanced persistency of resting and active periods of locomotor activity in schizophrenia. *PLoS ONE* (2012) 7:e0043539. doi: 10.1371/journal.pone.0043539
69. Casta,ñé A, Santana N, Artigas F. PCP-based mice models of schizophrenia: Differential behavioral, neurochemical and cellular effects of acute and subchronic treatments. *Psychopharmacology* (2015) 232:4085–97. doi: 10.1007/s00213-015-3946-6
70. Egerton A, Reid L, McKerchar CE, Morris BJ, Pratt JA. Impairment in perceptual attentional set-shifting following PCP administration: a rodent model of set-shifting deficits in schizophrenia. *Psychopharmacology* (2005) 179:77–84. doi: 10.1007/s00213-004-2109-y
71. Kalinichev M, Robbins MJ, Hartfield EM, Maycox PR, Moore SH, Savage KM, et al. Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague-Dawley rats: a locomotor activity and gene induction study. *Prog Neuro Psychopharmacol Biol Psychiatry* (2008) 32:414–22. doi: 10.1016/j.pnpbp.2007.09.008
72. Cohen B, Rosenbaum G, Luby E, Gottlieb J. Comparison of phencyclidine hydrochloride (sernyl) with other drugs: simulation of schizophrenic performance with phencyclidine hydrochloride (sernyl), lysergic acid diethylamide (lsd-25), and amobarbital (amytal) sodium; *ii.* symbolic and sequential think. *Arch Gen Psychiatry* (1962) 6:395–401. doi: 10.01710230063007
73. Javitt D, Zukin S. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* (1991) 148:1301–8. doi: 10.1176/ajp.148.10.1301
74. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, et al. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci.* (2003) 23:8692–700. doi: 10.1523/JNEUROSCI.23-25-08692.2003
75. Wheeler D, Knapp E, Bandaru VVR, Wang Y, Knorr D, Poirier C, et al. TNFα-induced neutral sphingomyelinase-2 modulates synaptic plasticity by controlling the membrane insertion of NMDA receptorsReceptors. *J Neurochem.* (2009) 109:1237–49. doi: 10.1111/j.1471-4159.2009.06038.x.TNF
76. Huang TL, Banion KO. Interleukin-1B and tumor necrosis factor-a suppress dexamethasone induction of glutamine synthetase in primary mouse astrocytes. *J Neurochem* (1998) 71:1436–42. doi: 10.1007/BF01866214
77. Evans T, Barkauskas D, Myers J, Hare E, You J, Ransohoff R, et al. High-resolution intravital imaging reveals that blood-derived macrophages but not resident microglia facilitate secondary axonal dieback in traumatic spinal cord injury. *Exp Neurol.* (2014) 254:109–20. doi: 10.1016/j.asieco.2008.09.006.EAST
78. Kroner A, Greenhalgh AD, Zarruk JG, PassosdosSantos R, Gaestel M, David S. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron* (2014) 83:1098–116. doi: 10.1016/j.neuron.2014.07.027
79. Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Kim K, et al. Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. *Immunity* (2014) 38:555–69. doi: 10.1016/j.immuni.2013.02.012.recruitment
80. Walsh JT, Hendrix S, Boato F, Smirnov I, Zheng J, Lukens JR, et al. MHCII-independent CD4+ T cells protect injured CNS neurons via IL-4. *J Clin Invest.* (2015) 125:699–714. doi: 10.1172/JCI76210
81. Korhonen P, Kanninen KM, Lehtonen Š, Lemarchant S, Puttonen KA, Oksanen M, et al. Immunomodulation by interleukin-33 is protective in stroke through modulation of inflammation. *Brain Behav Immun.* (2015) 49:322–36. doi: 10.1016/j.bbi.2015.06.013
82. Qian L, Yuanshao L, Wensi H, Yulei Z, Xiaoli C, Brian W, et al. Serum IL-33 is a novel diagnostic and prognostic biomarker in acute ischemic stroke. *Aging Dis.* (2016) 7:614–22. doi: 10.14336/AD.2016.0207
83. Luo Y, Zhou Y, Xiao W, Liang Z, Dai J, Weng X, et al. Interleukin-33 ameliorates ischemic brain injury in experimental stroke through promoting Th2 response and suppressing Th17 response. *Brain Res.* (2015) 1597:86–94. doi: 10.1016/j.brainres.2014.12.005
84. Jackson DJ, Makrinioti H, Rana BMJ, Shamji BWH, Trujillo-Torralbo MB, Footitt J, et al. IL-33-Dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med.* (2014) 190:1373–82. doi: 10.1164/rccm.201406-1039OC
85. Gadani SP, Smirnov I, Smith AT, Overall CC, Kipnis J. Characterization of meningeal type 2 innate lymphocytes and their response to CNS injury. *J Exp Med.* (2017) 214:285–96. doi: 10.1084/jem.20161982
86. Milligan E, Watkins L. Pathological and protective roles of glia in chronic pain. *Nat Rev Neurosci.* (2009) 10:23–36. doi: 10.1038/nrn2533
87. Verri WA, Guerrero ATG, Fukada SY, Valerio DA, Cunha TM, Xu D, et al. IL-33 mediates antigen-induced cutaneous and articular hypernociception in mice. *Proc Natl Acad Sci USA*. (2008) 105:2723–8. doi: 10.1073/pnas.0712116105
88. Zarpelon AC, Cunha TM, Alves-Filho JC, Pinto LG, Ferreira SH, McInnes IB, et al. IL-33/ST2 signalling contributes to carrageenin-induced innate inflammation and inflammatory pain: Role of cytokines, endothelin-1 and prostaglandin E2. *Br J Pharmacol.* (2013) 169:90–101. doi: 10.1111/bph.12110
89. Bertozzi MM, Rossaneis AC, Fattori V, Longhi-Balbinot DT, Freitas A, Cunha FQ, et al. Diosmin reduces chronic constriction injury-induced neuropathic pain in mice. *Chem Biol Interact.* (2017) 273:180–9. doi: 10.1016/j.cbi.2017.06.014
90. Longhi-Balbinot DT, Rossaneis AC, Pinho-Ribeiro FA, Bertozzi MM, Cunha FQ, Alves-Filho JC, et al. The nitroxyl donor, Angeli's salt, reduces chronic constriction injury-induced neuropathic pain. *Chem Biol Interact.* (2016) 27:617–30. doi: 10.1016/j.ccell.2015.04.006.SRSF2
91. Pinho-Ribeiro FA, Zarpelon AC, Fattori V, Manchope MF, Mizokami SS, Casagrande R, et al. Naringenin reduces inflammatory pain in mice. *Neuropharmacology* (2016) 105:508–19. doi: 10.1016/j.neuropharm.2016.02.019
92. Han P, Liu S, Zhang M, Zhao J, Wang Y, Wu G, et al. Inhibition of spinal interlukin-33/ST2 signaling and downstream ERK and JNK pathways in electroacupuncture analgesia in formalin mice. *PLoS ONE* (2015) 10:e0129576. doi: 10.1371/journal.pone.0129576
93. Rostan O, Arshad MI, Piquet-Pellorce C, Robert-Gangneux F, Gangneux JP, Samson M. Crucial and diverse role of the interleukin-33/ST2 axis in infectious diseases. *Infect Immun.* (2015) 83:1738–48. doi: 10.1128/IAI.02908-14
94. Tong X, Lu F. IL-33/ST2 involves the immunopathology of ocular toxoplasmosis in murine model. *Parasitol Res.* (2015) 114:1897–905. doi: 10.1007/s00436-015-4377-3
95. Jones LA, Roberts F, Nickdel MB, Brombacher F, McKenzie ANJ, Henriquez FL, et al. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with Toxoplasma gondii. *Eur J Immunol.* (2010) 40:426–36. doi: 10.1002/eji.200939705
96. Idro R, Jenkins NE, Newton CR. Pathogenesis, clinical features, and neurological outcome of cerebral malaria. *Lancet Neurol.* (2005) 4:827–40. doi: 10.1016/S1474-4422(05)70247-7

97. Palomo J, Reverchon F, Piotet J, Besnard AG, Couturier-Maillard A, Maillet I, et al. Critical role of IL-33 receptor ST2 in experimental cerebral malaria development. *Eur J Immunol.* (2015) 45:1354–65. doi: 10.1002/eji.201445206
98. Bergis D, Kassis V, Ranglack A, Koeberle V, Piiper A, Kronenberger B, et al. High serum levels of the interleukin-33 receptor soluble ST2 as a negative prognostic factor in hepatocellular carcinoma. *Transl Oncol.* (2013) 6:311–8. doi: 10.1593/tlo.12418
99. Hu L-A, Fu Y, Zhang D-N, Zhang J. Serum IL-33 as a diagnostic and prognostic marker in non- small cell lung cancer. *Asian Pac J Cancer Prev.* (2013) 14:2563–6. doi: 10.7314/APJCP.2013.14.4.2563
100. Milovanovic M, Volarevic V, Radosavljevic G, Jovanovic I, Pejnovic N, Arsenijevic N, et al. IL-33/ST2 axis in inflammation and immunopathology. *Immunol Res.* (2012) 52:89–99. doi: 10.1007/s12026-012-8283-9
101. Tong X, Barbour M, Hou K, Gao C, Cao S, Zheng J, et al. Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways. *Mol Oncol.* (2016) 10:113–25. doi: 10.1016/j.molonc.2015.06.004
102. Fang KM, Yang CS, Lin TC, Chan TC, Tzeng SF. Induced interleukin-33 expression enhances the tumorigenic activity of rat glioma cells. *Neuro Oncol.* (2014) 16:552–66. doi: 10.1093/neuonc/not234
103. Gramatzki D, Frei K, Cathomas G, Moch H, Weller M, Mertz KD. Interleukin-33 in human gliomas: Expression and prognostic significance. *Oncol Lett.* (2016) 12:445–52. doi: 10.3892/ol.2016.4626
104. Zhang JF, Wang P, Yan YJ, Li Y, Guan MW, Yu JJ, et al. IL-33 enhances glioma cell migration and invasion by upregulation of MMP2 and MMP9 via the ST2-NF-κB pathway. *Oncol Rep.* (2017) 38:2033–42. doi: 10.3892/or.2017.5926
105. Zhang J, Wang P, Ji W, Ding Y, Lu X. Overexpression of interleukin-33 is associated with poor prognosis of patients with glioma. *Int J Neurosci.* (2017) 127:210–7. doi: 10.1080/00207454.2016.1175441
106. Zhang F, Tossberg JT, Spurlock CF, Yao S-Y, Aune TM, Sriram S. Expression of IL-33 and its epigenetic regulation in Multiple Sclerosis. *Ann Clin Transl Neurol.* (2014) 1:307–18. doi: 10.1002/acn3.47
107. Barbosa IG, Rodrigues DH, Rocha NP, Sousa LF, da C, Vieira ELM, et al. Plasma levels of alarmin IL-33 are unchanged in autism spectrum disorder: a preliminary study. *J Neuroimmunol.* (2015) 278:69–72. doi: 10.1016/j.jneuroim.2014.11.021
108. Saresella M, Piancone F, Marventano I, Zoppis M, Hernis A, Zanette M, et al. Multiple inflammasome complexes are activated in autistic spectrum disorders. *Brain Behav Immun.* (2016) 57:125–33. doi: 10.1016/j.bbi.2016.03.009
109. Hamzaoui K, Borhani-Haghighi A, Kaabachi W, Hamzaoui A. Increased interleukin 33 in patients with neuro-Behcet's disease: correlation with MCP-1 and IP-10 chemokines. *Cell Mol Immunol.* (2014) 11:613–6. doi: 10.1038/cmi.2014.31
110. Franca RFO, Costa RS, Silva JR, Peres RS, Mendonça LR, Colón DF, et al. IL-33 signaling is essential to attenuate viral-induced encephalitis development by downregulating iNOS expression in the central nervous system. *J Neuroinflammation* (2016) 13:159. doi: 10.1186/s12974-016-0628-1
111. Onda H, Kasuya H, Takakura K, Hori T, Imaizumi T, Takeuchi T, et al. Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. *J Cereb Blood Flow Metab.* (1999) 19:1279–88. doi: 10.1097/00004647-199911000-00013
112. Gao Y, Ma L, Luo C, liang, Wang T, Zhang M, yang, Shen X, et al. IL-33 exerts neuroprotective effect in mice intracerebral hemorrhage model through suppressing inflammation/apoptotic/autophagic pathway. *Mol. Neurobiol.* (2017) 54:3879–92. doi: 10.1007/s12035-016-9947-6
113. Lin CY, Pfluger CM, Henderson RD, McCombe PA. Reduced levels of interleukin 33 and increased levels of soluble ST2 in subjects with amyotrophic lateral sclerosis. *J Neuroimmunol.* (2012) 249:93–5. doi: 10.1016/j.jneuroim.2012.05.001
114. Barbosa IG, Morato IB, De Miranda AS, Bauer ME, Soares JC, Teixeira AL. A preliminary report of increased plasma levels of IL-33 in bipolar disorder: further evidence of pro-inflammatory status. *J Affect Disord.* (2014) 157:1–4. doi: 10.1016/j.jad.2013.12.042

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Fairlie-Clarke, Barbour, Wilson, Hridi, Allan and Jiang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 18 December 2018
doi: 10.3389/fimmu.2018.03026

Inhibiting Glycolysis and ATP Production Attenuates IL-33-Mediated Mast Cell Function and Peritonitis

*Heather L. Caslin[1,2], Marcela T. Taruselli[2], Tamara Haque[2], Neha Pondicherry[2], Elizabeth A. Baldwin[2], Brian O. Barnstein[2] and John J. Ryan[2]**

[1] VCU Life Sciences, Virginia Commonwealth University, Richmond, VA, United States, [2] Department of Biology, Virginia Commonwealth University, Richmond, VA, United States

Cellular metabolism and energy sensing pathways are closely linked to inflammation, but there is little understanding of how these pathways affect mast cell function. Mast cells are major effectors of allergy and asthma, and can be activated by the alarmin IL-33, which is linked to allergic disease. Therefore, we investigated the metabolic requirements for IL-33-induced mast cell function, to identify targets for controlling inflammation. We found that IL-33 increases glycolysis, glycolytic protein expression, and oxidative phosphorylation (OX PHOS). Inhibiting OX PHOS had little effect on cytokine production, but antagonizing glycolysis with 2-deoxyglucose or oxamate suppressed inflammatory cytokine production *in vitro* and *in vivo*. ATP reversed this suppression. Glycolytic blockade suppressed IL-33 signaling, including ERK phosphorylation, NFκB transcription, and ROS production *in vitro*, and suppressed IL-33-induced neutrophil recruitment *in vivo*. To test a clinically relevant way to modulate these pathways, we examined the effects of the FDA-approved drug metformin on IL-33 activation. Metformin activates AMPK, which suppresses glycolysis in immune cells. We found that metformin suppressed cytokine production *in vitro* and *in vivo*, effects that were reversed by ATP, mimicking the actions of the glycolytic inhibitors we tested. These data suggest that glycolytic ATP production is important for IL-33-induced mast cell activation, and that targeting this pathway may be useful in allergic disease.

Keywords: IL-33, mast cells, glycolysis, ATP, metabolism, metformin

OPEN ACCESS

Edited by:
Fang-Ping Huang, The University of Hong Kong, Hong Kong

Reviewed by:
Philippe Deterre, Center for the National Scientific Research (CNRS), France
Silvia Piconese, Sapienza University of Rome, Italy

***Correspondence:**
John J. Ryan
jjryan@vcu.edu

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *22 August 2018*
Accepted: *06 December 2018*
Published: *18 December 2018*

Citation:
Caslin HL, Taruselli MT, Haque T, Pondicherry N, Baldwin EA, Barnstein BO and Ryan JJ (2018) Inhibiting Glycolysis and ATP Production Attenuates IL-33-Mediated Mast Cell Function and Peritonitis. Front. Immunol. 9:3026. doi: 10.3389/fimmu.2018.03026

INTRODUCTION

Cellular metabolism and energy sensing pathways control the breakdown of carbohydrates, fatty acids, and proteins when energy is required. While we know that cells require energy for homeostasis, maintenance, and proliferation, it is now understood that metabolism is closely linked to immune cell differentiation and activation, impacting cell phenotype, effector functions, and overall inflammatory conditions (1). Glycolysis is consistently noted as the primary energy production pathway used by inflammatory cells, such as T-helper (Th)1, Th17, M1 macrophages, and dendritic cells (DCs) during acute activation, while oxidative phosphorylation (OX PHOS) in the electron transport chain (ETC) is the primary energy production pathway used by regulatory cells such as T-regulatory (Treg), M2 macrophages, and myeloid derived suppressor cells (MDSC)

(2–5). Glycolysis is inefficient, with only 2 ATP produced per glucose molecule compared with 32 per glucose in OX PHOS, but the benefits of utilizing glycolysis during activation and proliferation are multi-fold. Glycolysis rapidly increases ATP availability, operates under low oxygen tension, and provides pentose phosphate pathway and Kreb's cycle intermediates to anabolic pathways producing nucleotides, amino acids, and lipids (2, 6). This has been most extensively studied in T cells, which undergo dynamic and complex metabolic reprogramming in response to activation, cytokine stimulation, and other changes in their microenvironment (4, 5, 7). In contrast, there is limited information on how metabolism is modulated in mast cells.

Mast cells are tissue resident myeloid cells that reside in both mucosal and connective tissue. These cells are typically recognized for their effector function in Th2 immunity, specifically their detrimental role in allergic disease and protective role against parasites and venoms (8–11). While much is known about mast cell activation, little data concerning the role of glucose metabolism in mast cell responses has been published. Studies from the 1990's suggest that adequate glucose and ATP are required for full mast cell function (12–14), and that lactate is released upon activation by compound 48/80 and polymyxin B in rat mast cells (15). Additionally, studies by Chakravarty showed that glycolytic blockade with 2-deoxyglucose (2-DG), iodoacetate, fluoride and oxamate (OX) suppressed compound 48/80 and antigen-induced histamine release in rat mast cells (16, 17). Recently, an extracellular flux analyzer (Seahorse device) was used to show that IgE XL rapidly increases glycolysis, while OX PHOS increases ∼2 h after stimulation (18). The same study showed that suppressing glycolysis with dichloroacetate (DCA) and inhibiting complex I of the electron transport chain (ETC) with rotenone suppressed cytokine production and degranulation. By contrast, inhibiting fatty acid oxidation with etomoxir had no effect (18). Additionally, OX PHOS activity has been shown to increase following IgE XL in mast cells via p-ERK and mitochondrial Stat3 (19). These results suggest that IgE-mediated activation requires glycolysis and ETC activity, however the metabolic requirements for other important mast cell activators have not been examined.

IL-33 is a cytokine mediator that is considered an alarmin. It is released by endothelial, epithelial, and fibroblast cells in response to damage, and by mast cells following activation (20–22). IL-33 activates many immune cell types, including mast cells, Th2, and innate-like lymphoid cell- (ILC)2. It augments IgE-induced inflammation (23, 24) and is elevated in asthma and atopic dermatitis (25–28). IL-33 administration promotes disease in animal studies, while anti-IL-33 or anti-ST2 antibody treatments can reduce inflammation (29, 30). Thus, understanding its function may be critical to allergic disease. We have recently shown that lactic acid, a byproduct of glycolysis, can suppress IL-33-induced mast cell activation (31). This prompted interest in how metabolism may contribute to IL-33 function, which has not been studied in immune cells.

Our purpose was to determine the metabolic requirements for IL-33 activation in mast cells and to examine potential targets for controlling IL-33-mediated inflammation. Our data suggest that IL-33-induced cytokine secretion requires glycolysis for ATP production and that glycolytic blockade suppresses inflammatory cytokine production *in vitro* and *in vivo*. To test proof of principle and suggest a clinically relevant way to modulate these pathways in humans, we report the effects of the FDA-approved drug metformin on IL-33 activation. AMPK induction by metformin, which suppresses glycolysis in immune cells, inhibited cytokine production *in vitro* and *in vivo*. These data suggest that suppressing glycolysis directly or via AMPK activation has therapeutic potential for IL-33-mediated inflammation.

METHODS

Animals

Mouse C57BL/6J and NFκB-luc breeding pairs were purchased from The Jackson Laboratory (Bar Harbor, ME), and colonies were maintained in a pathogen free facility. Bone marrow was extracted from mice at a minimum of 10 weeks old and IL-33-induced peritonitis studies were conducted at 10–16 weeks of age with both male and female mice with approval from the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Mast Cell Culture

Mouse bone marrow cells were differentiated in complete RPMI 1640 media supplemented with WEHI-3 cell supernatant containing IL-3 and BHK-MKL cell supernatant containing SCF as described to yield 90-99% FcεRI$^+$ and cKit$^+$ bone marrow derived mast cells (BMMC) at 21–28 days (31, 32). Following differentiation and expansion, BMMC were plated at 1x10^6/mL with IL-3 and SCF for all experiments (10 ng/mL). Cells were treated as described and activated ± IL-33 at 50 ng/mL unless otherwise stated.

Materials

Recombinant mouse IL-3, SCF, and IL-33 for *in vitro* experiments were purchased from Shenandoah Biotechnology (Warwick, PA). Sodium oxamate and 2-deoxyglucose (2DG) were purchased from Alfa Aesar (Tewksbury, MA). Etomoxir, rotenone, and SRT1720 were purchased from Cayman Chemical (Ann Arbor, MI). Antimycin A was purchased from Chem Cruz via Santa Cruz Biotechnology (Dallas, TX). ATP disodium salt was purchased from Tocris via Biotechne Corporation (Minneapolis, MN). Metformin was purchased from MP Biosciences (Santa Ana, CA). A769662 was purchased from Med Chem Express (Monmouth Junction, NJ).

In vivo Studies

Recombinant mouse IL-33 for *in vivo* experiments was purchased from Biolegend (San Diego, CA). Age- and sex- matched groups of mice (∼12 weeks old) were injected intraperitoneally (IP) with 2-DG (1 g/kg, ∼100 μl), sodium oxamate (15 mg/kg, ∼100 μl), metformin (100 mg/kg, ∼100 μl), or PBS (100 μl) 1 h prior to IL-33. IL-33 (1 μg/mouse in 100 μl PBS) was injected IP to elicit peritonitis, and mice were sacrificed after 4 h. Plasma from cardiac puncture was used to measure cytokines via ELISA, and

neutrophil recruitment was assessed from peritoneal lavage cells analyzed with flow cytometry as described below.

Cellular Metabolism

To measure the extracellular acidification rate (ECAR), proton production rate (PPR), and oxygen consumption rate (OCR) as surrogates for glycolysis and oxidative phosphorylation, a Seahorse XFp analyzer (Agilent, Santa Clara, CA) was used. Cells were plated in duplicate at 200,000/well on 4.6 μg/ml Cell-Tak™ in minimal DMEM containing 10 mM glucose, 1 mM sodium pyruvate, 2 mM L-glutamine, and 1% FBS. The protocol was as follows: initialization, 3 cycles baseline, inject IL-3/SCF (10 ng/ml final concentration), 3 cycles, inject IL-33 (100 ng/ml final concentration), 5 cycles. For each condition, an average was taken across all wells.

To determine glucose uptake and lactate export, cell supernatants were analyzed for glucose and lactate concentrations 16 h after activation, using the Glucose Assay Kit 1 and L-Lactate Assay Kit 1 from Eton Bioscience (San Diego, CA). Glucose uptake was calculated as [glucose in unactivated cell supernatant] – [glucose in activated cell supernatant]. Lactate export was calculated as [lactate in activated cell supernatant] – [lactate in unactivated cell supernatant].

Gel Electrophoresis and Western Blot

To determine protein concentration and protein phosphorylation, cell lysates were collected using Protease arrest (GBiosciences, Maryland Heights, MO) in cell lysis buffer (Cell Signaling Technology, Danvers, MA). Protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermofisher, Waltham, MA). 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels (Bio–Rad, Hercules, CA) were loaded with 30 μg protein, electrophoresed and transferred to nitrocellulose (Pall Corporation, Ann Arbor, MI), and membranes were blocked for 60 min in Blocker casein in tris-buffered saline (TBS) (from Thermofisher, Waltham, MA). Blots were incubated with primary antibodies overnight in block buffer + Tween20 (1:1,000) ± rabbit anti-p-AMPK (1:750), rabbit anti-HK2 (1:750), rabbit anti-actin (1:1,000, antibodies all purchased from Cell Signaling, Danvers, MA). Blots were washed six times for 5 min each in TBS-Tween-20, followed by incubation with secondary antibody (1:10,000) for 60 min at room temperature (Cell Signaling, Danvers, MA). Size estimates for proteins were obtained using molecular weight standards from Bio–Rad (Hercules, CA). Blots were visualized and quantified using a LiCor Odyssey CLx Infrared imaging system (Lincoln, NE). After background subtraction, fluorescence intensity for the protein of interest was normalized to the signal intensity for the relevant loading control and unactivated samples, using Image Studio 4.0 (LiCor).

ELISA

ELISA analysis was used to measure cytokine concentrations from the cell culture supernatant 16 h after activation and from the plasma 4 h after IL-33 induced peritonitis (described above). Murine IL-6, TNF, and MCP-1 (CCL2) ELISA kits were purchased from Biolegend; murine MIP-1α (CCL3) ELISA kits were purchased from Peprotech (Rocky Hill, NJ). Assays were performed in duplicate (plasma) or triplicate (cell supernatant) according to the manufacturers' protocols.

Flow Cytometry

For cell signaling studies, cells were activated for 15 min. Cells were collected with 1.6% paraformaldehyde fixation and permeablized with methanol for p-ERK analysis. Cells were stained with anti-CD16/32 (clone 2.4G2, BD Pharmingen via BD Biosciences, San Jose, CA) and APC-anti-H/M pERK1/2 (clone MILAN8R, eBioscience, via Thermofischer, Waltham, MA) or the isotype control (APC mouse IgG1; eBioscience) at 2 μg/mL for 30 min at 4°C, and analyzed by flow cytometry with the FACsCelesta (BD Biosciences). The gating strategy used doublet exclusion (FSC-A x FSC-H), and size vs. granularity (FSC x SSC). MFI was recorded for all samples.

For oxidative stress measures, cells were treated ± 2DG or OX for 1 h then activated with IL-33 for 2 h. Cells were then washed and re-suspended in Hank's buffered saline solution (HBSS) + 2′,7′ Diochlorofluorescin Diacetate (DCFH-DA, 5 μM, Millipore, Burlington, MA) ± 2DG or OX ± IL-33 for 30 min at 37°C. Cells were analyzed in the FITC channel by flow cytometry. The gating strategy used was doublet exclusion (FSC-A x FSC-H) and gating on size and granularity (FSC x SSC). MFI was recorded for all samples.

For ATP diffusion, cells were treated with AlexaFluor 647-labeled ATP (ThermoFisher, Waltham, MA) at 1, 2.5, 5, and 8 μM for 20 min in cRPMI at 37°C. Cells were then washed 2X and suspended in PBS for flow cytometric analysis. Percent positive cells were recorded for all samples.

Following IL-33-induced peritonitis (described above), peritoneal lavage cells were collected, red blood cells were lysed with ACK buffer, and rinsed pellets were stained with anti-CD16/32 (clone 2.4G2, BD Pharmingen), PE rat anti-mouse Ly6G (clone1A8, BD Pharmingen), APC-anti mouse CD45 (clone 30-F11, Biolegend) or the isotype controls PE rat IgG2a (BD Pharmingen) and APC rat IgG2b (Biolegend), all at 2 μg/mL for 30 min at 4°C, and analyzed by flow cytometry with the FACsCelesta (BD Biosciences). The gating strategy used was doublet exclusion (FSC-A x FSC-H), size and granularity (FSC x SSC), lymphocytes (CD45+), and neutrophils (Ly6G++). Percent positive was reported from total leukocyte (CD45+) events.

Luciferase

BMMC were differentiated from NFκB-luc transgenic bone marrow as above. Following treatment ± 2DG or OX for 1 h and IL-33 activation for 2 h, cells were lysed and luciferase activity was measured with the Promega Luciferase Assay Substrate and Glomax 20/20 Luminometer (Promega, Madison, WI). Luciferase expression is reported relative to protein concentration (Pierce BCA Protein Assay Kit, Thermofisher, Waltham, MA) and normalized to the unactivated control.

Statistical Analyses

Glucose uptake, lactate export, and enzyme expression (**Figures 1B,C**; comparison of two groups) were analyzed

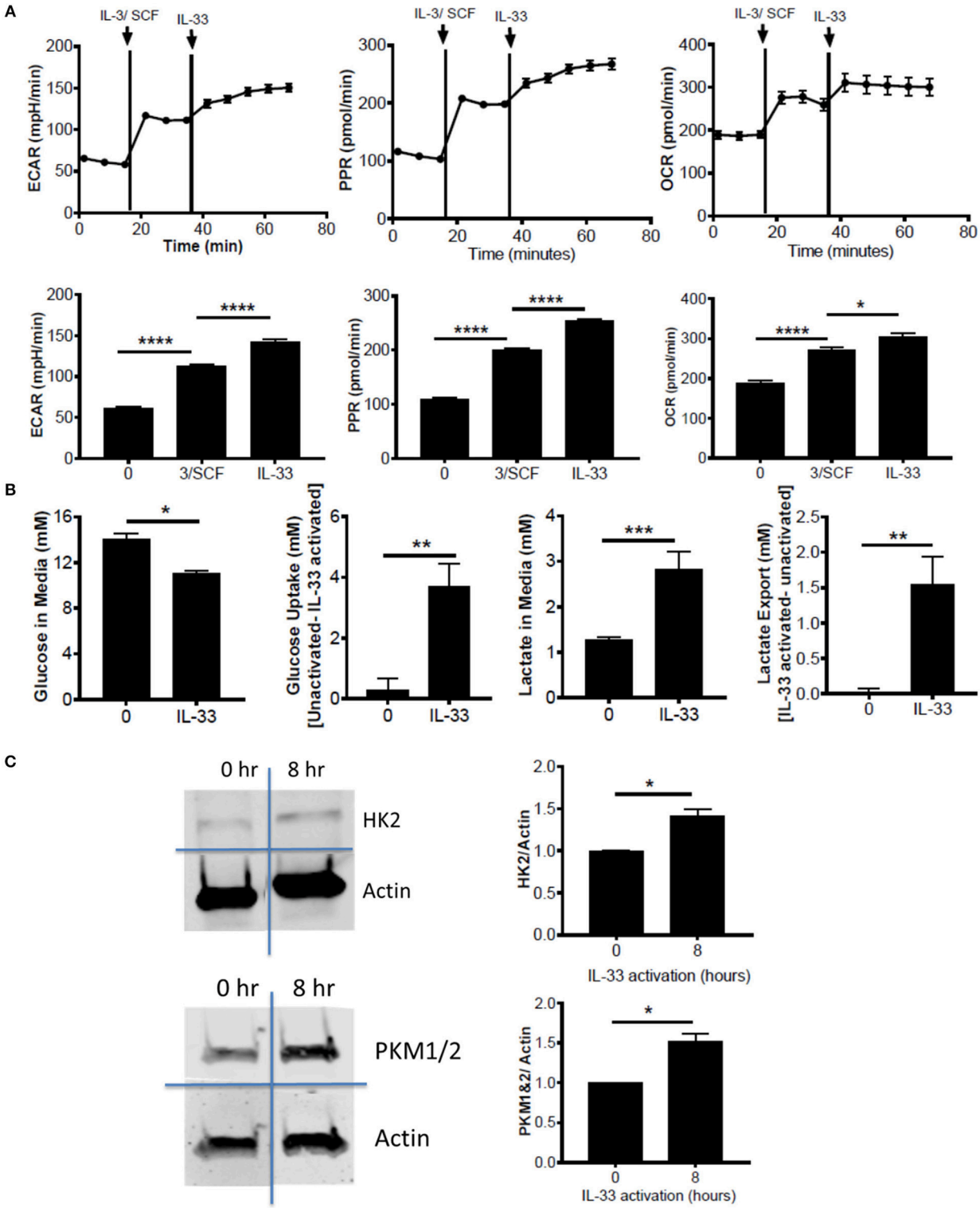


FIGURE 1 | IL-33 activation induces glycolysis and OX PHOS. **(A)** Seahorse metabolic analysis was used to measure basal metabolism in BMMC, followed by IL-3/SCF (10 ng/mL) and IL-33 (100 ng/mL). ECAR, PPR, and OCR measurements averaged over time are shown. **(B)** BMMC were activated for 16 h ± IL-33 (50 ng/ml). Glucose and lactate were measured in the cell supernatant by colorimetric assay. Uptake and export were determined by the difference between activated and unactivated groups. Above data are means ± SEM of 3 populations, representative of 3 independent experiments. **(C)** BMMC were activated with IL-33 (100 ng/ml) for 8 h. Lysates were analyzed by western blot for hexokinase, pyruvate kinase, and actin. Data are representative of 3 independent populations $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

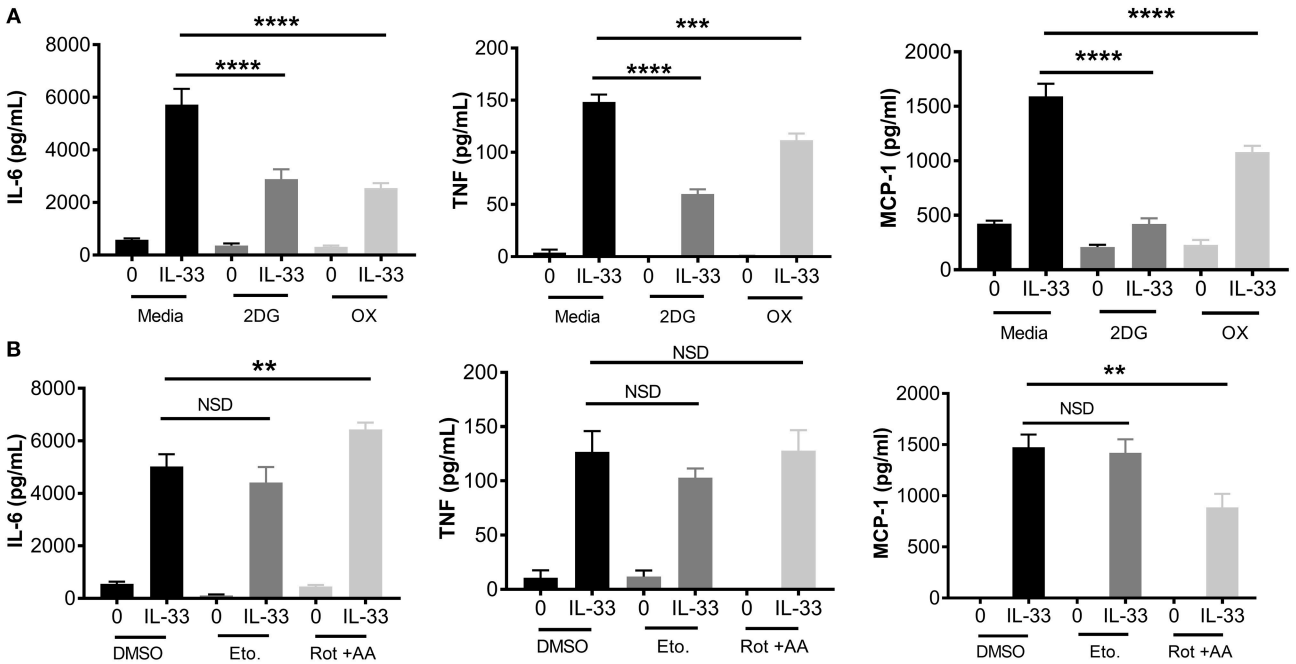


FIGURE 2 | Glycolytic inhibition suppresses cytokine production following IL-33 activation. **(A)** BMMC were treated with 2DG (1 mM) or OX (20 mM) for 1 h prior to IL-33 (50 ng/mL) activation for 16 h. Cytokines were measured in supernatant by ELISA. **(B)** BMMC were treated with DMSO (vehicle control), etomoxir (Eto, 200 μM), or rotenone+antimycin A (Rot+AA, 1 μM) for 1 h prior to IL-33 (50 ng/mL) activation for 16 h. Cytokines were measured in supernatant by ELISA. Data are means ± SEM of 3 populations analyzed in triplicate, representative of 3 independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, NSD, no significant difference.

by *t*-test. The remainder of the data (3+ groups) were analyzed by a one-way analysis of variance (ANOVA) to detect overall differences between groups. With F-statistic significance, Tukey's multiple comparisons were used as *post hoc* tests to determine which conditions were significantly different from the control. We have reported only the *post hoc* analyses between activated conditions, as unactivated were expected to be different from activated samples. Differences between unactivated conditions (*in vitro*) and between PBS controls (*in vivo*) were reported when significantly different. GraphPad Prism software was used for all statistical analyses. Data are expressed as mean ± standard error of mean (SEM) with statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, NSD, no significant difference.

RESULTS

IL-33 Activation Induces Glycolysis and OX PHOS

To determine the effects of IL-33 on mast cell metabolism, we analyzed extracellular acidification rate (ECAR) and proton production rate (PPR) as indicators of H^+ production and as a surrogate for glycolytic rate. Oxygen consumption rate (OCR) was used as an indicator of mitochondrial OX PHOS. Bone marrow derived mast cells (BMMC) were measured at baseline, following IL-3/SCF addition, and following IL-33 activation. ECAR, PPR, and OCR were all significantly elevated following growth factor addition and IL-33 activation (**Figure 1A**). We should note that these measures were only significantly elevated with IL-33 activation in the presence of IL-3 and SCF (data not shown). It is known that IL-33 co-stimulation with SCF is necessary for IL-33-induced cytokine production(33), and thus IL-33-mediated changes in glycolysis, like cytokine production, seem to be dependent upon SCF (and possibly IL-3) signaling. We confirmed the induction of glycolysis by IL-33 with measures of glucose uptake and lactate export. Following IL-33 activation for 16 h, glucose uptake and lactate export were calculated using concentrations in the cell supernatants from the IL-33 activated and control groups. IL-33 activation significantly increased glucose uptake and lactate export (**Figure 1B**), supporting enhanced glycolysis as measured by ECAR and PPR.

Lipopolysaccharide (LPS) is an innate activation signal that shares downstream signaling cascades with IL-33 (34). LPS has been shown to increase macrophage and dendritic cell glycolysis, similar to the IL-33 effects we observed in **Figures 1A,B** (2, 35, 36). LPS effects occur in 2 stages: a rapidly increased glycolytic enzyme activity and inhibition of OX PHOS, and a prolonged increase in enzyme expression (2, 35, 36). To determine if IL-33 has an effect on glycolytic enzyme expression, BMMC were activated for 8 h ± IL-33. IL-33 induced a modest but consistent increase in hexokinase (HK)2 and pyruvate kinase (PK)M1/2 expression (**Figure 1C**). These data suggest that IL-33 activation increases both glycolysis and OX PHOS.

Glycolytic Inhibition Suppresses Cytokine Production Following IL-33 Activation

To determine the importance of glycolysis for IL-33-mediated mast cell function, we employed chemical antagonists. BMMC were treated ± the glycolytic inhibitors 2DG (1 mM) or OX (20 mM) for 1 h prior to IL-33 activation for 16 h. Importantly, there was no detectable change in cell viability at these doses over the duration of the experiment (data not shown). Both 2DG and OX significantly suppressed IL-33-induced IL-6, TNF, and MCP-1 (**Figure 2A**). Additionally, we used chemical antagonists to determine the importance of OX PHOS. BMMC treated for 1 h ± etomoxir (Eto, 200 μM), inhibiting fatty acid oxidation, or rotenone and antimycin A (Rot+AA; 1 μM), inhibiting complex I and II of the ETC, had no effect on IL-6 and TNF production (**Figure 2B**). Rotenone and antimycin A did suppress MCP-1. Rotenone and antimycin A were used at the highest dose at which they did not increase cell death over 24 h, similar to concentrations published to suppress IgE-mediated signaling in mast cells (18). Together with data from **Figure 1**, these results suggest that the increase in glycolysis following IL-33 activation is functionally important for cytokine production in mast cells and that MCP-1 production may have slightly different signaling and metabolic controls.

Glycolytic Inhibition Suppresses ERK Phosphorylation, NFκB-Mediated Transcription, and ROS Production

While these data suggest that glycolytic inhibition reduces cytokine release following IL-33, it is unclear if early receptor signaling events are similarly affected. Therefore, ERK phosphorylation (p-ERK) and NFκB transcriptional activity were measured. BMMC were treated ± the glycolytic inhibitors 2DG or OX for 1 h prior to IL-33 activation for 15 min. Phosphorylation events were determined by flow cytometry. As shown in **Figure 3A**, 2DG or OX treatment significantly suppressed IL-33-mediated ERK activation. Furthermore, NFκB-luc BMMC bearing a luciferase gene driven by two copies of the NFκB regulatory element were also treated ± 2DG or OX for 1 h prior to IL-33 activation for 2 h. Luciferase expression was measured as a surrogate for NFκB-induced transcription. Similar to ERK activation, 2DG and OX significantly suppressed IL-33-mediated luciferase expression (**Figure 3B**), suggesting that glycolysis is required for IL-33-induced NFκB function.

We have previously published that IL-33 induced-cytokine production is suppressed by antioxidants such as n-acetylcysteine (37), but ROS production following IL-33 activation has not been investigated. Because glycolysis promotes ROS production by the pentose phosphate pathway (2, 38), we examined the effect of glycolytic inhibition on IL-33-induced ROS production. BMMC were treated ± 2DG or OX for 1 h and activated with IL-33 for 2 h. Oxidative stress was measured by DCFH-DA fluorescence by flow cytometry. IL-33 significantly increased DCFH-DA fluorescence, an effect suppressed by 2DG or OX (**Figure 3C**). Together, these data suggest that glycolysis contributes to IL-33-mediated signaling and ROS production required for optimal mast cell function.

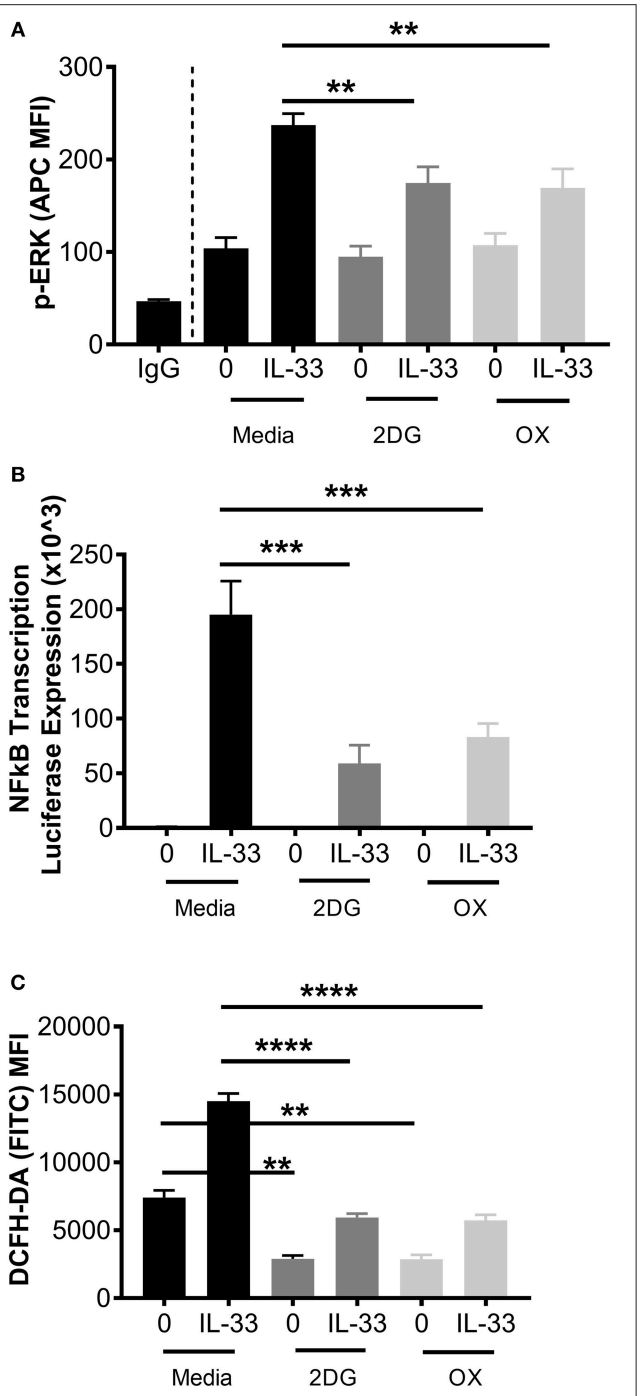


FIGURE 3 | Glycolytic inhibition suppresses ERK phosphorylation, NFκB transcription, and ROS production. **(A)** BMMC were treated with media, 2DG (1 mM) or OX (20 mM) for 1 h and activated +/– IL-33 (100 ng/mL) for 15 min. p-ERK was analyzed by flow cytometry. **(B)** NFκB-luc transgenic BMMC were treated with 2DG (1 mM) or OX (20 mM) for 1 h and activated for 2 h with IL-33 (100 ng/mL). Luciferase activity was measured with the Promega Luciferase Assay Substrate and Glomax Luminometer, then normalized to the unactivated media control. **(C)** BMMC were treated with 2DG (1 mM) or OX (20 mM) for 1 h and activated for 2 h with IL-33 (50 ng/mL). Oxidative stress was analyzed by DCFH-DA fluorescence by flow cytometry. Data are means ± SEM of 3 populations analyzed in duplicate, representative of 3 independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Increased ATP Availability Restores Cytokine Production Following Glycolytic Inhibition

ATP is required for kinase activity, tRNA synthetase function, ion transport, and chromatin remodeling, all of which play a role in cell signaling and cytokine production. In addition to providing ATP; glycolysis also generates pentose phosophate pathway and Kreb's cycle intermediates used for nucleotide, amino acid, and lipid synthesis (2). To determine if ATP alone is sufficient to restore cytokine production during glycolytic blockade, BMMC were treated for 1 h ± 2DG or OX prior to activation with IL-33 ± ATP (10 mM) for 16 h. While 2DG or OX significantly suppressed IL-6 and MCP production, cytokine production was not significantly suppressed with increased ATP availability (**Figure 4A**). ATP alone had no effect on cytokine secretion at the concentration used (**Figure 4A**), and diffused into the cell at low concentrations within 20 min (**Figure 4B**). Together, these data suggest that ATP availability can maintain cytokine production during glycolytic blockade, supporting the theory that IL-33-induced glycolysis yields ATP that is critical for inflammatory function.

Glycolytic Inhibitors Suppress IL-33-Induced Neutrophil Recruitment and Cytokine Production *in vivo*

To test the importance of IL-33-induced glycolysis *in vivo*, we used a model of IL-33-induced peritonitis in which neutrophil recruitment is mast cell-dependent (39). As shown in the schematic (**Figure 5A**), mice were IP injected with 2DG (750 mg/kg), OX (15 mg/kg), or PBS (control). After 1 h, mice were injected IP with IL-33 (1 μg) or PBS. After 4 h, peritoneal lavage and cardiac puncture were harvested. 2DG and OX significantly suppressed IL-33 induced neutrophil ($Ly6G^{hi}$) recruitment into the peritoneum compared with the PBS control group (**Figure 5B**). Similarly, 2DG and OX reduced plasma IL-6 and MIP-1α (**Figure 5C**). We should note that 2DG and OX induced neutrophil recruitment independent of IL-33 (**Figures 5B,C**). These data suggest the suppressive effects of glycolytic inhibition extend to IL-33 activation *in vivo,* but also point to limitations regarding their use.

IL-33 and AMPK-SIRT1 Are Antagonistic Pathways

While 2DG has been used in a Phase I trial for cancer treatment, there have been off-target effects reported. Additionally, the C_{MAX} reported for the recommended Phase II 2DG dose was 450 mM (for 45 mg/kg) (40), well below the doses used in our model. Fatigue, dizziness, and dose-dependent cardiac QTc prolongation were also observed in a few patients (40). Similarly, OX is known to have poor cell membrane permeability and concentrations sufficient for LDH suppression cannot be reached *in vivo* (41). Due to the limitations of these inhibitors for future clinical use, we became interested in AMPK as another way to target glycolysis in IL-33-related diseases.

AMPK is known for its role in energy sensing, activated in response to fasting and exercise. AMPK switches the cell from anabolic pathways to catabolic pathways, utilizing all potential energy in the form of glucose and lipids by both glycolysis and OX PHOS in liver, kidney, and skeletal muscle (2). Interestingly, AMPK increases OX PHOS but inhibits glycolysis in immune cells, perhaps because of the anabolic effects of glycolysis (2, 42). To determine the effects of AMPK on IL-33-induced cytokine production, BMMC were treated with A799662 (AMPK agonist; 100 μM) or SRT1720 (an agonist of the APMK target SIRT1; 5 μM) for 1 h prior to activation with IL-33 for 16 h. Both agonists significantly reduced IL-33-mediated IL-6 and TNF production (**Figure 6**). These data suggest that activating AMPK or its downstream signaling pathways can suppress IL-33-induced mast cell activation, mirroring the effects of glycolytic inhibition.

Metformin Suppresses IL-33-Induced BMMC Activation as Well as Neutrophil Recruitment and Cytokine Production *in vivo*

Metformin is an FDA-approved AMPK activator, widely prescribed for the treatment of diabetes. This offers a clinically-relevant means of assessing how AMPK activation affects IL-33 function. First, BMMC were treated with metformin at physiological doses (10, 50, 100 μM) (43, 44) for 24 h before IL-33 stimulation for 16 h. For all metformin doses, we observed significant suppressive effects (**Figure 7A**). Similar suppression was observed with 1-h treatment, albeit at higher doses (data not shown). To confirm that the effects of metformin were due to reduced glycolysis and ATP availability, BMMC treated with metformin (100 mM) for 24 h were activated with IL-33 ± ATP (10 mM) for 16 h. ATP reversed the suppression by metformin (**Figure 7B**). These results suggest that suppressing glycolysis and ATP production by increasing AMPK activity with metformin is an effective way to limit cytokine production following IL-33 activation.

To establish proof of principle *in vivo*, metformin treatment was used in the IL-33-induced peritonitis model. As shown in the schematic (**Figure 7C**), mice received metformin (IP, 100mg/kg, ∼100 μl) or PBS (control, 100 μl). After 1 h, both groups of mice were injected IP with IL-33 (1 μg, 100 μl) or PBS (100 μl). Peritoneal lavage and cardiac puncture were harvested after 4 h. Metformin significantly suppressed IL-33-mediated neutrophil ($Ly6G^{hi}$) recruitment into the peritoneum compared with the PBS control group (**Figure 7D**). Furthermore, metformin significantly reduced plasma MCP-1 (**Figure 7E**). These data support the theory that indirectly targeting glycolysis with an AMPK agonist can suppress IL-33-mediated inflammation *in vivo*.

DISCUSSION

Immune cell metabolism is closely linked to phenotype and effector functions. While T cell and macrophage metabolism have been highly studied over the past decade, mast cell metabolism and IL-33-mediated activation have received little attention. This study is the first to report that IL-33 increases glycolysis, generating ATP that is required for subsequent inflammatory

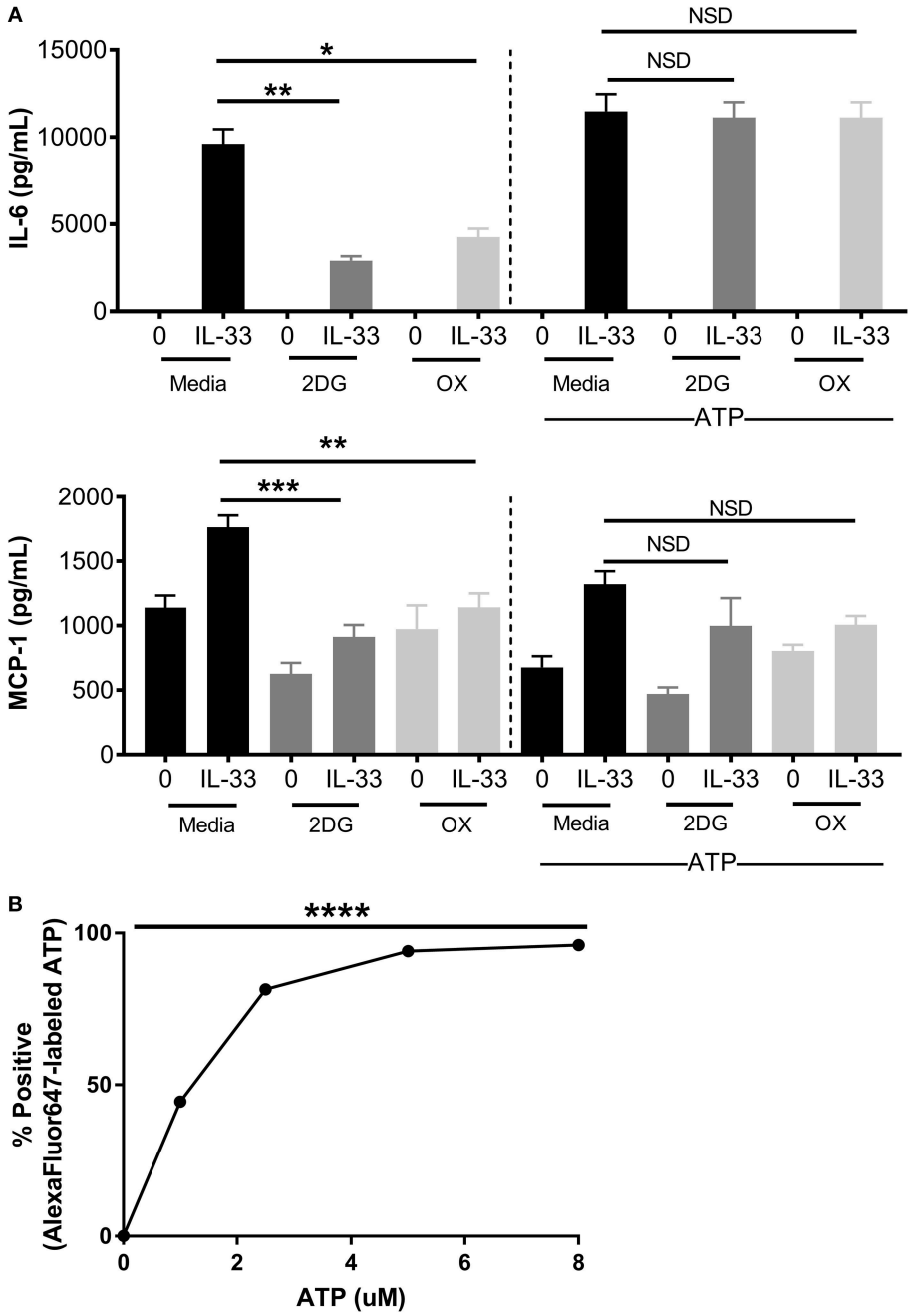


FIGURE 4 | Increased ATP availability restores cytokine production following glycolytic inhibition. **(A)** BMMC were treated with 2DG (1 mM) or OX (20 mM) for 1 h and activated for 16 h with IL-33 (50 ng/mL) and/or ATP (10 μM). Cytokines were measured in cell supernatant by ELISA. **(B)** BMMC were treated with AF647-labeled ATP for 20 min prior to flow cytometric analysis. Data are means ± SEM of 3 populations analyzed in triplicate, representative of 3 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, NSD, no significant difference.

cytokine production. Targeting glycolytic ATP production by inhibiting glycolysis with 2-DG and OX, or by activating AMPK with metformin was sufficient to reduce IL-33-mediated effects *in vitro* and *in vivo*. With increased ATP availability, these inhibitors had little effect on cytokine production, highlighting the importance of this glycolytic product. These data advance our understanding of IL-33 function and suggest that regulators of glycolysis, like AMPK, may be potential targets for treating inflammatory diseases involving IL-33.

Previous to this report, only one paper using small-cell lung cancer (SCLC) cells reported metabolic effects of IL-33, showing increased glucose uptake and lactate export (45). This

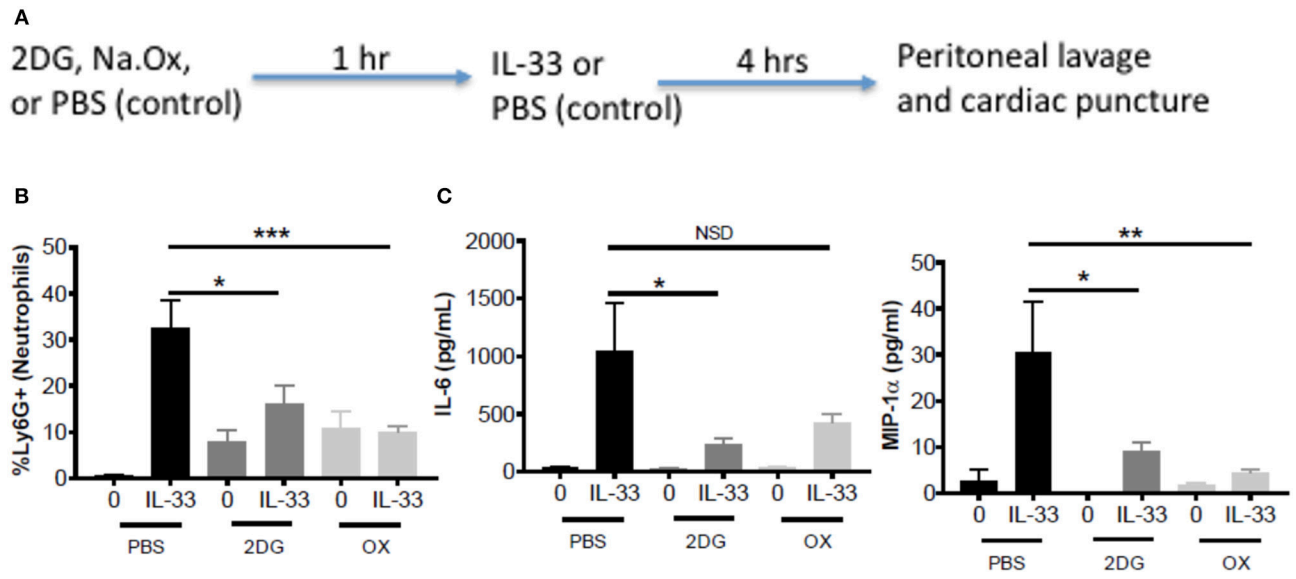


FIGURE 5 | Glycolytic inhibitors suppress IL-33-induced neutrophil recruitment and cytokine production *in vivo*. **(A)** Schematic diagram: C57BL/6J mice (age and sex matched males and females) were IP injected with 2DG (750 mg/kg), OX (15 mg/kg), or PBS. After 1 h, mice were injected IP with IL-33 (1 μg) or PBS. All groups were sacrificed after 4 h. **(B)** Peritoneal lavage was used to collect cell infiltrates and analyze neutrophil (Ly6G+) recruitment via flow cytometry. **(C)** Cardiac puncture was used to collect plasma for cytokine analysis via ELISA. Data are means ± SEM of 2 mice/control group and 5 mice/IL-33-peritonitis group analyzed in triplicate (flow cytometry) and duplicate (ELISA), representative of 2 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, NSD, no significant difference.

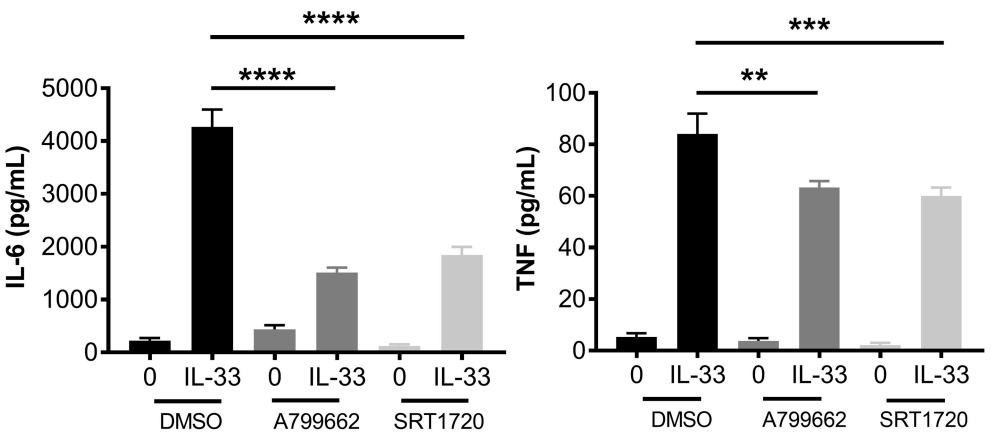


FIGURE 6 | IL-33 and AMPK-SIRT1 are antagonistic pathways. BMMC were treated with DMSO (vehicle control), A799662 (AMPK agonist, 100 μM) or SRT1720 (SIRT1 agonist, 5 μM) for 1 h and activated with IL-33 (50 ng/mL) for 16 h. Cytokines were measured in supernatant by ELISA. Data are means ± SEM of 3 populations analyzed in triplicate, representative of 3 independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

work is the first to note glycolytic requirements for IL-33 activation in immune cells. Additionally, we observed a slight increase in OX PHOS. We hypothesize that IL-33 signaling in other ST2^{+} cells, including Th2 and ILC2 cells, would also require glycolysis for optimal function, but this remains to be determined. Using the Seahorse analyzer, we observed increased glycolysis immediately upon IL-33 stimulation, likely due to increased enzyme activity. Additionally, metabolic enzyme expression was moderately elevated at 8 h, and glucose uptake and lactate export remained different at 16 h. While we noted an increase in HK2 and PKM1/2 expression, Wang et al. observed increased Glut1 surface expression in SCLC cells (45), suggesting that IL-33 may regulate the expression of many different proteins important for glycolysis.

Our data suggest a functional role for enhanced glycolysis following IL-33 activation. In BMMC, the glycolytic inhibitors 2DG and OX suppressed IL-33-mediated ERK activation, NFκB transcription, ROS production, and cytokine secretion. These data are consistent with other findings, as 2DG and OX inhibit IgE receptor-induced cytokine secretion in rat mast cells (16),

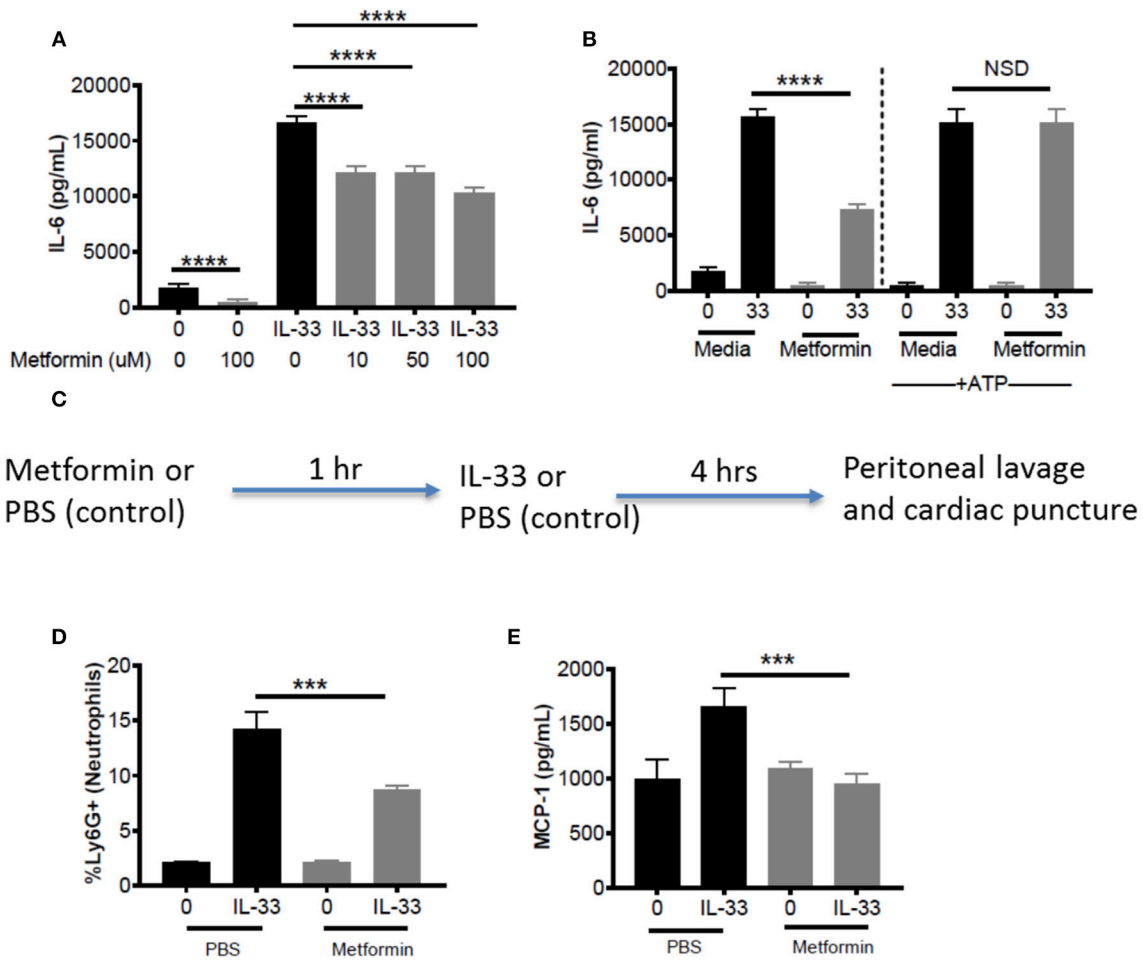


FIGURE 7 | Metformin suppress IL-33-induced BMMC activation as well as neutrophil recruitment and cytokine production *in vivo*. **(A)** BMMC were treated ± metformin (10, 50, 100 μM) for 24 h and activated with IL-33 (50 ng/mL) for 16 h. **(B)** BMMC were treated ± metformin (Met, 100 μM) for 1 h and activated with IL-33 (50 ng/mL) ± ATP (10 μM) for 16 h. Cytokines were measured in the supernatant by ELISA. Above data are means ± SEM of 3 populations run in triplicate, representative of 3 independent experiments. **(C)** Schematic diagram: C57BL/6 males and females were injected intraperitoneal (IP) with metformin (100 mg/kg) or PBS. After 1 h, mice were injected IP with IL-33 (1 μg) or PBS. All groups were sacrificed after 4 h. **(D)** Peritoneal lavage was used to collect cell infiltrates and analyze neutrophil (Ly6G+) recruitment via flow cytometry. **(E)** Cardiac puncture was used to collect plasma for cytokine analysis via ELISA. *In vivo* data are means ± SEM of 3 mice/control group and 8 mice/ peritonitis group analyzed in triplicate (flow cytometry) or duplicate (ELISA), representative of 2 independent experiments. ***$p < 0.001$, ****$p < 0.0001$.

cytokine mRNA and protein secretion, cytolytic activity, and cell cycle progression in CD8 T cells (46), and ERK phosphorylation in SCLC cells (47). It is important to note that inhibiting glycolysis also reduces glucose consumption and pyruvate availability for Kreb's cycle and the ETC, which is likely why others have noted OX PHOS suppression with 2DG (48). This effect may be greater with 2DG, as OX may push some pyruvate into the mitochondria for utilization, and should be accounted for with future studies. Thus, determining the importance of glycolysis vs. OX PHOS when analyzing inhibitor studies has challenges. Our interpretations of the data are that glycolytic ATP product is paramount for IL-33-induced mast cells responses. This conclusion is based on (1) ATP-mediated reversal of glycolytic suppression and (2) the consistent inhibitory effects of glycolytic inhibitors in contrast to inconsistent or lack of effects when blocking OX PHOS and ETC function. Because 2DG and OX suppress IL-33-mediated cytokine production and neutrophil recruitment *in vivo*, our data suggest that glycolytic ATP plays a critical role in IL-33-mediated inflammation.

ATP, the primary product of glycolysis, provides a phosphate group and energy for kinases (45, 49) and enzymes involved in signaling and transcription. Our data show that exogenous ATP can restore IL-33-induced cytokine production in the presence of glycolytic inhibitors. It is important to note that the effects on IL-6 appear more dramatic than MCP-1. Along with our data suggesting that OX PHOS blockade suppresses MCP-1, MCP-1 production likely utilizes different signaling cascades and transcription factors and may therefore require more ATP for transcription or additional products from glycolysis and subsequent pathways. Nevertheless, we see that ATP reverses

OX suppression from 35 to 23%, suggesting at least partial reversal. We hypothesize that glycolytic blockade reduces ATP availability and thus, ATP available for kinase phosphorylation and signaling through NFκB. Reduced signaling then leads to reduced cytokine production, suggesting that glycolytic blockade effects many downstream signaling events. We note here that low dose ATP was shown to diffuse into the cell with no concomitant IL-6 or MCP-1 production, suggesting this effect is independent of P2X receptors, which are activated at concentrations 10-fold higher (data not shown). Together with the above data, these results support the hypothesis that mast cells require glycolytic ATP production for IL-33-induced function, and suggest that glycolytic blockade can suppress IL-33-mediated inflammation *in vivo*.

Interestingly, we show here that IL-33 increases glycolysis in mast cells, increasing both lactate export and H^+ ion production, and we have previously published that lactic acid suppresses IL-33-mediated cytokine production in mast cells (31). From this we hypothesize that lactic acid increases with IL-33 activation and may act as a feedback regulator of IL-33 activation. There is evidence that glycolysis is elevated in both adaptive and innate immune cells with pro-inflammatory stimulation (1, 6), that lactic acid increases in inflammatory environments (50–54), and that lactic acid suppresses pro-inflammatory immune functions and shifts macrophages to a wound healing phenotype (55). Together, these results suggest that the feedback inhibition may play a larger role in inflammation, cancer, sepsis, asthma, and wound healing. Understanding these pathways and inherent feedback mechanisms may help us to better develop and dose drug treatments for use in different inflammatory diseases.

While 2DG and OX have been used in humans, there is little potential for their clinical use due to dose and side effect limitations. Our data suggest that activating AMPK, a mediator of cell metabolism, may be effective in IL-33-related diseases. There has been no evidence to directly link AMPK to IL-33 in immune cells, although systemic administration of an anti-ST2 antibody increased AMPK phosphorylation in the renal parenchyma of mice (56). We show that activating AMPK with metformin or the specific agonist A799662 suppressed IL-33-induced cytokine production. Furthermore, activating SIRT1, a deacetylase downstream of AMPK known to play a suppressive role in signal transduction and glycolysis (57–59), similarly suppressed cytokine production. This provides another possible clinical target for modulating IL-33-induced inflammatory responses and suggests that SIRT family members should be studied as a target in IL-33-related disease. ATP reversed metformin effects, supporting the theory that inhibiting glycolytic ATP production is the primary mechanism of action. With metformin use *in vivo*, we also provide proof of principle for its utility in IL-33-related pathologies. These data support the idea that metformin provides anti-inflammatory effects beyond lowering blood glucose, and suggest that AMPK is a rational target for suppressing IL-33-mediated cytokine production and effector functions *in vivo*.

Interestingly, IL-33 increased both glycolysis and OX PHOS in mast cells, similar to IgE signaling. However, blocking OX PHOS did not influence IL-6 or TNF production, in contrast to data reported with IgE-mediated activation (18). Lawrence Kane's group observed that rotenone (blocking complex I) suppressed IL-6 production and degranulation in IgE-mediated activation (18), yet in our studies with IL-33, rotenone and antimycin A (blocking both complex I and complex III) had no effect on IL-6 or TNF secretion. Interestingly, we do find effects on MCP-1. The dose of rotenone (1 mM) was the same in each study. This suggests important differences regarding the use and control of energy pathways in the IgE and IL-33 signaling cascades and potential differences in the signaling, transcription, and translation required for the production of different cytokines.

Future work should examine the signaling mechanisms directly responsible for changes in metabolism and cytokine transcription. Transcription of both glycolytic enzymes and cytokines are linked to HIF-1α following LPS activation. TLR4 signaling can induce NADPH oxidase activity and ROS production, which stabilizes HIF-1α (60). ASK1, p38, and ERK signaling can also contribute to HIF-1α accumulation (61–63), which suggests that similar pathways may play a role in IL-33-mediated changes. Furthermore, glycolysis induction is known to provide intermediates for lipid synthesis and histone acetylation (2, 6), in addition to generating ATP and ROS. The importance of these intermediates should be examined in the context of IL-33 activation.

This work emphasizes the importance of glucose metabolism in mast cell function, supporting recent publications by Ehud Razin's and Lawrence Kane's labs, which examined IgE activation of mast cells (18, 19). IL-33 activation increases glycolysis to provide ATP and ROS for optimal receptor signaling, cytokine production, and effector functions. Direct glycolytic blockade and metformin-induced AMPK activation were sufficient to reduce cytokine production both *in vitro* and *in vivo*. Together, these data advance our understanding of IL-33 activation and suggest that AMPK and glycolysis are potential targets for treating IL-33-mediated disease.

AUTHOR CONTRIBUTIONS

All authors assisted in experimental design. HC, MT, TH, NP, EB, and BB conducted experiments and performed initial analyses. HC and JR did detailed analyses and created most data figures. HC and JR wrote the manuscript with editing assistance from all co-authors.

FUNDING

Supported by grants from the National Institutes of Health (1R01AI59638, 1R01AI101153 , and 1R01AI138495 to JR).

ACKNOWLEDGMENTS

We would like to thank Patrick Paez and Dr. Xiang-Yang Wang for bone marrow cells harvested from NFκB-luc mice. We would like to thank Dr. Anthony Faber at VCU for providing access to the XFp Seahorse analyzer and Marissa Calbert for help with troubleshooting and scheduling. Additionally, we would like to thank Dr. Frank Fang at VCU for providing access to the Glomax 20/20 Luminometer.

REFERENCES

1. O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nature Rev Immunol.* (2016) 16:553–65. doi: 10.1038/nri.2016.70
2. Kelly B, O'neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* (2015) 25:771–84. doi: 10.1038/cr.2015.68
3. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and Quiescence. *Immunity* (2013) 38:633–43. doi: 10.1016/j.immuni.2013.04.005
4. Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol.* (2012) 33:168–73. doi: 10.1016/j.it.2012.01.010
5. Bantug GR, Galluzzi L, Kroemer G, Hess C. The spectrum of T cell metabolism in health and disease. *Nature Rev Immunol.* (2017) 18:19–34. doi: 10.1038/nri.2017.99
6. Loftus RM, Finlay DK. Immunometabolism: cellular metabolism turns immune regulator. *J Biol Chem.* (2016) 291:1–10. doi: 10.1074/jbc.R115.693903
7. Wahl DR, Byersdorfer CA, Ferrara JLM, Opipari AW, Glick GD. Distinct metabolic programs in activated T cells: opportunities for selective immunomodulation. *Immunol Rev.* (2012) 249:104–15. doi: 10.1111/j.1600-065X.2012.01148.x
8. Galli SJ. The mast cell-IgE paradox: from homeostasis to anaphylaxis. *Am J Pathol.* (2016) 186:212–24. doi: 10.1016/j.ajpath.2015.07.025
9. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med.* (2012) 18:693–704. doi: 10.1038/nm.2755
10. Galli SJ, Starkl P, Marichal T, Tsai M. Mast cells and IgE in defense against venoms: possible "good side" of allergy? *Allergol Int.* (2016) 65:3–15. doi: 10.1016/j.alit.2015.09.002
11. Hepworth MR, Danilowicz-Luebert E, Rausch S, Metz M, Klotz C, Maurer M, et al. Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. *Proc Natl Acad Sci.* (2012) 109:6644–9. doi: 10.1073/pnas.1112268109
12. Kitahata Y, Nunomura S, Terui T, Ra C. Prolonged culture of mast cells with high-glucose medium enhances the fcεri-mediated degranulation response and leukotriene candlt;subandgt;4andlt;/subandgt; production. *Int Arch Allergy Immunol.* (2010) 152:22–31. doi: 10.1159/000312122
13. Nagai K, Fukushima T, Oike H, Kobori M. High glucose increases the expression of proinflammatory cytokines and secretion of TNFα and β-hexosaminidase in human mast cells. *Eur J Pharmacol.* (2012) 687:39–45. doi: 10.1016/j.ejphar.2012.04.038
14. Takei M, Endo K. Histamine release and calcium concentrations in rat mast cells are dependent on intracellular ATP: effects of prostaglandin D2. *Prostaglandins Leukot Essent Fatty Acids* (1994) 50:357–62. doi: 10.1016/0952-3278(94)90247-X
15. Yoshizaki K, Arizono N, Hayano T, Watari H. Allergen-induced histamine secretion associated with lactate production in mast cells detected by 1H NMR. *Magn Reson Med.* (1993) 29:732–6. doi: 10.1002/mrm.1910290604
16. Chakravarty N. Further observations on the inhibition of histamine release by 2-deoxyglucose. *Acta Physiol.* (1968) 72:425–32. doi: 10.1111/j.1365-201X.1968.tb10852.x
17. Chakravarty N. Glycolysis in rat peritoneal mast cells. *J Cell Biol.* (1965) 25:123–8. doi: 10.1083/jcb.25.2.123
18. Phong B, Avery L, Menk AV, Delgoffe GM, Kane L. Cutting edge: murine mast cells rapidly modulate metabolic pathways essential for distinct effector functions. *J Immunol.* (2017) 198:640–4. doi: 10.4049/jimmunol.1601150
19. Erlich TH, Yagil Z, Kay G, Peretz A, Migalovich-Sheikhet H, Tshori S, et al. Mitochondrial STAT3 plays a major role in IgE-antigen–mediated mast cell exocytosis. *J Allergy Clin Immunol Pract.* (2014) 134:460–9.e10. doi: 10.1016/j.jaci.2013.12.1075
20. Saluja R, Khan M, Church MK, Maurer M. The role of IL-33 and mast cells in allergy and inflammation. *Clin Transl Allergy* 5:33. doi: 10.1186/s13601-015-0076-5
21. Hsu C-L, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS ONE* (2010) 5:e11944. doi: 10.1371/journal.pone.0011944
22. Cayrol C, Girard JP. IL-33, an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* (2014) 31:31–7. doi: 10.1016/j.coi.2014.09.004
23. Saluja R, Ketelaar ME, Hawro T, Church MK, Maurer M, Nawijn MC. The role of the IL-33/IL-1RL1 axis in mast cell and basophil activation in allergic disorders. *Mol Immunol.* (2015) 63:80–5. doi: 10.1016/j.molimm.2014.06.018
24. Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res.* (2010) 59:207–18. doi: 10.1007/s00011-009-0088-5
25. Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy* (2008) 38:1875–81. doi: 10.1111/j.1365-2222.2008.03114.x
26. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol.* (2009) 183:5094–103. doi: 10.4049/jimmunol.0802387
27. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet.* (2011) 43:887–92. doi: 10.1038/ng.888
28. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimaki S, et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J Invest Dermatol.* (2012) 132:1392–400. doi: 10.1038/jid.2011.446
29. Lee HY, Rhee CK, Kang JY, Byun JH, Choi JY, Kim SJ, et al. Blockade of IL-33/ST2 ameliorates airway inflammation in a murine model of allergic asthma. *Exp Lung Res.* (2014) 40:66–76. doi: 10.3109/01902148.2013.870261
30. Sjöberg LC, Gregory JAS, Dahlén E, Nilsson GP, Adner M. Interleukin-33 exacerbates allergic bronchoconstriction in the mice via activation of mast cells. *Allergy* (2015) 70:514–21. doi: 10.1111/all.12590
31. Abebayehu D, Spence AJ, Qayum AA, Taruselli MT, McLeod JJ, Caslin HL, et al. Lactic acid suppresses IL-33–mediated mast cell inflammatory responses via hypoxia-inducible factor-1α-dependent miR-155 suppression. *J Immunol.* (2016) 197:2909–17. doi: 10.4049/jimmunol.1600651
32. Qayum AA, Paranjape A, Abebayehu D, Kolawole EM, Haque TT, McLeod JJA, et al. IL-10–induced miR-155 targets SOCS1 to enhance IgE-mediated mast cell function *J. Immunol.* (2016) 196:4457–67. doi: 10.4049/jimmunol.1502240
33. Drube S, Heink S, Walter S, Löhn T, Grusser M, Gerbaulet A, et al. The receptor tyrosine kinase c-Kit controls IL-33 receptor signaling in mast cells. *Blood* (2010) 115:3899–906. doi: 10.1182/blood-2009-10-247411
34. Cohen, P. (2014). The TLR and IL-1 signalling network at a glance. *J Cell Sci.* 127:2383–90. doi: 10.1242/jcs.149831
35. Everts B, Amiel ESC, Huang C, Smith AMC, Chang H, Lam WY, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat Immunol.* (2014) 15:323–32. doi: 10.1038/ni.2833
36. Tan Z, Xie N, Banerjee S, Cui H, Fu M, Thannickal VJ, et al. The monocarboxylate transporter 4 is required for glycolytic reprogramming and inflammatory response in macrophages. *J Biol Chem.* (2015) 290:46–55. doi: 10.1074/jbc.M114.603589
37. Caslin HL, McLeod JJA, Spence AJ, Qayum AA, Kolawole EM, Taruselli MT, et al. Didox (3,4-dihydroxybenzohydroxamic acid) suppresses IL-33-induced cytokine production in primary mouse mast cells. *Cell Immunol.* (2017) 319:10–6. doi: 10.1016/j.cellimm.2017.04.013
38. Bedard K, Krause H. The NOX family of ROS-Generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev.* (2007) 87:245–313. doi: 10.1152/physrev.00044.2005
39. Enoksson M, Moller-Westerberg C, Wicher G, Fallon PG, Forsberg-Nilsson K, Lunderius-Andersson C, et al. Intraperitoneal influx of neutrophils in response to IL-33 is mast cell-dependent. *Blood* (2013) 121:530–6. doi: 10.1182/blood-2012-05-434209
40. Stein M, Lin H, Jeyamohan C, Dvorzhinski D, Gounder M, Bray K, et al. Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies. *Prostate* (2010) 70:1388–94. doi: 10.1002/pros.21172
41. Granchi C, Paterni I, Rani R, Minutolo F. Small-molecule inhibitors of human LDH(2015) 5. *Future Med Chem.* (2013) 5:1967–91. doi: 10.4155/fmc.13.151

42. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* (2010) 115:4742–9. doi: 10.1182/blood-2009-10-249540
43. Kamide Y, Ishizuka T, Tobo M, Tsurumaki H, Aoki H, Mogi C, et al. Acidic environment augments FcεRI-mediated production of IL-6 and IL-13 in mast cells. *Biochem Biophys Res Commun.* (2015) 464:949–55. doi: 10.1016/j.bbrc.2015.07.077
44. He L, Wondisford FE. Metformin action: concentrations matter. *Cell Metab.* (2015) 21:159–62. doi: 10.1016/j.cmet.2015.01.003
45. Wang C, Chen Z, Bu X, Han Y, Shan S, Ren T, et al. IL-33 signaling fuels outgrowth and metastasis of human lung cancer. *Biochem Biophys Res Commun.* (2016) 479:461–8. doi: 10.1016/j.bbrc.2016.09.081
46. Cham CM, Driessens G, O'Keefe JP, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur J Immunol.* (2008) 38:2438–50. doi: 10.1002/eji.200838289
47. Sun L, Liu X, Fu H, Zhou W, Zhong D. 2-Deoxyglucose suppresses ERK phosphorylation in LKB1 and ras wild-type non-small cell lung cancer cells. *PLoS ONE* (2016) 11:e0168793. doi: 10.1371/journal.pone.0168793
48. Wang F, Zhang S, Vuckovic I, Jeon R, Lerman A, Folmes CD, et al. Glycolytic stimulation is not a requirement for M2 macrophage differentiation. *Cell Metab.* (2018) 28:463–475.e4. doi: 10.1016/j.cmet.2018.08.012
49. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol Mol Biol Rev.* (2011) 75:50–83. doi: 10.1128/MMBR.00031-10
50. Bronte V. Tumor cells hijack macrophages via lactic acid. *Immunol Cell Biol.* (2014) 92:647. doi: 10.1038/icb.2014.67
51. Chen YD, Varasteh BB, Reaven GM. Plasma lactate concentration in obesity and type 2 diabetes. *Diabet Metab.* (1993) 19:348–54.
52. Hove H, Holtug K, Jeppesen PB, Mortensen PB. Butyrate absorption and lactate secretion in ulcerative colitis. *Dis Colon Rectum* (1995) 38:519–25. doi: 10.1007/BF02148853
53. Arnold RC, Shapiro NI, Jones AE, Schorr C, Pope J, Casner E, et al. Multicenter study of early lactate clearance as a determinant of survival in patients with presumed sepsis. *Shock* (2009) 32:35–9. doi: 10.1097/SHK.0b013e3181971d47
54. Löffler M, Zieker D, Weinreich J, Löb S, Königsrainer I, Symons S, et al. Wound fluid lactate concentration: a helpful marker for diagnosing soft-tissue infection in diabetic foot ulcers? Preliminary findings: marker for soft-tissue infection *Diabet Med.* (2011) 28:175–8. doi: 10.1111/j.1464-5491.2010.03123.x
55. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* (2014) 513:559–63. doi: 10.1038/nature13490
56. Park GH, Shinn HK, Kang JH, Na WJ, Kim YH, Park CS. Anti-interleukin-33 reduces ovalbumin-induced nephrotoxicity and expression of kidney injury molecule-1. *Int Neurourol J.* (2016) 20:114–21. doi: 10.5213/inj.1632578.289
57. Vachharajani VT, Liu T, Wang X, Hoth JJ, Yoza BK, McCall CE. Sirtuins link inflammation and metabolism. *J Immunol Res.* (2016) 2016:1–10. doi: 10.1155/2016/8167273
58. Yang Z, Kahn BB, Shi H, Xue BZ. Macrophage 1 AMP-activated protein kinase (1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. *J Biol Chem.* (2010) 285:19051–9. doi: 10.1074/jbc.M110.123620
59. Zhang R, Chen HZ, Liu JJ, Jia YY, Zhang ZQ, Yang RF, et al. SIRT1 suppresses activator protein-1 transcriptional activity and cyclooxygenase-2 expression in macrophages. *J Biol Chem.* (2010) 285:7097–110. doi: 10.1074/jbc.M109.038604
60. Liu L, Lu Y, Martinez J, Bi Y, Lian G, Wang T, et al. Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from Myc-dependent to HIF1 α -dependent. *Proc Natl Acad Sci.* (2016) 113:1564–9. doi: 10.1073/pnas.1518000113
61. Pchejetski D, Nunes J, Coughlan K, Lall H, Pitson SM, Waxman J, et al. The involvement of sphingosine kinase 1 in LPS-induced Toll-like receptor 4-mediated accumulation of HIF-1α protein, activation of ASK1 and production of the pro-inflammatory cytokine IL-6. *Immunol Cell Biol.* (2011) 89:268–74. doi: 10.1038/icb.2010.91
62. Sumbayev VV. LPS-induced Toll-like receptor 4 signalling triggers cross-talk of apoptosis signal-regulating kinase 1 (ASK1) and HIF-1α protein. *FEBS Lett.* (2008) 582:319–26. doi: 10.1016/j.febslet.2007.12.024
63. Sumbayev VV, Nicholas SA, Gibbs BF. Differential role of hypoxia-inducible factor 1 alpha in toll-like receptor-mediated and allergic inflammatory reactions. *World Allergy Org J.* (2010) 3:245. doi: 10.1097/WOX.0b013e3181f8daa5

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Caslin, Taruselli, Haque, Pondicherry, Baldwin, Barnstein and Ryan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 20 December 2018
doi: 10.3389/fimmu.2018.03051

Interleukin-33 in Malignancies: Friends or Foes?

Jia-Xin Shen[1,2], *Jing Liu*[1,3]* *and Guo-Jun Zhang*[1,4]*

[1] Chang Jiang Scholar's Laboratory, Shantou University Medical College, Shantou, China, [2] Department of Hematology, The First Affiliated Hospital of Shantou University Medical College, Shantou, China, [3] Department of Physiology, Shantou University Medical College, Shantou, China, [4] The Cancer Center and the Department of Breast-Thyroid Surgery, Xiang'an Hospital of Xiamen University, Xiamen, China

The human Interleukin-33 (IL-33), a member of the IL-1 family, is the cytokine as a cell endogenous alarmin, released by damaged or necrotic barrier cells (endothelial and epithelial cells). The signal transduction of IL-33 relies on recognition and interaction with specific receptor ST2, mainly expressed in immune cells. In both innate and adoptive immunity, IL-33 regulates the homeostasis in response to stress from within/out the microenvironment. Various, even negative biofunctions of IL-33 pathways have now been widely verified in pathogenesis among immunological mechanisms, like Th2-related immune-stimuli, inflammation/infection-induced tissue protectors. A larger versatility in studies of IL-33 on malignancies now focuses on: (1) promoting myeloid-derived suppressor cells (MDSC), (2) intervention toward $CD8^+$ T, Natural Killer (NK) cell infiltration, group 2 innate lymphoid cells (ILC2) proliferation, dendritic cells (DC) activation, and (3) inhibiting tumor growth and/or further metastasis as an immunoadjuvant. Although IL-33 functioned pro-tumorigenically in various cancers, for some cancer types the findings so far are controversial. This review begins from a summarized introduction of IL-33, to its remarkable implications and molecular transduction pathway in malignant neoplasms, ends with latest inspiration for IL-33 in treatment.

Keywords: IL-33, cytokine, cancer, immunology, therapy

OPEN ACCESS

Edited by:
Fang-Ping Huang,
The University of Hong Kong,
Hong Kong

Reviewed by:
Remo Castro Russo,
Universidade Federal de Minas Gerais,
Brazil
Angela Bonura,
Italian National Research Council, Italy

***Correspondence:**
Jing Liu
jliu12@stu.edu.cn
Guo-Jun Zhang
gjzhang@xah.xmu.edu.cn

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *01 June 2018*
Accepted: *10 December 2018*
Published: *20 December 2018*

Citation:
Shen J-X, Liu J and Zhang G-J (2018) Interleukin-33 in Malignancies: Friends or Foes? Front. Immunol. 9:3051. doi: 10.3389/fimmu.2018.03051

INTRODUCTION

Cytokines are central mediators between cells in the inflammatory tumor microenvironment, in which Interleukin-33 (IL-33) is considered as an alarmin released after cellular damage. IL-33 was discovered as a member of the IL-1 family of cytokines. The IL-1 gene family contains 11 members (IL-1α, IL-1β, IL-1RA, IL-18, IL-36RA, IL-36α, IL37, IL-36β, IL-36γ, IL-38, IL-33), which induces a complex network of pro-inflammatory cytokines, and regulates and initiates inflammatory responses, via expressing integrins on leukocytes and endothelial cells (1). IL-33 gene is constitutively located on the short arm of chromosome 9 at 9p24.1. Sequencing alignment toward human and murine IL-33 revealed two evolutionary conserved domains; a chromatin-binding motif, and a cleavage site for inflammatory proteases and apoptotic caspases (**Figure 1A**). The discovery of two ST2-binding sites further confirmed an exerted binding of IL-33 to a heterodimer, formed by the specific primary receptor ST2 and the IL-1 receptor accessory protein (2, 3).

The full-length IL-33 contains 270 amino acids in human and 266 in mice, which harbors a homeodomain-like helix-turn-helix domain presumably allowing to bind to DNA (4). The release

of IL-33 can be associated with mechanical and oxidative stress, necrotic cell death, or cell activation through ATP signaling in the absence of cell death (5). IL-33 is rapidly released from cells during necrosis or tissue injury, and signals through a cell surface receptor complex, ST2 (IL-1 receptor-like 1, IL1RL1) and IL1RAcP (IL-1 receptor accessory protein), to initiate inflammatory pathways in immune cells, such as type-2 innate lymphoid cells (ILC2), mast cells and natural killer (NK) cells (6). Although advances have been made, mechanisms regulating the alarmin activity of IL-33 remain poorly understood (**Figure 1B**).

Human nervous tissues, barrier structures with widespread of endothelial, epithelial and fibroblast-like cells that are exposed to the environment, were indicated high level of constitutive expression of IL-33. However, inducible IL-33 by inflammation catches more attention of researchers from classical cases with chronic obstructive pulmonary disease, inflammatory responses to suffered graft-vs.-host diseases after bone marrow transplantation. Most recently, tissue fibrosis, mucosal healing, and wound repairmen were as well found to be possible initiatives of IL-33 during inflammation. There are outcomes of cross-talks and interactions within DNA, mRNA, and protein levels. Strict regulation starts from nuclear localization and chromosome association of IL-33, where nuclear IL-33 functions as a transcriptional repressor when overexpressed in chronically in?amed tissues from patients with rheumatoid arthritis and Crohn's disease (7, 8).

The Roles of IL-33 in Malignancies

The multiple roles of IL-33 in malignancies were summarized in **Figure 2**. Most of the current studies on IL-33's multiple roles in cancers focus on tumor microenvironment, tumorigenesis and tumor-associated inflammatory responses. In head-and-neck-squamous cancers, cancer associated fibroblasts was found releasing IL-33, sequentially leading migration and invasion through epithelial-to-mesenchymal transition (9). Data from tongue cancer patients witnesses a worse prognosis with higher level of IL-33 or ST2. Well-designed tissue comparison assays showed an elevated IL-33 and IL1RL1 or ST2 in tissue of both human breast cancer and non-small-cell-lung cancer (NSCLC), compared to adjacent non-tumor tissues. Similarly, high level of serum IL-33 also indicated a poor prognosis in patients with these two types of cancers (10–13). Related mechanism includes genomic instability and mutation, epigenetic modification, apoptosis resistance to cancer-initiated cells and increases of cancer metastasis.

Immunity and Associated Microenvironment

The process of tumor development can trigger anti-tumor immune responses. The type 1 immune response is a critical component of cell-mediated immunity, which includes tumor-induced IFN-γ-producing Th1 cells, cytotoxic T lymphocytes, NK T cells, and γδ T cells, to limit tumor growth and metastasis (14). Since inflammation is another important component in malignancies, it drove more studies on how IL-33 plays roles in improving cancerous surveillance and immunity against tumor.

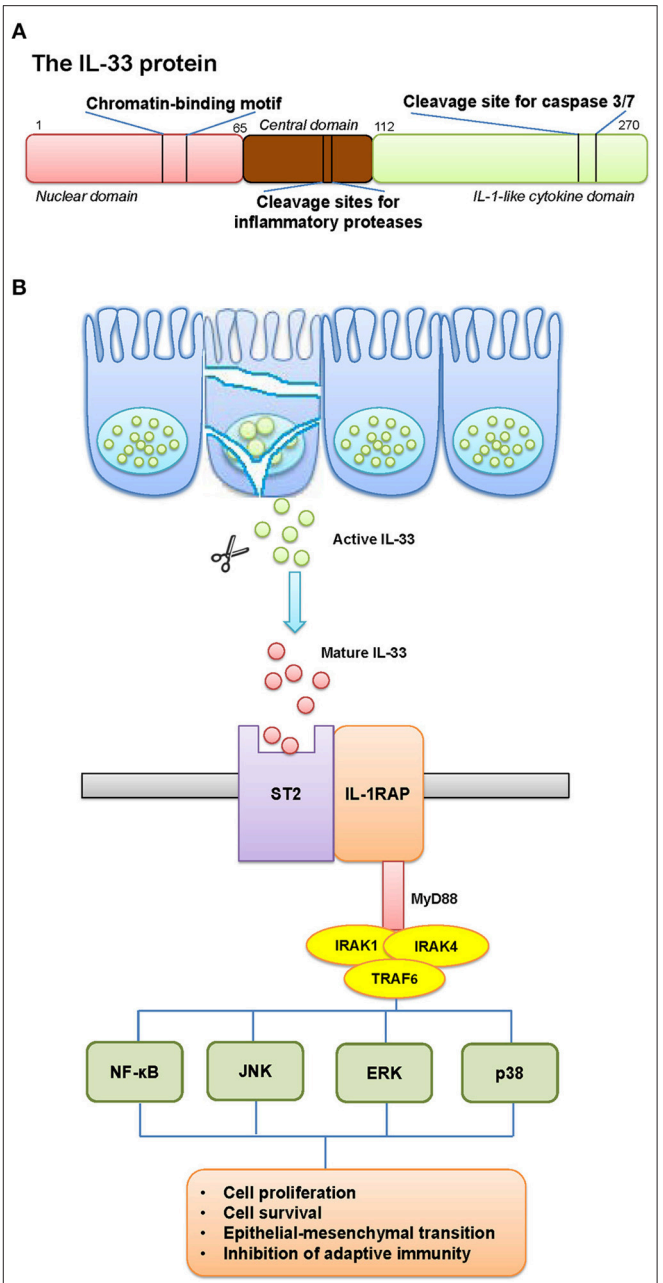


FIGURE 1 | The schematic elucidation of IL-33. **(A)** The schematic structure of IL-33 protein and its critical domains. **(B)** The pathway involved IL-33 and its receptor. **(A)** IL-33 gene is constitutively located on the short arm of chromosome 9 at 9p24.1. The structure of IL-33 protein contains two evolutionary conserved domains: a chromatin-binding motif, and a cleavage site for inflammatory proteases and apoptotic caspases. **(B)** The release mechanisms of IL-33 can occur by mechanical and oxidative stress, necrotic cell death, or cell activation that functions as an alarmin. IL-33 is rapidly released from cells during necrosis or tissue injury, and signals through a cell surface receptor complex, ST2 (IL-1 receptor-like 1, IL1RL1) and IL1RAcP (IL-1 receptor accessory protein), to initiate inflammatory pathways in immune cells. This clustered domain recruits signaling adaptor and kinases (including MyD88, IRAK1/4, TRAF6) in order to activate the transcription factors in tumor cells. The possible results would be a generated cancer-related inflammatory microenvironment, with tumor-promoting effects.

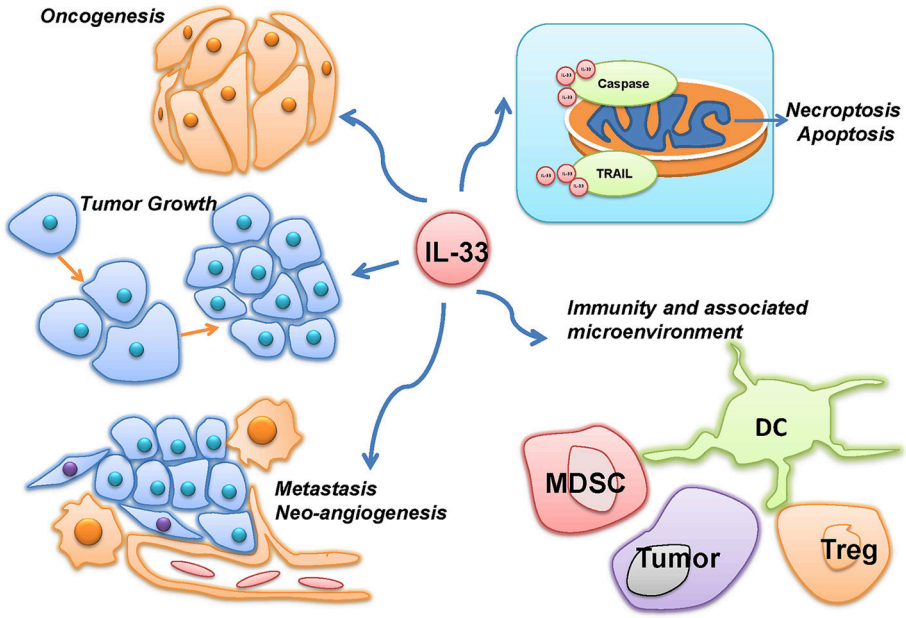


FIGURE 2 | The multiple roles of IL-33 in malignancies. IL-33 was reported to be involved in the different hallmarks of cancer, such as oncogenesis, tumor growth, metastasis, neo-angiogenesis, and evading programmed cell death. However, as an immune-associated factor, IL-33 was also demonstrated to affect the immunity and associated microenvironment of cancer through immune cells, such as myeloid-derived suppressor cells (MDSC), Dendritic cells (DC), and Regulatory T cells (Treg).

Gao et al. successfully gained the increased innate immunity of CD8(+) and NK cells by overexpressing IL-33 in tumor-bearing mice. The metastatic potential in models of B16 melanoma and Lewis lung carcinoma were significantly attenuated by transgenic expression of IL-33 (15). When depleting CD8(+) T cells and NK cells, pulmonary metastasis was significantly increased, indicating that IL-33 can mediate the anti-tumor immunity of CD8(+) T cells and NK cells.

However, other researches considered IL-33 promoted cancer progression through diminishing innate anti-tumor immunity and increasing intra-tumor accumulation of immunosuppressive cells in transgenic mice with breast cancer. Jovanovic et al. detected a time-dependent increase of endogenous IL-33 at both mRNA and protein levels in 4T1 breast cancer model and pulmonary metastasis during cancer progressions. In IL-33-treated mice, intra-tumor NKp46(+) NKG2D(+) and NKp46(+) FasL(+) cells were markedly reduced, while PBS-treated ST2-deficient mice had increased frequencies of these tumoricidal NK cells compared to that in untreated wild-type mice (16). Similarly, IL-33 increased IFN-γ by CD8(+) T and NK cells in tumor tissues, thereby inducing the microenvironment accessible to tumor eradication (17).

The tumor microenvironment, in which the tumor exists, constantly interacts with tumor cells. The developing tumor itself can promote anti-tumor immune responses by recruiting cytotoxic T lymphocytes, T helper 1 cells, and NK cells; so as to influence the microenvironment by releasing extracellular signals, promoting tumor angiogenesis and/or peripheral immune tolerance (14, 18). At the same time, the immune cells involved can affect the growth and evolution of tumor cells (19). Immune suppression specific to tumor and inflammatory stimuli may therefore display a cancer-initiated action, where cytokines are the central mediators (20, 21). IL-33 was proved to be a robust inducer of T helper 2 cells (releasing cytokines like IL-4/5/6/10/13) in tumor microenvironment, but a suppressor to the secretion of T helper 1 cells (cytokines like IL-12, IFN-γ) (22). Recent data revealed the role of IL-33 in several cancers, indicating dual functions as a damage-associated molecular pattern, or nuclear factors mediating gene expression (23).

Oncogenesis

Environmental factors (nicotiana or alcoholic use), and infections (hepatitis B virus), were indicated as triggering factors for minor changes in lungs, heart and livers (24). These were later proved to initiate a higher rate of neoplastic growth, followed by chronic inflammation to targeted organs and/or tissues. Therefore, the tumor-associated inflammatory pathways to cancer development are research hotspots.

Tumors often locally accumulated Treg cells, preventing tumor clearance. In intestinal tumors from $APC^{Min/+}$ mice, researchers found that a high level of cytokine IL-33 were preferentially expanded in $KLRG1^{+}$ $GATA3^{+}$ Treg cells, sequentially to activate E-cadherin ablation and increase β-catenin signals in epithelial cells (25, 26). In another remarkable study of patients with metastatic colon cancer, higher expression of IL-33 in cancer tissues was significantly associated with poorer

survival. Similarly, the study pointed out that IL-33 activated core stem cell genes via ST2 signaling pathway, recruited macrophages and stimulated stem-like characters to promote carcinogenesis of colon cancer cells (27, 28). Another new function of IL-33 discovered from non-basophilic leukemia, a rare subtype of acute myeloblastic leukemia, indicated IL-33 enhanced the basophilic differentiation of MYB-GATA1 expression, demonstrating a new role of IL-33 in leukemic cells and CD34-positive primary cells (29).

Tumor Growth

IL-33 is a cytokine implicated in mutual modulation of not only anti-tumor immunity but tumor growth. Inflammatory responses can be triggered by tumor growth through releasing danger signals and expression of tumor antigens (14). Nevertheless, tumors progress by enlisting and driving the dominance of immune suppressive cell types such as Treg and myeloid-derived suppressor cells, as well as myeloid cells that produce cancer-promoting factors (18, 30, 31). Moreover, IL-33 also promoted the growth and metastasis of solid cancers, such as gastric cancer, colorectal cancer, ovarian cancer, and breast cancer (32, 33). Wang et al. reported blocking IL-33 activities restricted tumor growth of NSCLC xenografts, indicating IL-33 blockade as a novel therapeutics for NSCLC patients (34).

Conversely, other studies pointed out anti-cancer activities of IL-33 to inhibit tumor progression in cellular levels and animal models. Qin et al. showed that recombinant IL-33 dramatically repressed the leukemia growth and prolonged the survival of leukemia-bearing mice by increasing IFN-γ production of leukemia-reactive CD8+ T cells (35). Tumor expression of IL-33 was also reported to inhibit tumor growth and favor tumor eradication by modifying the tumor microenvironment through $CD8^+$ T cells (17). Put together, it is proposed that IL-33 might exert the anti-cancer activities of suppressing tumor growth under certain circumstances.

Metastasis and Neo-Angiogenesis

Cancer metastasis is one of the severe outcomes in most patients with malignancies. It is characterized by rapid and uncontrolled proliferation with high ATP demands, which requires a high rate of glucose uptake. In the surface of NSCLC cells, IL-33/ST2 pathway upregulated membrane glucose transporter 1 to enhance their glucose uptake and glycolysis to meet the ATP switch of metastasis (36).

Saranchova et al. described a new mechanism of immune-surveillance in cancer. They found metastatic carcinomas expressed low levels IL-33 and antigen processing machinery (APM), compared to syngeneic primary tumors. Supplementation of IL-33 in metastatic tumors restrained tumor growth rates and frequencies of circulating tumor cells by upregulating APM and functionality of major histocompatibility complex (MHC)-molecules, indicating the inhibition function of IL-33 to primary tumors in cancer immune-surveillance and losing that function during metastatic transition as immune escape (37). Evidences pointed out IL-33 as a possible inducer and prognostic marker of cancer metastasis by way of mechanisms like immune regulation, with intensive cytotoxic activities of NK cells and increased systemic Th1 and Th17 cytokines (38).

Other malignancies witness proof of IL-33 to metastatic process. The soluble form of the IL-33 receptor (sST2), resisting IL-33-induced angiogenesis, is downregulated in metastatic cells compared with low-metastatic colorectal cancer cells (39). Wang et al. determined that IL-33 overexpression enhances robust outgrowth and metastasis *in vitro*/*in vivo*, while genetic knockdown of IL-33 limited the progression of NSCLC (40). In lines with these findings, epithelial ovarian cancer knocked down of IL-33 gene had reduced metastatic potential, while ectopic IL-33 promoted the migratory and invasive capacity. Underlying mechanisms clarified sST2 might block the ERK and JNK signaling pathways, where IL-33 in return, was regarded as a prognosis markers and targets for ovarian cancers (41). In gastric cancer cells, IL-33 promoted cancer migration and invasion through stimulating the secretion of MMP-3 and IL-6 via aberrant activation of ST2-ERK1/2 pathway (42). Taken together, IL-33 may promote cancer development and metastasis through different pathways.

Cancer Cell Death

Different modes of cell death regulate inflammation by modulating professional phagocyte activation (43). The topic concerning IL-33 initiated cancer cell death is still emerging. It is likely to influence a range of diseases, but evidence for IL-33 limited to only a few examples.

Apoptosis is one of the classically understood processes of cell death that denotes a specific caspase-8–dependent programmed cellular death (44). Ye et al. released a recent study that IL-33 prevent cancer cells against platinum-induced apoptosis via the JNK pathway in gastric cancer cells (45). Similarly, in colon cancer cells, IL-33 stimulated cell sphere formation and prevented chemotherapy-induced tumor apoptosis (28). One exception was noticed in MIA PaCa-2, a pancreatic cancer cell line, that not only colonies and proliferations rate, but relative caspase-3 activities were attenuated in the presence of IL-33. Subsequent anti-proliferative effect on cancer cells even correlated with down-regulated anti-apoptotic molecule FLIP and up-regulated pro-apoptotic molecule TRAIL (46). Since unidentified mechanisms of IL-33 in basic research, we need further experiments to clarify the possibly opposite effects in different situations, like inflammatory stages, cell types or even the concentration of IL-33.

Based on signaling pathway studies, IL-33 may also be involved in co-activation of receptor-interacting protein 1/3 (RIP1/3) kinases, subsequently to damage, press and chronic inflammation (47, 48). This discovery shed light on another versatility of IL-33, an atypical cell death named necroptosis. Unlike apoptosis, this caspase-8-independence cell death allows the cell to bypass caspase activation (49, 50). In liver malignancies, inflammatory stimuli are predominantly driven to tissue-resident Kupffer cell from by bacterial and viral infections. Necroptosis of Kupffer cells released the alarmin IL-33 to trigger basophil IL-4, which successively recruited macrophages proliferation. This ultimately activated the macrophages and thereby achieved to liver homeostasis (51). While in cervical

cancer, IL-33 related necroptosis could work as a possible pioneer for immunotherapy in cervical cancer. This HPV-induced cancer was found a rather low level of cytokine, indicating a stimulated releasing of IL-1α for an induced necroptotic cell death (52). Still, potential mechanisms on these studies need further clarified.

Therapeutic Strategies for IL-33 in Cancers

Understanding of direct and indirect effects of IL-33 would be important for profound therapeutic implications, especially in the realm of cancer immunotherapy. By using what scientists learn about the immune system, several synthetic molecules are created to attack a tumor more precisely and effectively (53). Current cancer immunotherapies include cytokines, monoclonal antibodies, and lymphocytes that will enhance existing antitumor immune responses (54). Cytokine-based immunotherapy has been extensively investigated in the treatment of malignancies. These immune-modulating effects allow interleukin-related treatment to provoke an immune response to chronic rheumatic diseases and attenuate disease progression of pathogenic conditions. However, only a few agents, such as interferon and IL-2, have proven to have sufficient clinical benefits to justify their more widespread use (55). Many preclinical studies demonstrated the antitumor effects of Th1 cytokines, to which IL-33 belongs, while clinical efficacy still limited.

Since IL-33 dually drives the immune system in either responsiveness/activation or tolerance/inhibition, IL-33 administration to the status of immune system dynamics controversially direct immune response and determine outcome (56). Any underlying natural bimodal homeostatic dynamic will be critical principal determinant of clinical efficacy. It is suspected that cancer therapy will be to analyze accurately the patient's underlying tumor immune response in a serial manner, then appropriately and accurately synchronize therapy with immune fluctuation (57). This time-dependent dynamic aspect of the immune response in the cancer patient has been largely overlooked in the past and has prevented us from being able to observe how our *in vivo* therapeutic approaches are influencing the immune response to produce the observed clinical effects.

IL-33 has been possibly considered as an immune adjuvant for vaccine therapy. Indeed, IL-33 functions as a promoter to memory T cell immunity in transgenic melanoma mice, thereby mediating a microenvironment that favors tumor rejection. Based on insights from Villarreal, this immunoadjuvant effects in an HPV-associated model is critical for protective immunity (58). Besides, increased IL-33 in pathological settings including tumor immunotherapy, viral infection and graft-vs.-host diseases, suggest that IL-33 overexpression might elicit potent antitumor immunity (59). Thus far, mouse models provide most of the information at hand. To what degree these observations are applicable to real patients is still unclear. Experimental evidences are currently lacking that clearly indicate the suitability of such an approach.

As a tumor biomarker, IL-33 could be versatile serving as a therapeutic target in patients. In breast cancers, estrogen receptor expressions account for most clinical cases. Thanks to hormone therapy with tamoxifen, prognoses of ER-positive breast cancer are significantly improved. While on the other hand, endocrine resistance to tamoxifen led to commonly-found compromised efficacy of hormone therapy where IL-33 played crucially. Knockdown of IL-33 is likely to correct this problem and resistance to tamoxifen-induced tumor growth inhibition could therefore be reversed (60). Similar cases derived from previous studies in lung and gastric cancer, in metastatic prostate carcinomas, and glioblastoma (61). These findings point out new connection betweenIL-33 and cancer pathogenesis and pinpoint IL-33 promising to optimize therapy in clinical practice.

Future Perspectives

The role of IL-33 in inflammatory diseases has been widely discussed since decades ago (62). This review highlights the remarkable span and diversity of its modulatory potency in tumors. Interestingly, our perspectives in IL-33 has now extended beyond its previous identification as an inducer of immune responses to that of a potency in chronic inflammation and timely activation by malignancies. However, many questions remain unraveled, such as the regulatory elements, the bioactive forms, and the inner homeostasis of IL-33. A study of network map insights on IL-33-mediated crosstalk in the pathogenesis of acute and chronic inflammatory diseases (63). It revealed various roles of classical signaling modules like ERK1/2, NK-κB, and PI3K/AKT, etc. that played in disease development, which in no doubt left us with new inspirations to tumor-related development. In therapeutic practices, IL-33-targeted antibody has only been put into clinical trials for asthma. Other approaches were newly recommended, but cautions should be exercised due to the many immune responses and the potential for driving cancer development.

AUTHOR CONTRIBUTIONS

JL and G-JZ contributed conception and design of the study. JL and J-XS organized the database, searched literatures, structured, and drafted the manuscript carefully. JL and G-JZ revised the original manuscript critically. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 81501539 and 81320108015) and the Natural Science Foundation of Guangdong Province (Nos. 2015A030310211 and 2016A030312008). G-JZ is a recipient of the Chang Jiang Scholar's award granted by the Ministry of Education of China. Special appreciation would be given to Miss Yang Wu for figure editing.

REFERENCES

1. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* (2011) 117:3720–32. doi: 10.1182/blood-2010-07-273417
2. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
3. Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol.* (2003) 163:69–79. doi: 10.1016/S0002-9440(10)63631-0
4. Scott IC, Majithiya JB, Sanden C, Thornton P, Sanders PN, Moore T, et al. Interleukin-33 is activated by allergen- and necrosis-associated proteolytic activities to regulate its alarmin activity during epithelial damage. *Sci Rep.* (2018) 8:3363. doi: 10.1038/s41598-018-21589-2
5. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity* (2013) 39:1003–18. doi: 10.1016/j.immuni.2013.11.010
6. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Ann Rev Immunol.* (2009) 27:519–50. doi: 10.1146/annurev.immunol.021908.132612
7. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, 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 USA.* (2007) 104:282–7. doi: 10.1073/pnas.0606854104
8. Bessa J, Meyer CA, de Vera Mudry MC, Schlicht S, Smith SH, Iglesias A, et al. Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. *J Autoimmun.* (2014) 55:33–41. doi: 10.1016/j.jaut.2014.02.012
9. Chen SF, Nieh S, Jao SW, Wu MZ, Liu CL, Chang YC, et al. The paracrine effect of cancer-associated fibroblast-induced interleukin-33 regulates the invasiveness of head and neck squamous cell carcinoma. *J Pathol.* (2013) 231:180–9. doi: 10.1002/path.4226
10. Ishikawa K, Yagi-Nakanishi S, Nakanishi Y, Kondo S, Tsuji A, Endo K, et al. Expression of interleukin-33 is correlated with poor prognosis of patients with squamous cell carcinoma of the tongue. *Auris Nasus Larynx* (2014) 41:552–7. doi: 10.1016/j.anl.2014.08.007
11. Liu J, Shen JX, Hu JL, Huang WH, Zhang GJ. Significance of interleukin-33 and its related cytokines in patients with breast cancers. *Front Immunol.* (2014) 5:141. doi: 10.3389/fimmu.2014.00141
12. Kim JY, Lim SC, Kim G, Yun HJ, Ahn SG, Choi HS. Interleukin-33/ST2 axis promotes epithelial cell transformation and breast tumorigenesis via upregulation of COT activity. *Oncogene* (2015) 34:4928–38. doi: 10.1038/onc.2014.418
13. Yang ZP, Ling DY, Xie YH, Wu WX, Li JR, Jiang J, et al. The association of serum IL-33 and sST2 with breast cancer. *Dis Markers* (2015) 2015:516895. doi: 10.1155/2015/516895
14. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* (2006) 313:1960–4. doi: 10.1126/science.1129139
15. Gao K, Li X, Zhang L, Bai L, Dong W, Gao K, et al. Transgenic expression of IL-33 activates CD8(+) T cells and NK cells and inhibits tumor growth and metastasis in mice. *Cancer Lett.* (2013) 335:463–71. doi: 10.1016/j.canlet.2013.03.002
16. Jovanovic IP, Pejnovic NN, Radosavljevic GD, Pantic JM, Milovanovic MZ, Arsenijevic NN, et al. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. *Int J Cancer J.* (2014) 134:1669–82. doi: 10.1002/ijc.28481
17. Gao X, Wang X, Yang Q, Zhao X, Wen W, Li G, et al. Tumoral expression of IL-33 inhibits tumor growth and modifies the tumor microenvironment through $CD8^+$ T and NK cells. *J Immunol.* (2015) 194:438–45. doi: 10.4049/jimmunol.1401344
18. Chen LJ, Zheng X, Shen YP, Zhu YB, Li Q, Chen J, et al. Higher numbers of T-bet(+) intratumoral lymphoid cells correlate with better survival in gastric cancer. *Cancer Immunol Immunother.* (2013) 62:553–61.
19. Korneev KV, Atretkhany KN, Drutskaya MS, Grivennikov SI, Kuprash DV, Nedospasov SA. TLR-signaling and proinflammatory cytokines as drivers of tumorigenesis. *Cytokine* (2017) 89:127–35. doi: 10.1016/j.cyto.2016.01.021
20. Candido J, Hagemann T. Cancer-related inflammation. *J Clin Immunol.* (2013) 33(Suppl 1):S79–84. doi: 10.1007/s10875-012-9847-0
21. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* (2008) 454:436–44. doi: 10.1038/nature07205
22. Cui G, Florholmen J. Polarization of cytokine profile from Th1 into Th2 along colorectal adenoma-carcinoma sequence: implications for the biotherapeutic target? *Inflamm Allergy Drug Targets* (2008) 7:94–7. doi: 10.2174/187152808785107589
23. Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* (2014) 31:31–7. doi: 10.1016/j.coi.2014.09.004
24. Mathurin P, Lucey MR. Alcohol, liver disease, and transplantation: shifting attitudes and new understanding leads to changes in practice. *Curr Opin Organ Transplant.* (2018) 23:175–9. doi: 10.1097/MOT.0000000000000517
25. Meinicke H, Bremser A, Brack M, Akeus P, Pearson C, Bullers S, et al. Tumour-associated changes in intestinal epithelial cells cause local accumulation of $KLRG1^+$ $GATA3^+$ regulatory T cells in mice. *Immunology* (2017) 152:74–88. doi: 10.1111/imm.12750
26. He Z, Chen L, Souto FO, Canasto-Chibuque C, Bongers G, Deshpande M, et al. Epithelial-derived IL-33 promotes intestinal tumorigenesis in Apc (Min/+) mice. *Sci Rep.* (2017) 7:5520. doi: 10.1038/s41598-017-05716-z
27. O'Donnell C, Mahmoud A, Keane J, Murphy C, White D, Carey S, et al. An antitumorigenic role for the IL-33 receptor, ST2L, in colon cancer. *Br J Cancer* (2016) 114:37–43. doi: 10.1038/bjc.2015.433
28. Fang M, Li Y, Huang K, Qi S, Zhang J, Zgodzinski W, et al. IL33 promotes colon cancer cell stemness via JNK activation and macrophage recruitment. *Cancer Res.* (2017) 77:2735–45. doi: 10.1158/0008-5472.CAN-16-1602
29. Ducassou S, Prouzet-Mauleon V, Deau MC, Brunet de la Grange P, Cardinaud B, Soueidan H, et al. MYB-GATA1 fusion promotes basophilic leukaemia: involvement of interleukin-33 and nerve growth factor receptors. *J Pathol.* (2017) 242:347–57. doi: 10.1002/path.4908
30. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* (2009) 16:183–94. doi: 10.1016/j.ccr.2009.06.017
31. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res.* (2011) 17:4550–7. doi: 10.1158/1078-0432.CCR-11-0116
32. Zhang Y, Davis C, Shah S, Hughes D, Ryan JC, Altomare D, et al. IL-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis. *Mol Carcinogenesis* (2017) 56:272–87. doi: 10.1002/mc.22491
33. Schwartz C, O'Grady K, Lavelle EC, Fallon PG. Interleukin 33: an innate alarm for adaptive responses beyond Th2 immunity-emerging roles in obesity, intestinal inflammation, and cancer. *Eur J Immunol.* (2016) 46:1091–100. doi: 10.1002/eji.201545780
34. Wang K, Shan S, Yang Z, Gu X, Wang Y, Wang C, et al. IL-33 blockade suppresses tumor growth of human lung cancer through direct and indirect pathways in a preclinical model. *Oncotarget* (2017) 8:8571–82. doi: 10.18632/oncotarget.19786
35. Qin L, Dominguez D, Chen S, Fan J, Long A, Zhang M, et al. Exogenous IL-33 overcomes T cell tolerance in murine acute myeloid leukemia. *Oncotarget* (2016) 7:61069–80. doi: 10.18632/oncotarget.11179
36. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* (2011) 11:85–95. doi: 10.1038/nrc2981
37. Saranchova I, Han J, Huang H, Fenninger F, Choi KB, Munro L, et al. Discovery of a metastatic immune escape mechanism initiated by the loss of expression of the tumour biomarker interleukin-33. *Sci Rep.* (2016) 6:30555. doi: 10.1038/srep30555
38. Jovanovic I, Radosavljevic G, Mitrovic M, Juranic VL, McKenzie AN, Arsenijevic N, et al. ST2 deletion enhances innate and acquired immunity to murine mammary carcinoma. *Eur J Immunol.* (2011) 41:1902–12. doi: 10.1002/eji.201141417
39. Akimoto M, Maruyama R, Takamaru H, Ochiya T, Takenaga K. Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by

modifying the tumour microenvironment. *Nat Commun.* (2016) 7:13589. doi: 10.1038/ncomms13589
40. Wang C, Chen Z, Bu X, Han Y, Shan S, Ren T, et al. IL-33 signaling fuels outgrowth and metastasis of human lung cancer. *Biochem Biophys Res Commun.* (2016) 479:461–8. doi: 10.1016/j.bbrc.2016.09.081
41. Tong X, Barbour M, Hou K, Gao C, Cao S, Zheng J, et al. Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways. *Mol Oncol.* (2016) 10:113–25. doi: 10.1016/j.molonc.2015.06.004
42. Yu XX, Hu Z, Shen X, Dong LY, Zhou WZ, Hu WH. IL-33 Promotes gastric cancer cell invasion and migration via ST2-ERK1/2 pathway. *Digest Dis Sci.* (2015) 60:1265–72. doi: 10.1007/s10620-014-3463-1
43. Mora J, Schlemmer A, Wittig I, Richter F, Putyrski M, Frank AC, et al. Interleukin-38 is released from apoptotic cells to limit inflammatory macrophage responses. *J Mol Cell Biol.* (2016) 8:426–38. doi: 10.1093/jmcb/mjw006
44. Seifert L, Miller G. Molecular pathways: the necrosome-a target for cancer therapy. *Clin Cancer Res.* (2017) 23:1132–6.
45. Ye XL, Zhao YR, Weng GB, Chen YC, Wei XN, Shao JP, et al. IL-33-induced JNK pathway activation confers gastric cancer chemotherapy resistance. *Oncol Rep.* (2015) 33:2746–52. doi: 10.3892/or.2015.3898
46. Fang Y, Zhao L, Xiao H, Cook KM, Bai Q, Herrick EJ, et al. IL-33 acts as a foe to MIA PaCa-2 pancreatic cancer. *Medical Oncol.* (2017) 34:23. doi: 10.1007/s12032-016-0880-3
47. Kaiser WJ, Upton JW, Long AB, Livingston-Rosanoff D, Daley-Bauer LP, Hakem R, et al. RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature* (2011) 471:368–72. doi: 10.1038/nature09857
48. Trichonas G, Murakami Y, Thanos A, Morizane Y, Kayama M, Debouck CM, et al. Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. *Proc Natl Acad Sci USA.* (2010) 107:21695–700. doi: 10.1073/pnas.1009179107
49. Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nat Rev Mol Cell Biol.* (2014) 15:135–47. doi: 10.1038/nrm3737
50. Mocarski ES, Upton JW, Kaiser WJ. Viral infection and the evolution of caspase 8-regulated apoptotic and necrotic death pathways. *Nat Rev Immunol.* (2011) 12:79–88. doi: 10.1038/nri3131
51. Bleriot C, Dupuis T, Jouvion G, Eberl G, Disson O, Lecuit M. Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. *Immunity* (2015) 42:145–58. doi: 10.1016/j.immuni.2014.12.020
52. Schmidt SV, Seibert S, Walch-Ruckheim B, Vicinus B, Kamionka EM, Pahne-Zeppenfeld J, et al. RIPK3 expression in cervical cancer cells is required for PolyIC-induced necroptosis, IL-1alpha release, and efficient paracrine dendritic cell activation. *Oncotarget* (2015) 6:8635–47. doi: 10.18632/oncotarget.3249
53. Kojima R, Scheller L, Fussenegger M. Nonimmune cells equipped with T-cell-receptor-like signaling for cancer cell ablation. *Nat Chem Biol.* (2018) 14:42–9. doi: 10.1038/nchembio.2498
54. Qu X, Tang Y, Hua S. Immunological approaches towards cancer and inflammation: a cross talk. *Front Immunol.* (2018) 9:563. doi: 10.3389/fimmu.2018.00563
55. Markley JC, Sadelain M. IL-7 and IL-21 are superior to IL-2 and IL-15 in promoting human T cell-mediated rejection of systemic lymphoma in immunodeficient mice. *Blood* (2010) 115:3508–19. doi: 10.1182/blood-2009-09-241398
56. Eissmann MF, Dijkstra C, Wouters MA, Baloyan D, Mouradov D, Nguyen PM, et al. Interleukin 33 signaling restrains sporadic colon cancer in an interferon-gamma-dependent manner. *Cancer Immunol Res.* (2018) 6:409–21. doi: 10.1158/2326-6066.CIR-17-0218
57. Poh AR, Ernst M. Targeting macrophages in cancer: from bench to bedside. *Front Oncol.* (2018) 8:49. doi: 10.3389/fonc.2018.00049
58. Villarreal DO, Wise MC, Walters JN, Reuschel EL, Choi MJ, Obeng-Adjei N, et al. Alarmin IL-33 acts as an immunoadjuvant to enhance antigen-specific tumor immunity. *Cancer Res.* (2014) 74:1789–800. doi: 10.1158/0008-5472.CAN-13-2729
59. Lu B, Yang M, Wang Q. Interleukin-33 in tumorigenesis, tumor immune evasion, and cancer immunotherapy. *J Mol Med.* (2016) 94:535–43. doi: 10.1007/s00109-016-1397-0
60. Hu H, Sun J, Wang C, Bu X, Liu X, Mao Y, et al. IL-33 facilitates endocrine resistance of breast cancer by inducing cancer stem cell properties. *Biochem Biophys Res Commun.* (2017) 485:643–50. doi: 10.1016/j.bbrc.2017.02.080
61. Wasmer MH, Krebs P. The role of IL-33-dependent inflammation in the tumor microenvironment. *Front Immunol.* (2016) 7:682. doi: 10.3389/fimmu.2016.00682
62. Foo YL, Jean-PG, Heth RT. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
63. Pinto SM, Subbannayya Y, Rex DAB, Raju R, Chatterjee O, Advani J, et al. A network map of IL-33 signaling pathway. *J Cell Commun Signal.* (2018) 12:615–24. doi: 10.1007/s12079-018-0464-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Shen, Liu and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 01 February 2019
doi: 10.3389/fimmu.2019.00127

Deficiency in IL-33/ST2 Axis Reshapes Mitochondrial Metabolism in Lipopolysaccharide-Stimulated Macrophages

Huadan Xu[1], Liankun Sun[1], Yichun He[2], Xiaofeng Yuan[3], Junqi Niu[4], Jing Su[1] and Dong Li[4,5]**

[1] Key Laboratory of Pathobiology, Ministry of Education, Department of Pathophysiology, College of Basic Medical Sciences, Jilin University, Changchun, China, [2] Department of Neurosurgery, China-Japan Union Hospital, Jilin University, Changchun, China, [3] Department of Pediatrics, Affiliated Hospital of Changchun University of Chinese Medicine, Changchun, China, [4] Department of Hepatology, The First Hospital of Jilin University, Changchun, China, [5] Department of Immunology, College of Basic Medical Sciences, Jilin University, Changchun, China

OPEN ACCESS

Edited by:
Jose Carlos Alves-Filho,
University of São Paulo, Brazil

Reviewed by:
Angela Bonura,
Italian National Research Council (CNR), Italy
Marco De Andrea,
University of Turin, Italy

***Correspondence:**
Jing Su
sujing@jlu.edu.cn
Dong Li
lidong1@jlu.edu.cn

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: 22 May 2018
Accepted: 15 January 2019
Published: 01 February 2019

Citation:
Xu H, Sun L, He Y, Yuan X, Niu J, Su J and Li D (2019) Deficiency in IL-33/ST2 Axis Reshapes Mitochondrial Metabolism in Lipopolysaccharide-Stimulated Macrophages. Front. Immunol. 10:127. doi: 10.3389/fimmu.2019.00127

The polarization and function of macrophages play essential roles in controlling immune responses. Interleukin (IL)-33 is a member of the IL-1 family that has been shown to influence macrophage activation and polarization, but the underlying mechanisms are not fully understood. Mitochondrial metabolism has been reported to be a central player in shaping macrophage polarization; previous studies have shown that both aerobic glycolysis and oxidative phosphorylation uniquely regulate the functions of M1 and M2 macrophages. Whether IL-33 polarizes macrophages by reshaping mitochondrial metabolism requires further investigation. In this work, we examined the mitochondrial metabolism of bone marrow-derived macrophages (BMDMs) from either wild type (WT), *Il33*-overexpressing, or IL-33 receptor knockout ($St2^{-/-}$) mice challenged with lipopolysaccharide (LPS). We found that after LPS stimulation, compared with WT BMDMs, $St2^{-/-}$ BMDMs had reduced cytokine production and increased numbers and activity of mitochondria *via* the metabolism regulator peroxisome proliferator-activated receptor-C coactivator-1 α (PGC1α). This was demonstrated by increased mitochondrial DNA copy number, mitochondria counts, mitochondria fission- and fusion-related gene expression, oxygen consumption rates, and ATP production, and decreased glucose uptake, lactate production, and extracellular acidification rates. For *Il33*-overexpressing BMDMs, the metabolic reprogramming upon LPS stimulation was similar to WT BMDMs, and was accompanied by increased M1 macrophage activity. Our findings suggested that the pleiotropic IL-33/ST2 pathway may influence the polarization and function of macrophages by regulating mitochondrial metabolism.

Keywords: IL-33, ST2, macrophage, PGC1α, ATP

INTRODUCTION

Macrophages play important role in every stage of immune responses, in both healthy and disease settings. Macrophages can be polarized to different phenotypes according to their surrounding microenvironment, and each phenotype has its own properties and unique functions. Generally, macrophages are cataloged into two major phenotypes based on their glucose metabolism and functions: lipopolysaccharide (LPS)—or IFNγ-stimulated inflammatory M1 type macrophages, which convert arginine into nitric oxide by inducible nitric oxide synthase (iNOS); and Interleukin (IL)-4-stimulated anti-inflammatory and pro-resolution M2 type macrophages, which convert arginine to ornithine by arginase-1 (1, 2). In addition to these differences in arginine metabolism, different subsets of macrophages have distinguishable mitochondrial activities.

It has been reported that mitochondrial metabolism is a central player in shaping macrophage polarization. Previous reports have shown that glycolysis is reprogrammed in LPS-stimulated M1 type macrophages due to impaired mitochondrial function, leading to a Warburg-like effect (aerobic glycolysis), which can be swiftly activated (3). M1 type macrophages not only use glucose to generate ATP, but also use the energy and metabolites (e.g., pyruvate) generated from glycolysis to fuel the pentose phosphate pathway (PPP) and fatty acid acetyl coenzyme A (acetyl-CoA) synthesis, eventually resulting the stabilization of hypoxia inducible factor 1α (HIF1α) and the production of pro-inflammatory cytokines (4). Conversely, IL-4-stimulated M2 type macrophages are supported by mitochondrial oxidative phosphorylation (OXPHOS) (2). As the different glucose metabolism pathways determine macrophage functions, reshaping them might alter these functions and even change immune responses from detrimental to beneficial or *vice versa* (5).

IL-33, which belongs to the IL-1 cytokine family and bind to the receptor ST2, was discovered in 2005 and has been extensively researched since (6). Because IL-33 is a pleiotropic cytokine, it can activate or polarize many types of immune cells, promoting either pro-inflammatory or anti-inflammatory immune responses depending on the specific microenvironment. The interaction of IL-33 and macrophages has been reported to be essential for all stages of immune responses, including the initiation (7), lasting (8–10), and final resolution stages (11, 12).

IL-33 can contribute to macrophage polarization in both pro-M1 and pro-M2 settings (13). Although the underlying mechanisms are not fully understood, IL-33 may polarize macrophages through its canonical ST2/MYD88/IRAK1/4 pathway, or potentially through the binding of full-length IL-33 with transcription factors that alter macrophage phenotypes. Our group previously found that the IL-33/ST2 pathway influenced macrophages proliferation and activity (Li et al. (11) and unpublished data), both of which are known to be closely associated with mitochondrial metabolism. We also found that peroxisome proliferator-activated receptor-coactivator 1α (PGC1α) played a key role in altering mitochondrial metabolism *via* promoting mitochondrial biogenesis (14). Thus, whether IL-33/ST2 signaling can sufficiently alter mitochondrial metabolism to change macrophage functions is worth investigating.

In this study, we used bone marrow-derived macrophages (BMDMs) from wild-type (WT), $St2^{-/-}$, and *Il33*-overexpressing mice, and we stimulated these macrophages with LPS to investigate the role of the IL-33/ST2 pathway in mitochondrial metabolism and macrophage function. We found that the IL-33/ST2 pathway was required for the LPS-induced metabolic reprogramming of macrophages. These results might provide further insight into how macrophages initiated proper responses after encountering stimuli.

MATERIALS AND METHODS

Mice

Specific pathogen-free 6–9-week-old male BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed in specific pathogen-free conditions at Jilin University (15). $St2^{-/-}$ mice were kindly provided by Prof. Weihua Xiao from the University of Science and Technology of China (Hefei, China), and *Il33* transgenic mice were kindly provided by Prof. Ying Sun from Capital Medical University (Beijing, China). Both strains were in the BALB/c background (11). All animal experiments were performed in accordance with the National Guidelines for Experimental Animal Welfare and with approval of the Animal Welfare and Research Ethics Committee at Jilin University (Changchun, China).

Cell Culture

Primary BMDMs were generated as previously described (11). Briefly, murine bone marrow cells were harvested and cultured in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.05 M 2-ME, and 10 ng/ml macrophage colony-stimulating factor (M-CSF; PeproTech, Rocky Hill, NJ, US) for 6 d in a humidified cell culture incubator containing 5% CO_2 at 37°C. All tissue culture reagents and lipopolysaccharide (LPS, L6529) were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from cultured BMDMs using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, US). Genomic DNA digestion and reverse transcription were performed using the EasyScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. For qPCR analyses, cDNA were amplified using a TransStart Green qPCR SuperMix (TransGen Biotech). The cycling parameters were 94°C for 5 s, 50°C—60°C for 15 s and 72°C for 10 s for 40 cycles. A melting-curve analysis was then performed to check PCR specificity. CT values were measured during the exponential amplification phase. Relative expression levels (defined as fold change) of target genes were determined using the 2–ΔΔCT method. *Actb* was used as an internal control. Expression levels were normalized to the fold change detected in the corresponding control cells, which was

defined as 1.0. The primers used were as follows: *Il1a* forward 5′-ACG GCT GAG TTT CAG TGA GAC C-3′ and reverse 5′-CAC TCT GGT AGG TGT AAG GTG C-3′; *Il1b* forward 5′-TGG ACC TTC CAG GAT GAG GAC A-3′ and reverse 5′-GTT CAT CTC GGA GCC TGT AGT G-3′; *Nos2* forward 5′-GCC TCG CTC TGG AAA GA-3′ and reverse 5′-TCC ATG CAG ACA ACC TT-3′; *Ifng* forward 5′-CAG CAA CAG GCA AGG CGA AAA AGG-3′ and reverse 5′-TTT CCG CTT CCT GAG GCT GGA T-3′. *Mfn1* forward 5′-CCT ACT GCT CCT TCT AAC CCA-3′ and reverse 5′-AGG GAC GCC AAT CCT GTG A-3′; *Mfn2* forward 5′-GTG GGC TGG AGA CTC ATC G-3′ and reverse 5′-CTC ACT GGC GTA TTC CGC AA-3′; *Opa1* forward 5′-ACA GCA AAT TCA AGA GCA CGA-3′ and reverse 5′-TTG CGC TTC TGT TGG GCA T-3′; *Dnm1l* forward 5′-ACC GGG AAT GAC CAA AGT ACC-3′ and reverse 5′-TGG GAT TAC TGA TGA ACC GAA GA-3′; and *Fis1* forward 5′- AGA GCA CGC AAT TTG AAT ATG CC-3′ and reverse 5′-ATA GTC CCG CTG TTC CTC TTT-3′.

Relative Mitochondrial Copy Number

Mitochondrial copy numbers were measured as previously described (14). Briefly, BMDMs were cultured on coverslips for 24 h, and then treated with LPS for 72 h. Relative mitochondrial DNA (mtDNA) copy number was measured by qPCR on total DNA extracted using the TIANamp Genomic DNA Kit (Tiangen, Beijing, China). Primer sequences for the mitochondrial segment were: *mt-Nd1* forward 5′-CAC CCA AGA ACA GGG TTT GT-3′ and reverse 5′-TGG CCA TGG GAT TGT TGT TAA-3′. Primer sequences for the single-copy nuclear control were: *18S* forward 5′-TAG AGG GAC AAG TGG CGT TC-3′ and reverse 5′-CGC TGA GCC AGT CAG TGT-3′. Mitochondrial copy number was calculated relative to nuclear DNA using the following equations:

$$\Delta CT = \text{Mitochondrial CT} - \text{NuclearCT} \quad (1)$$

$$\text{Relative mitochondrial DNA content} = 2^{-\Delta CT} \quad (2)$$

Determining Glucose Uptake and Lactate Production

BMDMs cells were treated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 72 h, and then the culture medium was collected for glucose and lactate measurements with glucose and lactate assay kits (Beyotime, Haimen, Jiangsu, China), respectively. Data were normalized to the corresponding total protein amounts from each sample, as previously described (16).

Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) Analysis

A total of 8×10^4 BMDMs were seeded into 96-well plates and incubated overnight to allow adherence. The following day, different concentrations of LPS were added into the indicated wells for 24 h. Each treatment was repeated in three wells. OCR and ECAR were measured using oxygen-sensitive (Mito-Xpress) and pH-sensitive (pH-Xtra) fluorescent probes (Luxcel Bioscience, Cork, Ireland) as previously described (16).

Determining Intracellular ATP Production

Intracellular ATP production was measured using the Enhanced ATP Test Kit (Beyotime). Briefly, BMDMs were treated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 72 h, and then cells were collected and the assay was performed according to the manufacturer's instructions. Data were normalized to the corresponding total protein amounts from each sample, as previously described (17).

Measuring Mitochondrial Membrane Potential

Mitochondrial membrane potential (MMP) in BMDM was determined using a JC-1 probe contained within the Mitochondrial Membrane Potential Assay Kit (Beyotime). At 6 h post-LPS treatment, cells were incubated with 1 ml of 1 × JC-1 for 30 min at 37°C in the dark, and the ratio of cells positive for red fluorescence (JC-1 polymer-positive, indicating intact MMP) to those positive for green fluorescence (monomeric JC-1, indicating loss of MMP) was determined by flow cytometry using a BD Accuri C6 (BD Biosciences, Franklin Lakes, NJ, US) and then analyzed with FlowJo software (Version 10.0.7; FlowJo, LLC, OR, US) as previously described (18).

Mitochondrial Imaging by Confocal Microscopy

BMDMs were cultured on coverslips for 24 h, and then treated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 72 h. The fluorescent dye MitoTracker RED (Thermo Fisher Scientific) was used to monitor mitochondrial content in living cells according to the manufacturer's instructions. Then cells were imaged with an Olympus FV 1000 laser-scanning confocal microscope (Olympus, Tokyo, Japan).

Cytokine Measurements

The concentrations of cytokines in cell culture media were determined using ELISA kits (Thermo Fisher Scientific) according to manufacturers' instructions.

Western Blotting Analysis

BMDMs were treated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 24 h or 48 h, then the cells were lysed in RIPA buffer (Thermo Scientific) containing protease inhibitors (Roche, Basel, Switzerland). Protein concentrations were estimated by the BCA protein assay (Thermo Scientific). Proteins were then incubated at 70°C for 10 min in reducing SDS sample buffer and 30 μg of protein lysate per lane was run through NuPAGE® Novex® 4–12% Bis-Tris Protein Gels (Thermo Scientific) and transferred to Hybond ECL membranes (GE Healthcare, Chicago, IL, US). Membranes were blocked for 1 h in 5% non-fat dried milk in double distilled PBS (DPBS) and incubated overnight with the appropriate primary antibody at 4°C. Membranes were then washed in DPBS/Tween 20 (Bio-Rad Laboratories, Hercules, CA, US) and incubated with the appropriate secondary antibody. Detection was performed by ECL Western Blotting Detection Reagents (Bio-Rad Laboratories). Antibody against PGC1, DRP1, MFN1, MFN2, OPA1, and FIS1 were obtained from Santa Cruz Biotechnology (Dallas, TX, US); β actin and all secondary

antibodies were obtained from Proteintech (Wuhan, Hubei, China).

Statistical Analysis

Data are expressed as means ± standard error (SEM). Statistical significance between two groups was analyzed by One-way ANOVA followed by Student's *t*-test using Prism software (GraphPad Software, La Jolla, CA, US). N.S. represents no statistical difference between the compared groups; * represents $P < 0.05$ and was considered statistically significant. All experiments were repeated at least three times.

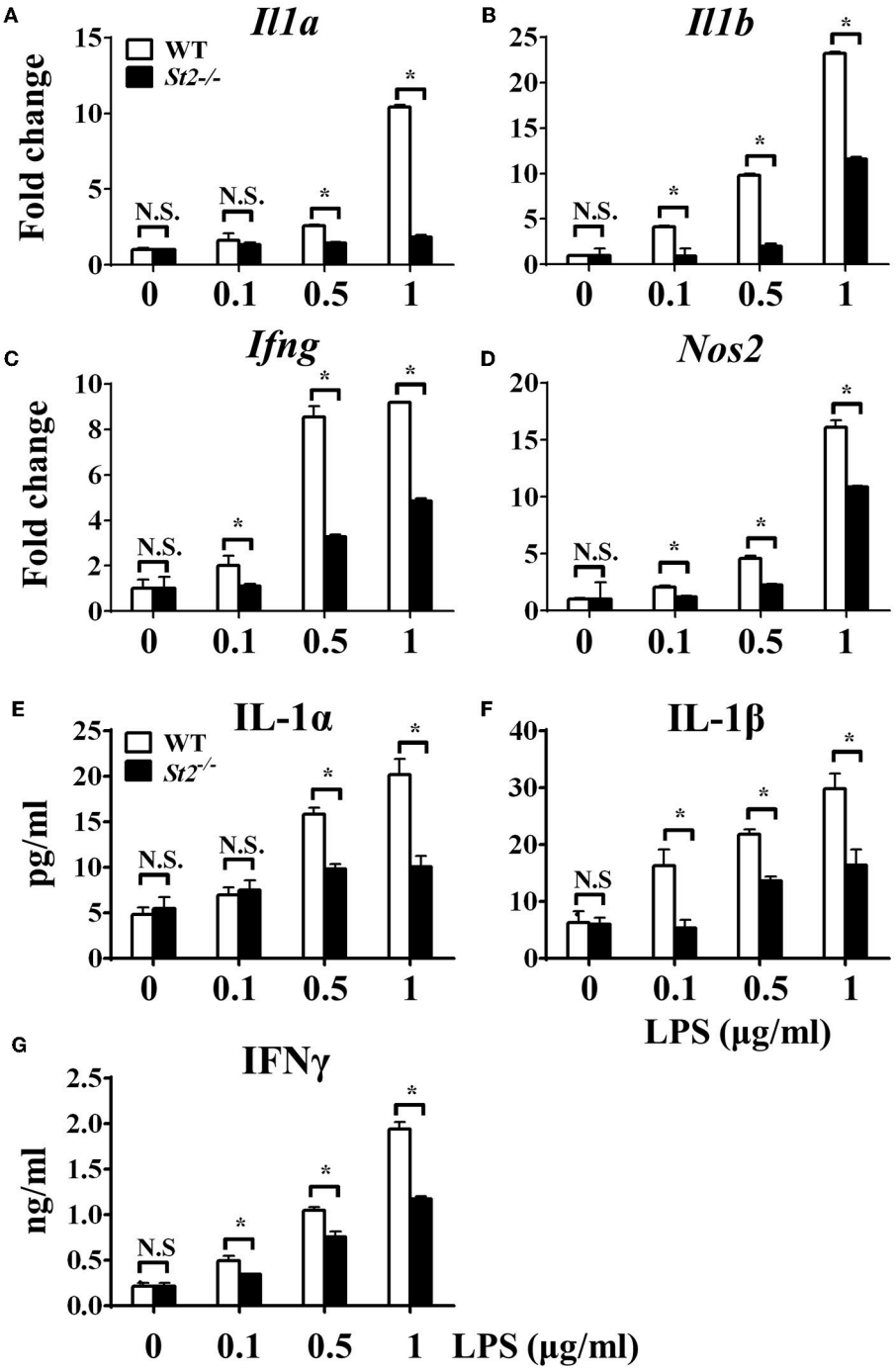


FIGURE 1 | ST2 deficiency impaired macrophages responses upon LPS stimulation. BMDMs from BALB/c or *St2*$^{-/-}$ mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 72 h. Total RNA was isolated and the expression of *Il1a* **(A)**, *Il1b* **(B)**, *Ifng* **(C),** and *Nos2* **(D)** was evaluated by qPCR; the concentration of IL-1α **(E)**, IL-1β **(F)**, and IFNγ **(G)** in the supernatant was evaluated by ELISA. Vertical bars = SEMs ($n = 3$ per group per experiment). N.S., no significant difference; *$p < 0.05$.

RESULTS

ST2 Deficiency Impaired Macrophage Responses Upon LPS Stimulation With Less Glucose Uptake and Lactic Acid Generation

To investigate the role of IL-33/ST2 signaling in LPS-stimulated macrophages, BMDMs were exposed to different LPS doses, and metabolic characteristics and cytokine production were monitored. As reported before, *St2*-deficient BMDMs were not as responsive as WT BMDMs, as demonstrated by decreased *Il1a*, *Il1b*, *Nos2* and *Ifng* expression as measured by qPCR (**Figures 1A–D**) and the concentration of IL-1α, IL-1β, IFNγ in supernatant by ELISA (**Figures 1E–G**). *St2* deficiency increased the OCR (**Figure 2A**) of BMDMs, while reducing their ECAR (**Figure 2B**), lactate acid generation (**Figure 2C**), and glucose consumption (**Figure 2D**). These results indicated that macrophages undergo aerobic glycolysis (a Warburg-like effect) after they have been active by LPS; however, in the absence of IL-33/ST2 signaling, macrophages increase OXPHOS after LPS stimulation. Subsequent experiments showed that this Warburg-like effect was not induced by mitochondrial damage (**Supplementary Figure 1**).

ST2 Deficiency Was Associated With Enhanced Mitochondrial Function

To investigate the mechanism underlying the metabolic reprogramming of macrophages that lacked IL-33/ST2 signaling,

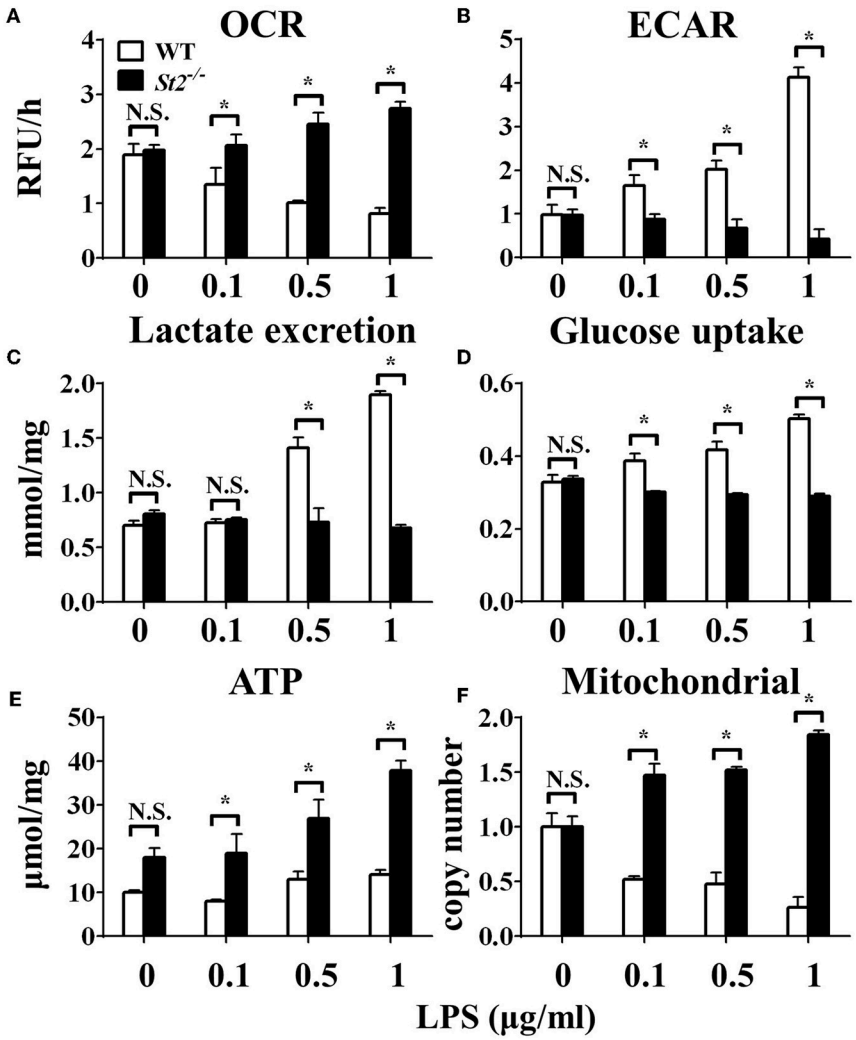


FIGURE 2 | ST2 deficiency affected the metabolism of macrophages upon LPS stimulation. BMDMs from BALB/c or $St2^{-/-}$ mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 24 h and the **(A)** extracellular oxygen consumption rate (OCR) was immediately measured by fluorescence intensity of a typical oxygen probe; **(B)** extracellular acidification rate (ECAR) was measured by incubation at 37°C for 3 h. The lactate excretion **(C)**, glucose uptake **(D)**, ATP **(E)**, and relative mitochondrial DNA copy numbers **(F)** of BMDMs were measured after 72 h stimulation of LPS (0, 0.1, 0.5, 1.0 μg/ml). Vertical bars = SEMs ($n = 3$ per group per experiment). N.S., no significant difference; $^*p < 0.05$.

we evaluated mitochondrial activity. Both the number and activity of mitochondria were increased in *St2*-deficient BMDMs, as shown by more mitochondrial gene copies and ATP production in $St2^{-/-}$ BMDMs compared with WT BMDMs (**Figures 2E,F**). The expression of *Ppargc1a*, which encodes PGC-1α, a master regulator of mitochondrial biogenesis, was also measured; the results showed that LPS increased PGC-1α expression only in $St2^{-/-}$ BMDMs (**Figure 3A**). Next, the induction of mitochondrial fission- and fusion-associated genes (*Fis1*, *Dnm1l* and *Mfn1*, *Mfn2*, *Opa1*, respectively) was

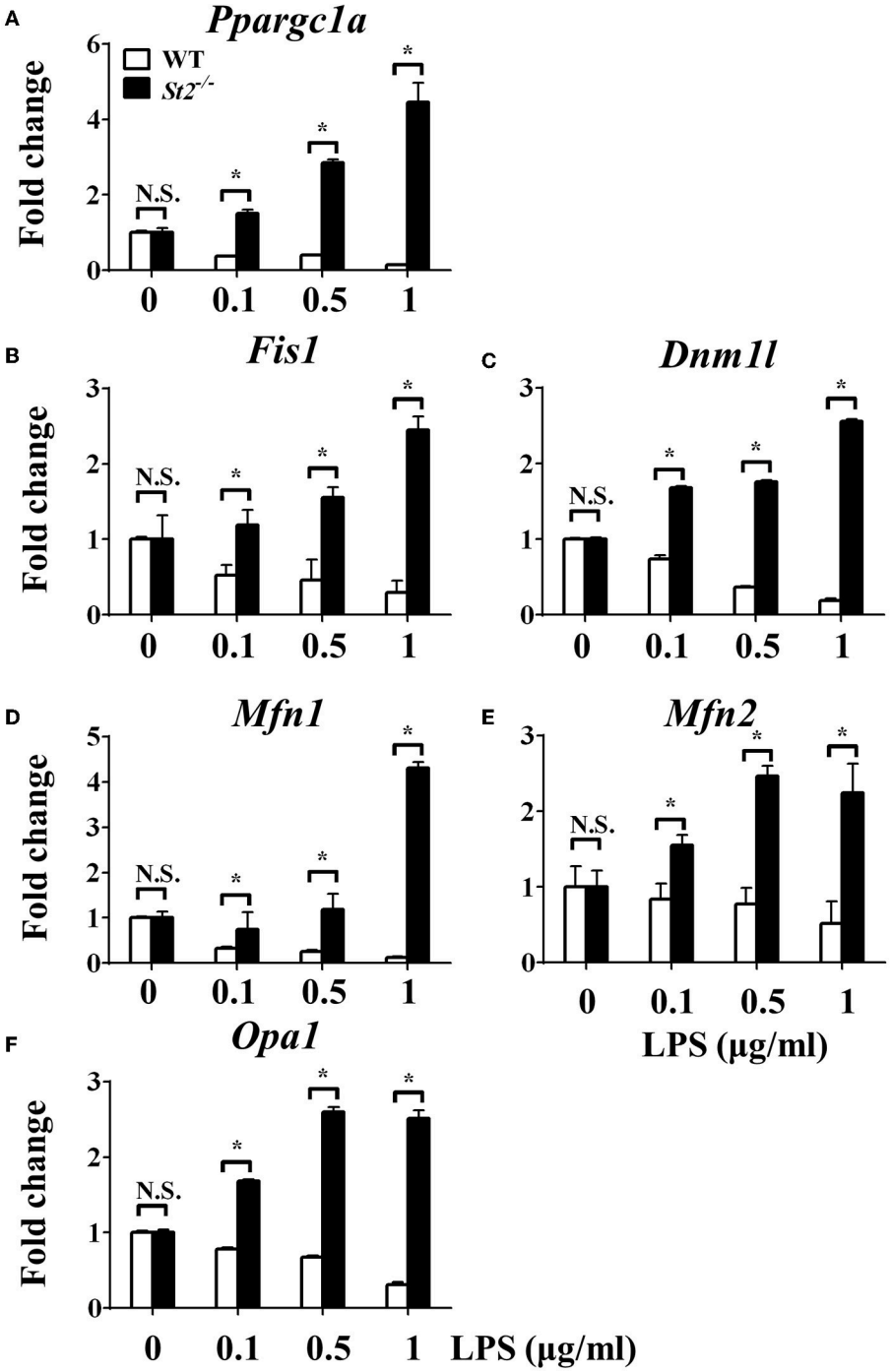


FIGURE 3 | ST2 deficiency was associated with enhanced mitochondrial biogenesis**.** BMDMs from BALB/c or $St2^{-/-}$ mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml). After 72 h, total RNA was isolated and the expression of *Ppargc1a* **(A)**, *Fis1* **(B)**, *Dnm1l* **(C)**, *Mfn1* **(D)**, *Mfn2* **(E)**, and Opa1 **(F)** were evaluated by qPCR, respectively. Vertical bars = SEMs ($n = 3$ per group per experiment). N.S., no significant difference; $^*p < 0.05$.

determined by qPCR (**Figures 3B–F**) and western blotting (**Supplementary Figure 2**). LPS only enhanced the expression of these genes in *St2*$^{-/-}$ BMDMs but not in WT. These changes in mitochondria were also confirmed by fluorescent staining. Mitochondrial numbers were reduced by LPS in WT but increased in *St2*$^{-/-}$ BMDMs (**Figure 4**).

Overexpressing IL-33 Promoted Macrophage Responses Upon LPS Stimulation With More Glucose Uptake and Lactic Acid Generation

After we established that LPS induced OXPHOS in macrophages in the absence of IL-33/ST2 signaling by increasing the proliferation, fission, and fusion of mitochondria, possibly due to the induction of PGC-1α, we next determined whether IL-33 overexpression could alter these effects. After both WT and *Il33*-overexpressing BMDMs were stimulated with different doses of LPS, IL-1α, IL-1β, iNOS, and IFNγ production were measured and metabolic changes in the cells were monitored. IL-33 overexpression enhanced the production of pro-inflammatory cytokines and iNOS in LPS-stimulated macrophages, as measured by qPCR and ELISA (**Figures 5A–G**). Furthermore, IL-33 overexpression reduced the OCR of macrophages and increased the ECAR (**Figures 6A,B**), lactate acid production (**Figure 6C**), and glucose uptake (**Figure 6D**). These results indicated that, in contrast to ST2 deficiency, IL-33 overexpression was associated with enhanced macrophage

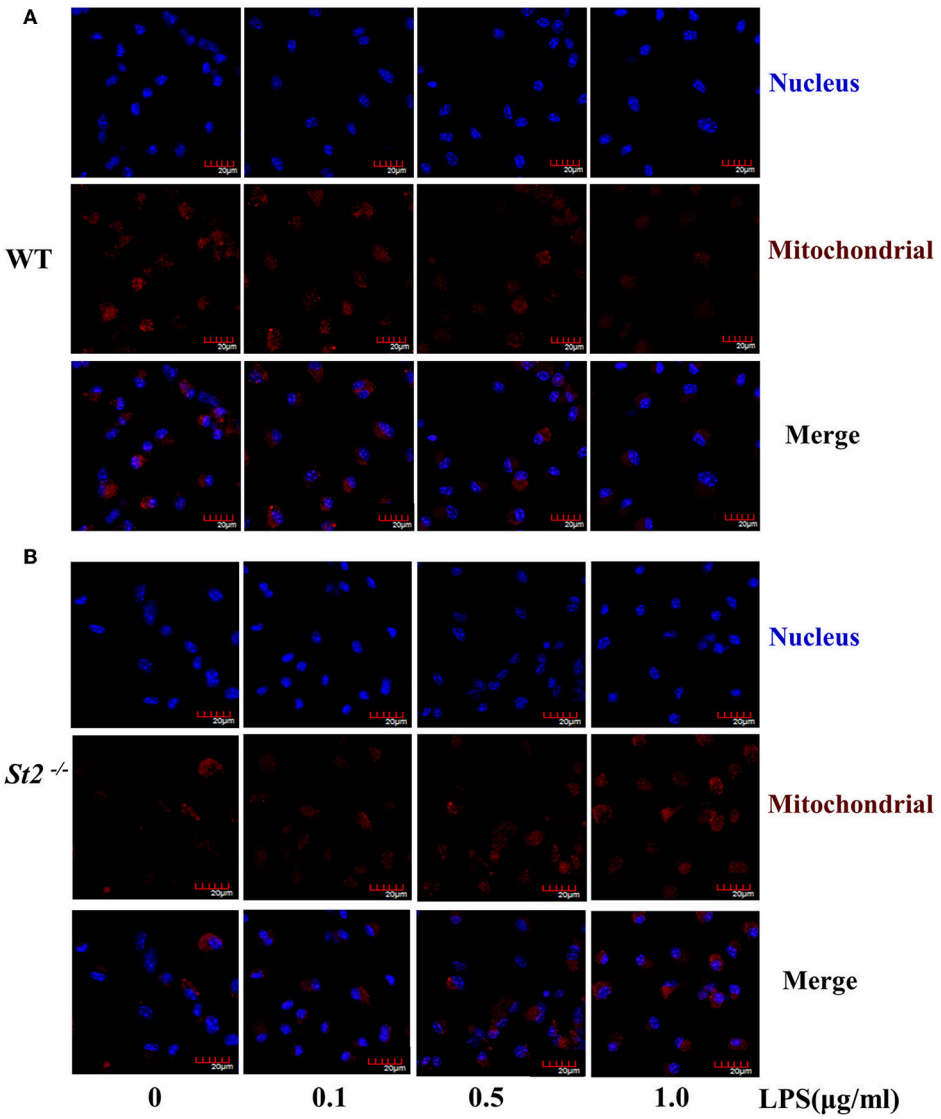


FIGURE 4 | ST2 deficiency was associated with increased mitochondria mass. BMDMs from WT **(A)** or *St2*$^{-/-}$ **(B)** mice stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 72 h. Mitochondrial mass was observed with confocal laser microscopy in BMDMs stained with MitoTracker Red (scale bar: 20 μm). Data are representative of three experiments.

function by enhancing the Warburg-like effects that were triggered by LPS.

IL-33 Overexpression Was Associated With Reduced Mitochondrial Fission and Fusion

We further investigated whether IL-33 overexpression changed the metabolism of LPS-stimulated macrophages through a similar mechanism as ST2 deficiency. IL-33 overexpression was associated with fewer mitochondrial gene copies (**Figure 6F**), less fission (**Figures 7B,C**) and fusion (**Figures 7D–F**; **Supplementary Figure 2**), but was still associated with higher ATP production (**Figure 6E**). The changes in PGC-1α expression in LPS-stimulated *Il33*-overexpressing BMDMs were similar to WT BMDMs (**Figure 7A**). These results indicated that IL-33

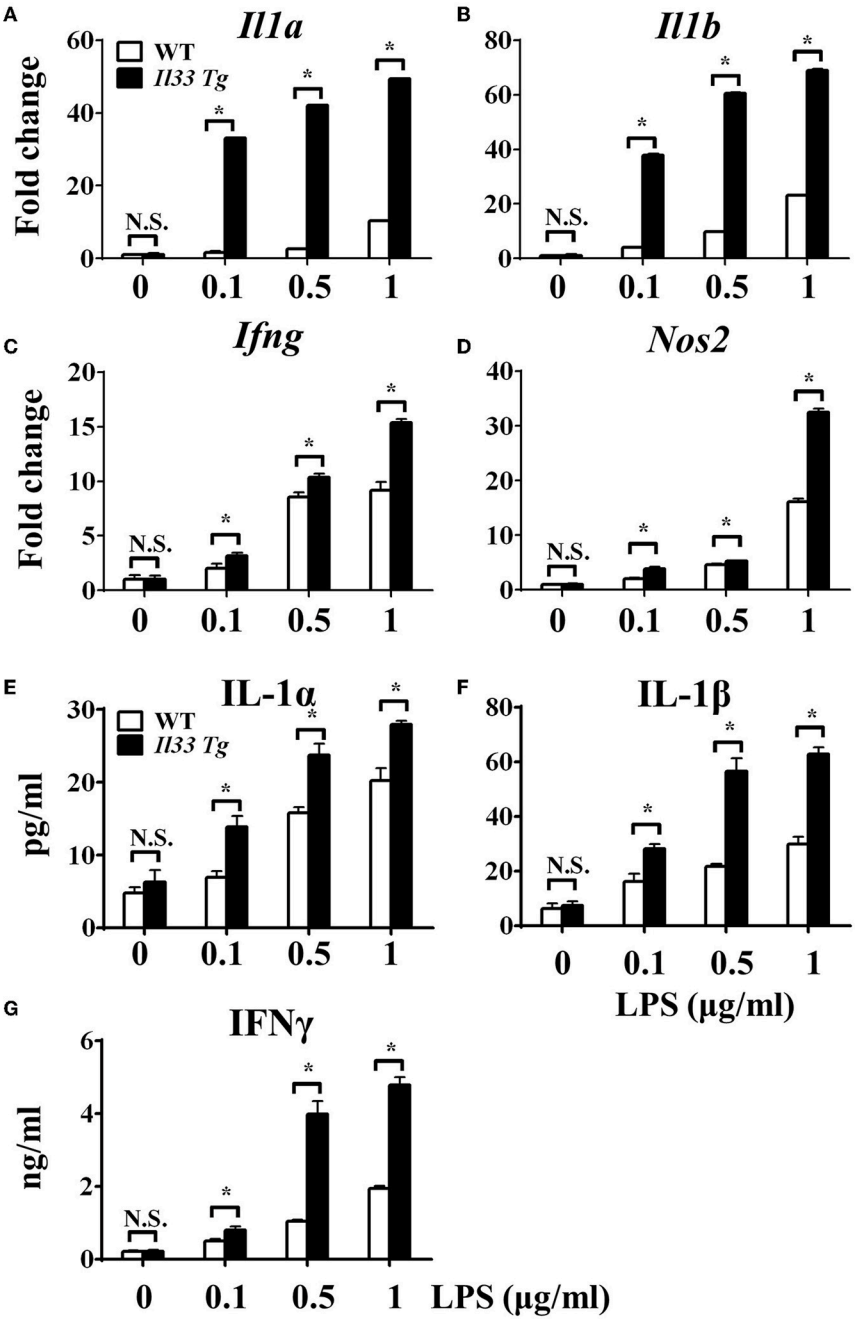


FIGURE 5 | IL-33 over-expression promoted the macrophages responses upon LPS stimulation**.** BMDMs from BALB/c or *Il33* over-expressing (*Il33 Tg*) mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 72 h. Total RNA was isolated and the expression of *Il1a* **(A)**, *Il1b* **(B)**, *Ifng* **(C)**, and *Nos2* **(D)** was evaluated by qPCR; the concentration of IL-1α **(E)**, IL-1β **(F)**, and IFNγ **(G)** in the supernatant was evaluated by ELISA. Vertical bars = SEMs ($n = 3$ per group per experiment). N.S., no significant difference; *$p < 0.05$.

enhanced the metabolic changes in macrophages following LPS stimulation *via* decreasing mitochondrial proliferation, fission, and fusion.

DISCUSSION

The metabolic changes in macrophages upon contacting different stimuli are essential for macrophage polarization and function in both physiological and pathological conditions (4). The IL-33/ST2 pathway is known to direct macrophages toward different phenotypes when combined with different stimuli *via* previously unknown mechanisms (19, 20). Here, we showed for the first time that the IL-33/ST2 pathway may directly reshape central carbon metabolism in macrophages.

IL-33 plays pleiotropic role in human immunopathology (21, 22). For example, it can be beneficial for sepsis (7, 12), malaria (23), obesity related inflammation (24), autoimmune-related uveitis (25), and experimental autoimmune encephalomyelitis (10). In these settings, IL-33 had protective effects by inducing neutrophils, type 2 innate lymphoid cells, regulatory T cells and the production of IL-17 and IFNγ, depending on the specific settings. Conversely, IL-33 is a detrimental factor in other settings, such during autoantibody-induced arthritis (26–29), eosinophilic asthma (8, 9), cancer (30, 31), early-stage colitis (32), and lung fibrosis (11). IL-33 might exacerbate these

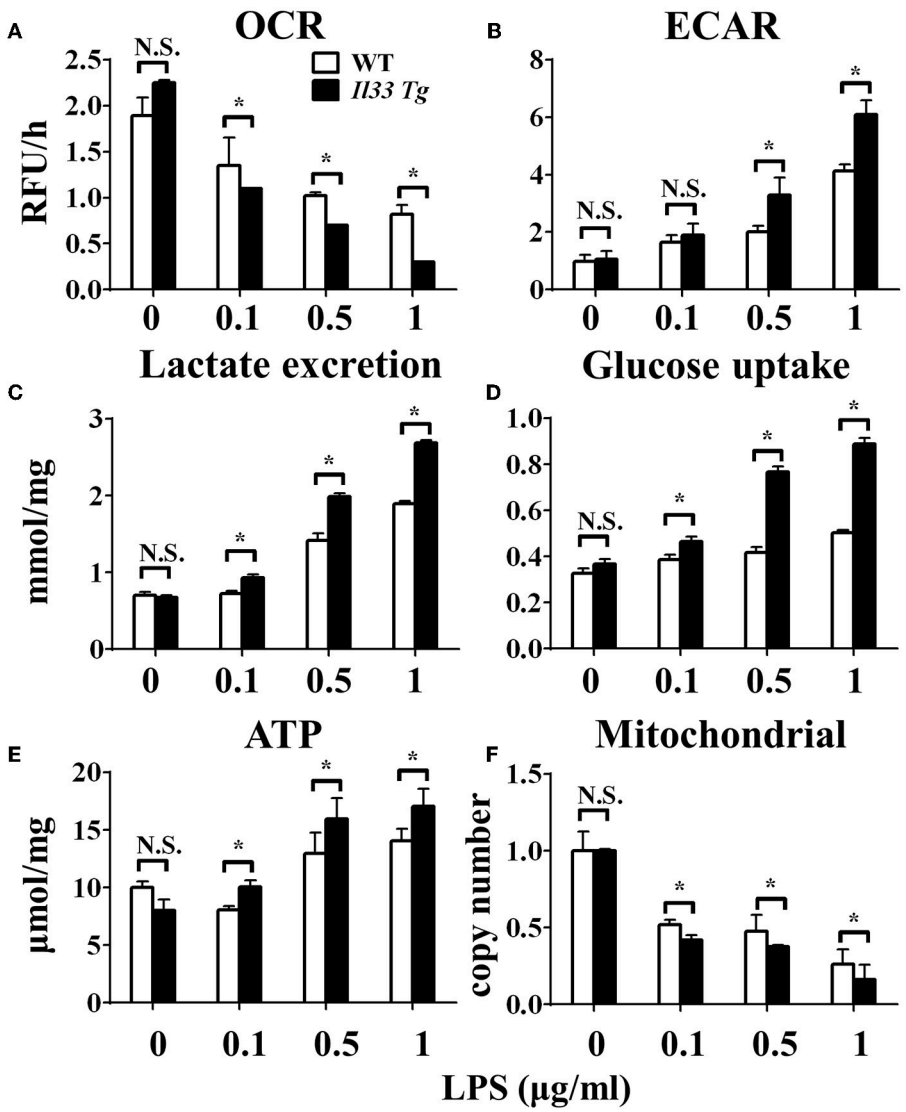


FIGURE 6 | IL-33 over-expression affected the metabolism of macrophages upon LPS stimulation**.** BMDMs from BALB/c or *Il33* over-expressing (*Il33 Tg*) mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 24 h and the **(A)** extracellular oxygen consumption rate (OCR) was immediately measured by fluorescence intensity of a typical oxygen probe; **(B)** extracellular acidification rate (ECAR) was measured by incubation at 37°C for 3 h. The lactate excretion **(C)**, glucose uptake **(D)**, ATP **(E)** and relative mitochondrial DNA copy numbers **(F)** of BMDMs were measured after 72 h stimulation of LPS (0, 0.1, 0.5, 1.0 μg/ml). Vertical bars = SEMs ($n = 3$ per group per experiment). N.S., no significant difference; $^*p < 0.05$.

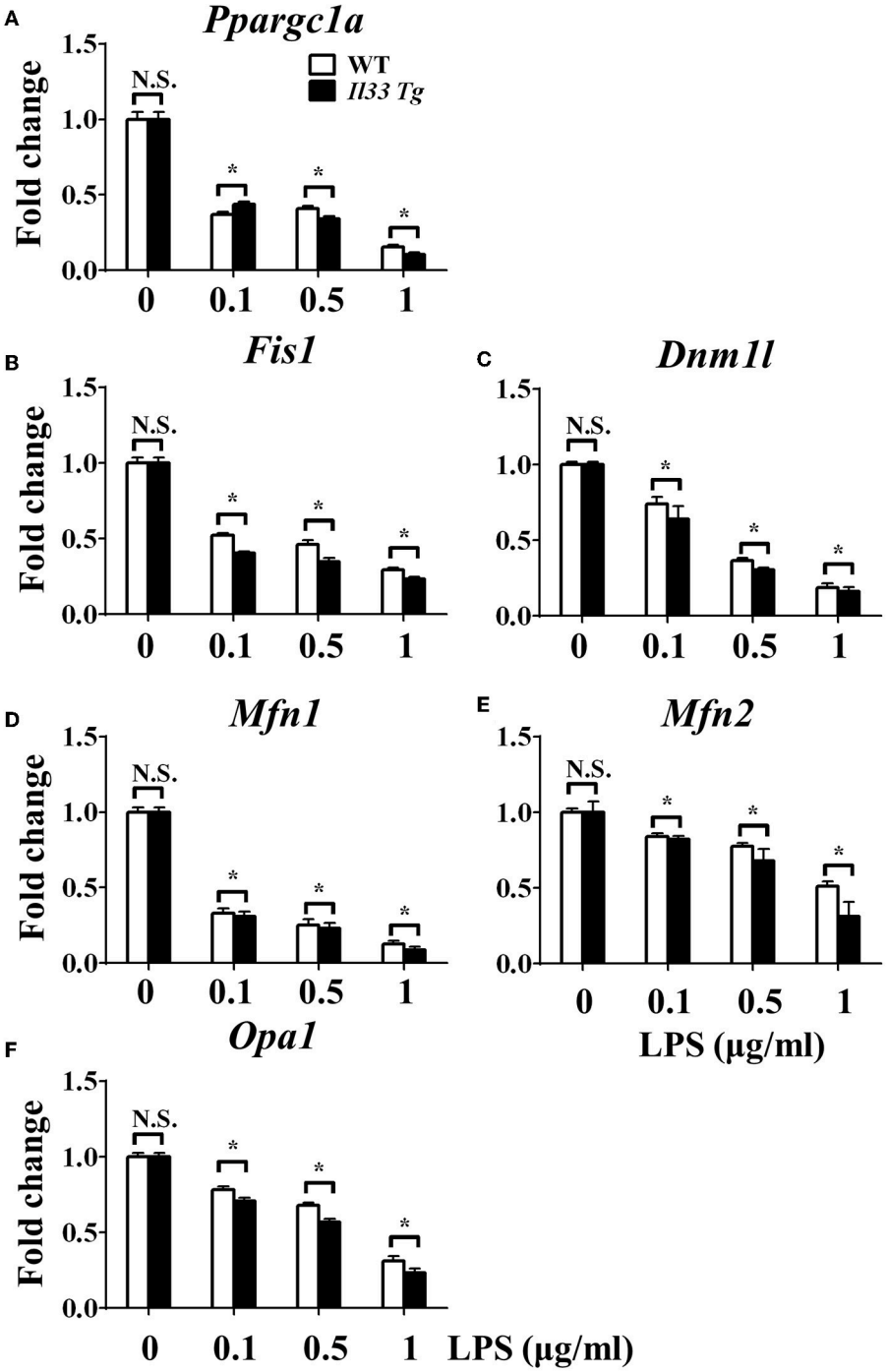


FIGURE 7 | IL-33 over-expression was associated with reduced mitochondrial biogenesis. BMDMs from BALB/c or *Il33* over-expressing (*Il33 Tg*) mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml). After 72 h, total RNA was isolated and the expression of *Ppargc1a* **(A)**, *Fis1* **(B)**, *Dnm1l* **(C)**, *Mfn1* **(D)**, *Mfn2* **(E)**, and Opa1 **(F)** was evaluated by qPCR, respectively. Vertical bars = SEMs ($n = 3$ per group per experiment). N.S., no significant difference; $^*p < 0.05$.

diseases through the induction of eosinophils, type 2 innate lymphoid cells, mast cells, the production of pro-inflammatory and pro-fibrotic cytokines, inducing mucositis, or directly promoting the proliferation and metastasis of cancer cells. These examples highlight the multitude of roles and underlying mechanisms downstream of IL-33/ST2 signaling in both healthy and disease settings. Further studies designed at understanding these mechanisms are required and could possibly provide insight into how to manipulate the immune system to treat these diseases.

One of the possible reasons for the controversial roles of the IL-33/ST2 pathway in inflammation could be explained by interactions between IL-33/ST2 signaling and different macrophages. It is well established that macrophages have distinguished functions in shaping immune responses (33), and previous reports have shown that IL-33 can polarize macrophages to the pro-inflammatory M1-like subset or the anti-inflammatory and pro-resolution M2-like subset. Furthermore, blocking IL-33/ST2 signaling inhibited macrophage responses after LPS stimulation (34, 35), while exogenous IL-33 enhanced the M1-like polarization of LPS-stimulated macrophages (35). These results are similar to what we showed in this work (**Figures 1**, **4**). Furthermore, macrophages generate IL-33 in response to LPS stimulation (36, 37), and exogenous IL-33 enhances the polarization of macrophages to a M2-like phenotype when combined with other type 2 cytokines (8). These studies prove the close relationship between IL-33/ST2 signaling and macrophage activation and polarization.

It has been reported that the activation and polarization of macrophages requires metabolic reprogramming (2). IL-33 has been shown to upregulate hypoxia- HIF-1α (29), which in-turn modulates glucose metabolism and macrophage function. IL-33 can also signal in an autocrine manner, which can create a positive-feedback loop for the IL-33/ST2 pathway (38). But the underlying mechanisms remained undiscovered, which might be due to the different nuclear functions of full length IL-33 compared with the mature cytokine form of IL-33 (39, 40). Our groups' previously work proved that PGC1α produced metabolic changes in cells *via* promoting mitochondrial proliferation and activity (14), which were also closely related to macrophage responses to LPS stimulation. WT macrophages could down-regulate PGC1α to limit mitochondrial proliferation, which promotes glycolysis over OXPHOS. Aerobic glycolysis or the Warburg effect is less efficient at ATP production compared with OXPHOS, but glycolysis generates several metabolites that are useful for protein synthesis and the reactive oxygen species generated by NADPH oxidase (41). When PGC1α is upregulated, mitochondrial proliferation is promoted, and cells use OXPHOS as the primary method of generating ATPs. OXPHOS is so efficient at ATP generation that might deplete substrates for other important biosynthetic reactions inside the cell.

In this work, we investigated the metabolic reprogramming of LPS-stimulated macrophages in the absence or excess of IL-33/ST2 signaling. We found that the IL-33/ST2 pathway played an important role in the metabolic switch, from OXPHOS to glycolysis (Warburg effect), in LPS-stimulated macrophages by altering PGC1α levels. We also determined that this metabolic reprogramming did not result from mitochondrial damage, as MMP was not significantly changed by LPS stimulation or *St2* knockout. These results could provide further insight into the interactions between IL-33/ST2 and macrophages, and might help in future pharmaceutical approaches to treat immune dysfunctions.

AUTHOR CONTRIBUTIONS

JS and DL contributed to experimental design, securing funds, and manuscript preparation. LS and JN contributed to the supervision of the study and manuscript preparation. HX, YH, and XY contributed experimentation and data analysis.

FUNDING

This work was supported by the National Natural Science Foundation of China (Nos. 81501423 and 81672948), the Norman Bethune Program of Jilin University (No. 2015223) and Jilin Provincial Research Foundation for the Development of Science and Technology Projects (20160414005GH, 20190103095JH). The funding sources had no role in the design or conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

ACKNOWLEDGMENTS

We thank Prof. Weihua Xiao from the University of Science and Technology of China (Hefei, China) and Prof. Ying Sun from Capital Medical University (Beijing, China) for providing us genetically modified animals. We also thank all the staff from the animal units at the College of Basic Medical Sciences for taking care of the animals. Finally, we thank James P. Mahaffey, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00127/full#supplementary-material

Supplementary Figure 1 | ST2 deficiency reduced the mitochondrial membrane potential of LPS stimulated macrophages. BMDMs from BALB/c **(A)** or *St2*$^{-/-}$ **(B)** mice were stimulated with LPS (0, 0.1, 0.5, and 1.0 μg/ml) for 6 h. Mitochondrial membrane potential staining with JC-1 was detected by flow cytometry. Upper panel showed representative histogram figures and lower panel showed representative dot plots. Data are representative of three experiments.

Supplementary Figure 2 | IL-33/ST2 pathway affected the fusion and fission of mitochondria in LPS stimulated macrophages. BMDMs from BALB/c, *St2*$^{-/-}$ or *Il33* over expression (*Il33 Tg*) mice were stimulated with LPS (0 and 1.0 μg/ml) for 24 or 48 h. The expression of mitochondrial fission and fusion-related proteins (PGC1, DRP1, MFN2, MFN1, OPA1, and FIS1) were analyzed by western blot. Data are representative of three experiments.

REFERENCES

1. Morris SM Jr. Arginine metabolism: boundaries of our knowledge. *J Nutr*. (2007) 137:1602S–9S. doi: 10.1093/jn/137.6.1602S
2. Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (Going)? *Trends Immunol*. (2017) 38:395–406. doi: 10.1016/j.it.2017.03.001
3. Nagy C, Haschemi A. Time and demand are two critical dimensions of immunometabolism: the process of macrophage activation and

the pentose phosphate pathway. *Front Immunol*. (2015) 6:164. doi: 10.3389/fimmu.2015.00164
4. Palsson-McDermott EM, Curtis AM, Goel G, Lauterbach MA, Sheedy FJ, Gleeson LE, et al. Pyruvate kinase M2 regulates Hif-1alpha activity and IL-1beta induction and is a critical determinant of the warburg effect in LPS-activated macrophages. *Cell Metab*. (2015) 21:65–80. doi: 10.1016/j.cmet.2014.12.005
5. Galvan-Pena S, O'Neill LA. Metabolic reprograming in macrophage polarization. *Front Immunol*. 5:420. doi: 10.3389/fimmu.2014.00420
6. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
7. Alves-Filho JC, Sonego F, Souto FO, Freitas A, Verri WA Jr, Auxiliadora-Martins M, et al. Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nat Med*. (2010) 16:708–12. doi: 10.1038/nm.2156
8. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol*. (2009) 183:6469–77. doi: 10.4049/jimmunol.0901575
9. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. *J Immunol*. (2010) 185:3472–80. doi: 10.4049/jimmunol.1000730
10. Jiang HR, Milovanovic M, Allan D, Niedbala W, Besnard AG, Fukada SY, et al. IL-33 attenuates EAE by suppressing IL-17 and IFN-gamma production and inducing alternatively activated macrophages.*Eur J Immunol*. (2012) 42:1804–14. doi: 10.1002/eji.201141947
11. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, et al. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J Allergy Clin Immunol*. (2014) 134:1422–32 e11. doi: 10.1016/j.jaci.2014.05.011
12. Nascimento DC, Melo PH, Pineros AR, Ferreira RG, Colon DF, Donate PB, et al. IL-33 contributes to sepsis-induced long-term immunosuppression by expanding the regulatory T cell population. *Nat Commun*. 8:14919. doi: 10.1038/ncomms14919
13. Joshi AD, Oak SR, Hartigan AJ, Finn WG, Kunkel SL, Duffy KE, et al. Interleukin-33 contributes to both M1 and M2 chemokine marker expression in human macrophages. *BMC Immunol*. 11:52. doi: 10.1186/1471-2172-11-52
14. Shen L, Sun B, Sheng J, Yu S, Li Y, Xu H, et al. PGC1alpha promotes cisplatin resistance in human ovarian carcinoma cells through upregulation of mitochondrial biogenesis. *Int J Oncol*. (2018) 53:404–16. doi: 10.3892/ijo.2018.440
15. Li D, Gao X, Gu J, Tian Y, Liu Y, Jin Z, et al. A novel application of Ti-substituted polyoxometalates: anti-inflammatory activity in ova-induced asthma murine model. *Bioinorg Chem Appl*. (2016) 2016:3239494. doi: 10.1155/2016/3239494
16. Qu X, Sheng J, Shen L, Su J, Xu Y, Xie Q, et al. Autophagy inhibitor chloroquine increases sensitivity to cisplatin in QBC939 cholangiocarcinoma cells by mitochondrial ROS. *PLoS ONE* (2017)12:e0173712. doi: 10.1371/journal.pone.0173712
17. Xiang XY, Kang JS, Yang XC, Su J, Wu Y, Yan XY, et al. SIRT3 participates in glucose metabolism interruption and apoptosis induced by BH3 mimetic S1 in ovarian cancer cells. *Int J Oncol*. (2016) 49:773–84. doi: 10.3892/ijo.2016.3552
18. Zhang W, Su J, Xu H, Yu S, Liu Y, Zhang Y, et al. Dicumarol inhibits PDK1 and targets multiple malignant behaviors of ovarian cancer cells. *PLoS ONE* (2017) 12:e0179672. doi: 10.1371/journal.pone.0179672
19. Pei C, Barbour M, Fairlie-Clarke KJ, Allan D, Mu R, Jiang HR. Emerging role of interleukin-33 in autoimmune diseases. *Immunology* (2014) 141:9–17. doi: 10.1111/imm.12174
20. Fattori V, Hohmann MSN, Rossaneis AC, Manchope MF, Alves-Filho JC, Cunha TM, et al. Targeting IL-33/ST2 signaling: regulation of immune function and analgesia. *Expert Opin Ther Targets* (2017) 21:1141–52. doi: 10.1080/14728222.2017.1398734
21. Murphy GE, Xu D, Liew FY, McInnes IB. Role of interleukin 33 in human immunopathology. *Ann Rheum Dis*. (2010) 69(Suppl. 1):i43–7. doi: 10.1136/ard.2009.120113
22. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol*. (2016) 16:676–89. doi: 10.1038/nri.2016.95
23. Besnard AG, Guabiraba R, Niedbala W, Palomo J, Reverchon F, Shaw TN, et al. IL-33-mediated protection against experimental cerebral malaria is linked to induction of type 2 innate lymphoid cells, M2 macrophages and regulatory T cells. *PLoS Pathog*. (2015) 11:e1004607. doi: 10.1371/journal.ppat.1004607
24. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circ Res*. (2010) 107:650–8. doi: 10.1161/CIRCRESAHA.110.218867
25. Barbour M, Allan D, Xu H, Pei C, Chen M, Niedbala W, et al. IL-33 attenuates the development of experimental autoimmune uveitis. *Eur J Immunol*. (2014) 44:3320–9. doi: 10.1002/eji.201444671
26. Mu R, Huang HQ, Li YH, Li C, Ye H, Li ZG. Elevated serum interleukin 33 is associated with autoantibody production in patients with rheumatoid arthritis. *J Rheumatol*. (2010) 37:2006–13. doi: 10.3899/jrheum.100184
27. Xu D, Jiang HR, Li Y, Pushparaj PN, Kurowska-Stolarska M, Leung BP, et al. IL-33 exacerbates autoantibody-induced arthritis. *J Immunol*. (2010) 184:2620–6. doi: 10.4049/jimmunol.0902685
28. Tang S, Huang H, Hu F, Zhou W, Guo J, Jiang H, 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). 2013:985301. doi: 10.1155/2013/985301
29. Hu F, Shi L, Mu R, Zhu J, Li Y, Ma X, et al. Hypoxia-inducible factor-1alpha and interleukin 33 form a regulatory circuit to perpetuate the inflammation in rheumatoid arthritis. *PLoS ONE* (2013) 8:e72650. doi: 10.1371/annotation/032b026d-5bcf-4c1b-aa15-65c226a53818
30. Tong X, Barbour M, Hou K, Gao C, Cao S, Zheng J, et al. Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways. *Mol Oncol*. (2016) 10:113–25. doi: 10.1016/j.molonc.2015.06.004
31. Guabiraba R, Besnard AG, Menezes GB, Secher T, Jabir MS, Amaral SS, et al. IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice. *Mucosal Immunol*. (2014) 7:1079–93. doi: 10.1038/mi.2013.124
32. Pushparaj PN, Li D, Komai-Koma M, Guabiraba R, Alexander J, McSharry C, et al. Interleukin-33 exacerbates acute colitis via interleukin-4 in mice. *Immunology* (2013) 140:70–7. doi: 10.1111/imm.12111
33. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity Mar*. (2016) 44:450–62. doi: 10.1016/j.immuni.2016.02.015
34. Sweet MJ, Leung BP, Kang D, Sogaard M, Schulz K, Trajkovic V, et al. A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of toll-like receptor 4 expression. *J Immunol*. (2001) 166:6633–9. doi: 10.4049/jimmunol.166.11.6633
35. Espinassous Q, Garcia-de-Paco E, Garcia-Verdugo I, Synguelakis M, von Aulock S, Sallenave JM, et al. IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating lipopolysaccharide receptor complex. *J Immunol*. (2009) 183:1446–55. doi: 10.4049/jimmunol.0803067
36. Polumuri SK, Jayakar GG, Shirey KA, Roberts ZJ, Perkins DJ, Pitha PM, et al. Transcriptional regulation of murine IL-33 by TLR and non-TLR agonists. *J Immunol*. (2012) 189:50–60. doi: 10.4049/jimmunol.1003554
37. Samuchiwal SK, Balestrieri B, Raff H, Boyce JA. Endogenous prostaglandin E2 amplifies IL-33 production by macrophages through an E prostanoid (EP)2/EP4-cAMP-EPAC-dependent pathway. *J Biol Chem*. (2017) 292:8195–206. doi: 10.1074/jbc.M116.769422

38. Tominaga S, Tago K, Tsuda H, Komine M. Dual function of IL-33 on proliferation of NIH-3T3 cells. *Cytokine* (2015) 72:105–8. doi: 10.1016/j.cyto.2014.12.004
39. Bessa J, Meyer CA, de Vera Mudry MC, Schlicht S, Smith SH, Iglesias A, et al. Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. *J Autoimmun*. (2014) 55:33–41. doi: 10.1016/j.jaut.2014.02.012
40. Haraldsen G, Balogh J, Pollheimer J, Sponheim J, Kuchler AM. Interleukin-33—cytokine of dual function or novel alarmin? *Trends Immunol*. (2009) 30:227–33. doi: 10.1016/j.it.2009.03.003
41. Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: beyond ATP. *Nat Rev Immunol*. (2017) 17:608–20. doi: 10.1038/nri.2017.66

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Xu, Sun, He, Yuan, Niu, Su and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 04 March 2019
doi: 10.3389/fimmu.2019.00364

IL33: Roles in Allergic Inflammation and Therapeutic Perspectives

Ben C. L. Chan[1], *Christopher W. K. Lam*[2], *Lai-Shan Tam*[3] *and Chun K. Wong*[1,4]*

[1] State Key Laboratory of Research on Bioactivities and Clinical Applications of Medicinal Plants, Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, [2] State Key Laboratory of Quality Research in Chinese Medicines, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Taipa, Macau, [3] Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong, [4] Department of Chemical Pathology, The Chinese University of Hong Kong, Shatin, Hong Kong

Interleukin (IL)-33 belongs to IL-1 cytokine family which is constitutively produced from the structural and lining cells including fibroblasts, endothelial cells, and epithelial cells of skin, gastrointestinal tract, and lungs that are exposed to the environment. Different from most cytokines that are actively secreted from cells, nuclear cytokine IL-33 is passively released during cell necrosis or when tissues are damaged, suggesting that it may function as an alarmin that alerts the immune system after endothelial or epithelial cell damage during infection, physical stress, or trauma. IL-33 plays important roles in type-2 innate immunity via activation of allergic inflammation-related eosinophils, basophils, mast cells, macrophages, and group 2 innate lymphoid cells (ILC2s) through its receptor ST2. In this review, we focus on the recent advances of the underlying intercellular and intracellular mechanisms by which IL-33 can regulate the allergic inflammation in various allergic diseases including allergic asthma and atopic dermatitis. The future pharmacological strategy and application of traditional Chinese medicines targeting the IL-33/ST2 axis for anti-inflammatory therapy of allergic diseases were also discussed.

Keywords: IL-33, allergic inflammation, signal transduction, eosinophils, mast cells, innate lymphoid cells (ILC), Chinese herbal medicine, therapeutics

OPEN ACCESS

Edited by:
Rong Mu,
Peking University People's Hospital, China

Reviewed by:
Remo Castro Russo,
Federal University of Minas Gerais, Brazil
Dong Li,
Jilin University, China

***Correspondence:**
Chun K. Wong
ck-wong@cuhk.edu.hk

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *18 May 2018*
Accepted: *12 February 2019*
Published: *04 March 2019*

Citation:
Chan BCL, Lam CWK, Tam L-S and Wong CK (2019) IL33: Roles in Allergic Inflammation and Therapeutic Perspectives. Front. Immunol. 10:364. doi: 10.3389/fimmu.2019.00364

INTRODUCTION

Interleukin33 (IL-33) is a member of the IL-1 cytokine family that includes IL-1α, IL-1β, and IL-18 (1) and constitutively expressed in structural and lining cells including fibroblasts, endothelial, and epithelial cells of skin, gastrointestinal tract, and lungs that are exposed to the environment (2). IL-33 lacks a secretory signal peptide encoded by the *Il1rl1* gene (1), an IL-1 family trait for releasing via the classical endoplasmic reticulum and Golgi pathway (1). Under the inactive state, IL-33 is harbored in the cell nuclei and associated with chromatin by a chromatin-binding motif, belonging to the cellular homeostasis and acting as a transcriptional repressor (2, 3). The N-terminus of IL-33 contains a nuclear localization sequence, a homeodomain-like helix-turn-helix DNA-binding domain and a chromatin-binding domain (3). Different from most cytokines that are actively secreted from cells, IL-33 is released passively in its full length form (amino acids 1–270, IL-33_{FL}) during cell necrosis, cellular activation through ATP signaling without cell death or when tissues are damaged, suggesting that it may function as an alarmin that alerts the immune system after endothelial or epithelial cell damage during infection, physical stress or trauma (4, 5). IL-33 activates signaling pathways depending on the myeloid differentiation primary response gene 88 (Myd88) of immune cells expressing the cytokine receptor interleukin 1 receptor-like 1 (ST2) and

signals through a heterodimeric receptor complex comprising an IL-33-specific ST2 coupled with the co-receptor IL-1 receptor accessory protein (IL-1 RAcP) (6, 7). ST2 is selectively and stably expressed on the cell surface of Th2 cells (8), CD4+ T cells, group 2 innate lymphoid cells (ILC2s) and also other immune cells such as mast cells, basophils, eosinophils, macrophages, dendritic cells and natural killer cells (9–18). Signaling of IL-33 can be activated through nuclear factor kappa-B (NF-κB), c-Jun N-terminal kinase (JNK), and p38 mitogen activated protein kinase (MAPK) cascades (19).

In humans, both IL-33 mRNA and protein are substantially elevated in the inflamed skin lesions of atopic dermatitis (AD) patients when compared with non-inflamed skin (20). IL-33 is a Th2-oriented cytokine which enhances the production of Th2 cytokines, particularly IL-5 and IL-13 (21). In addition, IL-33 is also a chemoattractant for Th2 cells *in vitro* and *in vivo*, indicating the importance of IL-33 in Th2 cells mobilization (22). In large-scale genome-wide association studies, genes encoding IL-33 and its receptors have been identified as susceptibility loci in asthma (23–26).The alarmin activities of IL-33 are regulated at multiple levels (6, 7, 27). Several hours after its extracellular release, IL-33_{FL} is transiently inactivated by oxidation of critical cysteine residues (28). Inflammatory proteases from immune cells such as neutrophils (cathepsin G and elastase) and mast cells (chymase and tryptase) degrade IL-33_{FL} into shorter mature forms containing the C-terminal IL-1-like cytokine domain with much higher activity than IL-33_{FL} (29). A recent study has further shown that IL-33_{FL} functions as a protease sensor that detects proteolytic activities associated with various environmental allergens including house dust mite, pollens, bacteria and fungi (30). When exposed to allergen proteases, IL-33_{FL} is rapidly cleaved in its central "sensor" domain, which leads to the activation of the generation of ILC2s, and allergic inflammation can be reduced by preventing the IL-33_{FL} cleavage (30, 31). In this review, we focus on the recent advances of the underlying intercellular and intracellular mechanisms by which IL-33 can regulate various key immune cells in the allergic inflammatory diseases including allergic asthma and AD, and the future pharmacological strategy and the potential application of traditional Chinese medicines targeting the IL-33/ST2 axis for the treatment of allergic inflammatory diseases.

EFFECTS OF IL-33 ON IMMUNE CELLS ACTIVATION IN ALLERGIC INFLAMMATIONS

Eosinophils

IL-33 potently induces eosinophilia in *in vivo* murine models (32, 33) and activates eosinophils, the principal effector cells in allergic inflammation, to produce superoxide (34), upregulates the expression of adhesion molecules and enhances eosinophil survival (35), suggesting that it can play an important role in the exacerbation of inflammation in allergic diseases mediated by the activation of eosinophils. Polymorphism of human IL-33 and ST2 genes has been shown to associate with increased numbers of eosinophils (36). In our previous studies, we have shown the activation of eosinophils, by different stimuli and its interactions with structural cells in atopic dermatitis (AD) and allergic asthma (37–44). Such findings showed that intercellular interaction of eosinophils and dermal fibroblasts could provoke the release of pro-inflammatory cytokines and chemokines, implying the pathogenic effects of eosinophils infiltration in the inner dermal fibroblast layer in AD skin lesions.

In our study of allergic inflammation, IL-33 significantly promote eosinophil survival and cell surface expression of the adhesion molecule intercellular adhesion molecule (ICAM)-1, but ICAM-3, and L-selectin expressions were suppressed. In addition, IL-33 stimulates significant release of pro-inflammatory cytokine IL-6 and the chemokines CXCL8 and CCL2 from eosinophils (41).The release of cytokines and chemokines were differentially regulated by the activation of nuclear factor (NF)-kB, p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathways in eosinophils (41, 45). In our study of IL-33 in AD using eosinophils and fibroblasts co-culture, we found that there was significant increase in the production of pro-inflammatory cytokines such as IL-6 and AD-associated chemokines CXCL1, CXCL10, CCL2, and CCL5 (45). Such increase was further upregulated by IL-33 stimulation, and significant production of CXCL8 from eosinophils and fibroblasts co-culture was observed (42). The main source in co-culture for the release of CCL5, and IL-6, CXCL1, CXCL8, CXCL10, and CCL2 was eosinophils and fibroblasts, respectively, and direct contact between eosinophils and fibroblasts was essential for the release of AD-related chemokine CXCL1, CXCL10, CXCL8, and CCL5. IL-33 stimulation also upregulated the cell surface expression of intercellular adhesion molecule-1 (ICAM-1) on both eosinophils and fibroblasts in co-culture, with differential activation of ERK, JNK, p38 MAPK, NF-kB, and phosphatidylinositol 3-kinase–Akt (PI3K/Akt) pathways (42).

T Cells and ILC2

Besides eosinophils, IL-33 has also been shown to be an active and soluble co-stimulator of T cells, by promoting the expansion and functional differentiation of both effector T cells and GATA-3+ regulatory T cells (9, 46). Study of IL-33 signaling-deficient mice has also demonstrated the crucial role of IL-33 in protective anti-viral T cell immunity (9). Activated Th1 and CD8+ T cells have been shown to transiently express lower amounts of IL-33 receptor ST2, when compared with Th2 cells. However, IL-33 signaling can induce the expression of the lineage-specific transcription factors FOXP3, GATA-3, and T-bet for the positive activation of ST2 expression on Th1 cells (47). For regulatory T cells in the intestine, high level of ST2 are constitutively expressed and associated to the pathogenesis of eosinophilic pneumonia (48, 49).

In the mucosal barrier sites, IL-33 has been shown to coordinate the type 2 immune response through the activation of ST2-positive immune cells, such as ILC2s and CD4+ T cells (50). ILC2s are primarily localized at mucosal surfaces of lung, skin, gut and adipose tissues (51, 52) and play an important role in IL-33 associated allergic inflammatory diseases. Although ILC2s lack antigen receptors, they can be rapidly activated by

the epithelial derived cytokines IL-33, IL-25, and thymic stromal lymphopoietin (TSLP), prostaglandin D2 from mast cells or cysteinyl leukotrienes secreted by activated hematopoietic cells (53–55). Activated ILC2 cells proliferate rapidly and act as an early innate source by producing large amount of the Th2 cytokines IL-5 and IL-13 in a synergistic manner (56). Bone marrow ILC2s has been shown to be a local source of IL-5 in IL-33-driven eosinophilia (57). Impaired Th2 cell differentiation was observed in ILC2 knockout mice (48), and the differentiation is in a cell-contact manner through major histocompatibility complex class II (49).

Mast Cells and Basophils

Mast cells and its blood counterpart basophils, both play an important roles in allergic inflammation by generation and release of a panel of inflammatory mediators, such as histamine (58). Allergens can cross link with IgE sensitized mast cells to activate and release of large amounts of preformed and newly formed mediators: histamine, heparin, and proteases such as carboxypeptidase A3, chymase and tryptase (59–61). Most of these active proteases in the granule can cleave targets in nearby tissue compartments upon secreted from the activated mast cells (61). In human, mucosal mast cells express only tryptase and connective tissue mast cells express tryptase, chymase, and carboxypeptidase A3 (62). In mouse, mucosal mast cells express 2 chymase subtypes, mast cell protease (MCPT) 1 and MCPT2, whereas connective tissue type mast cells express the chymase MCPT4 and the elastase MCPT5, the tryptases MCPT6 and MCPT7, and carboxypeptidase A3 (62). IL-33 associated mast cell functions are involved in the pathogenesis of different allergic inflammations such as food anaphylaxis (63) and respiratory allergy induced by house dust mite or aspirin (54, 64, 65). Since the IL-33 receptor ST2 is constitutively expressed on mast cells, basophils and their progenitors cells, it is a critical amplifiers of IL-33–mediated allergic inflammation with the capacity in secreting a wide array of inflammatory cytokines and mediators (66). Antigen or IL-33 activated human mast cells can also release soluble ST2, which may further modulate the biologic effects of IL-33 (67). IL-33 has been demonstrated to enhance the adhesion of mast cells onto laminin, fibronectin, and vitronectin, increase the expression of adhesion molecules, such as ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, thus promoting mast cell adhesion to blood vessel walls (59). Mast cell survival, growth, development and maturation can be enhanced by IL-33 via the ST2/Myd88 pathway (68). Mast cell-derived tryptase and chymase have been demonstrated to cleave extracellular IL-33 into mature active forms (29) and IL-33 isoforms may have additional abilities to activate mast cells, thereby further provoking inflammation (69). Human mast cell chymase (HC) seems to be substrate specific. In a study using 51 active recombinant cytokines and chemokines (70), only 3 of them were substantially cleaved (IL-15 and two IL-1–related alarmins: IL-18 and IL-33) by HC.

The roles of mast cells proteases are not only associated with pro-inflammatory activities. In an *in vivo* study using ovalbumin (OVA)-sensitized mice lacking mouse MC protease 4 (mMCP4) (71), a chymase that is functionally equivalent to human chymase, the airway hyperresponsiveness when challenged with OVA was significantly higher in mMCP-$4^{(-/-)}$ mice when compared with wild type mice. The thickness of the smooth muscle cell (SMC) layer was more pronounced in mMCP-$4^{(-/-)}$ mice than in wild type control mice, thus indicating that chymase may have a modulating effect on airway SMCs. Taken together, the regulating role of chymase present in the upper airways could protect the animals against allergic airway responses. Therefore, the pro-inflammatory and anti-inflammatory effects of mast cells proteases may occur during different time frames, for example, initial activities that promote the airway response being followed by the mounting of protective activities that down-regulate the initial pro-inflammatory activities. Similar to mast cells, IL-33 mediates activation of human basophils and enhances their effector functions (58–60). Compared to mast cells, human basophils seem to have less amounts of proteases (61, 72). IL-33 also promotes asthma-related IL-4 and IL-13 production from basophils via MyD88-signaling pathway (73).

Macrophages

With abundant localization in lung tissue, macrophages are the important innate immune cells participating in allergic asthmatic inflammation (74). Th2 cytokines (IL-4 and IL-13) can polarize macrophage into alternative activated macrophage (AAM) phenotypes (75). Depletion of alveolar macrophages in murine acute allergic lung inflammation model demonstrated that Th2-immunity of allergic lung inflammation and airway remodeling were attenuated (76). IL-33 has been shown to promote the polarization of AAM that expressed mannose receptor and secreted CCL17 and CCL24 in an IL-13-dependent manner, thereby contributing to the airway inflammation in mice (77). IL-33 can enhance the lipopolysaccharide-mediated *in vitro* activation of macrophages, with the upregulation of the expression of toll-like receptor (TLR)4, myeloid differentiation protein 2, soluble CD14, and MyD88 (78). The updated effects of IL-33 on the activation of eosinophils, basophils, mast cells, macrophages, ILC2 cells and T cells in allergic inflammation is summarized in **Figure 1**.

IL-33 in the Development of Allergy During Early Life

Recent murine studies have reported that there is a spontaneous accumulation of ILC2s, eosinophils, basophils and mast cells in the developing lung soon after birth, which is IL-33 dependent (79). Moreover, IL-33 is produced from type 2 airway epithelial cells (AEC2) during postnatal lung inflation (80). Large amount of IL-13 secreted from IL-33-activated ILC2 has been shown to polarize alveolar macrophages (AM) to anti-inflammatory M2 phenotype in newborn mice and contributed to lung quiescence in homeostasis with a delay in antibacterial effector responses for lifetime (80). On the other hand, exposure of allergen house dust mite during postnatal lung alveolarization further enhanced subsequent IL-33-induced Th2 cytokine production in activated ILC2s and CD11b+ dendritic cells (79, 81). Moreover, IL-33 inhibited IL-12 production and stimulated OX40L in neonatal dendritic cells, thereby promoting Th2 cell predominant for lung remodeling (79). House dust mites (HDM)-induced long-lasting

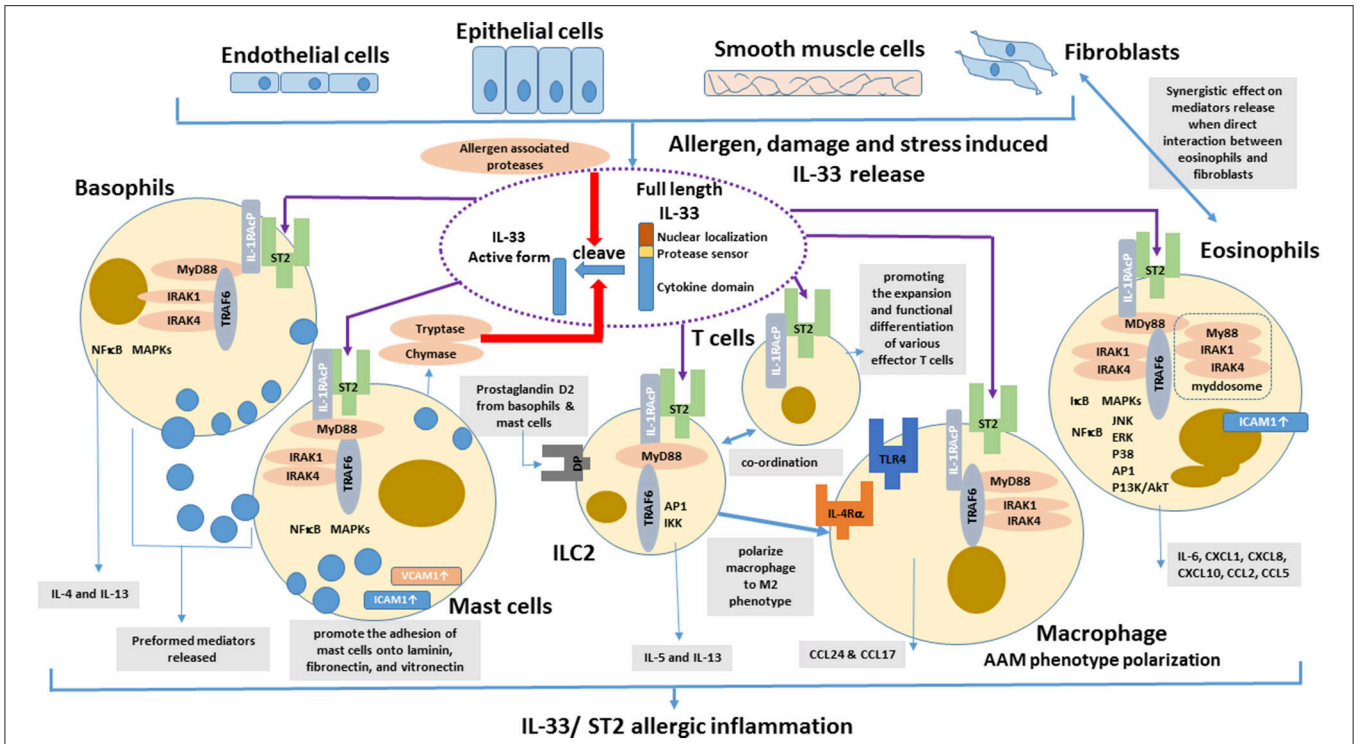


FIGURE 1 | Effects of IL-33 on the activation of eosinophils, basophils, mast cells, DCs, ILC2 cells, and T cells in allergic inflammation. IL-33 is normally sequestered in the nucleus of various cells via nuclear-localization and chromatin-binding motifs in its amino terminus. After the cells are damaged, under stress, or stimulated by allergens, full-length IL-33 is released extracellularly, but it has low activity as a cytokine. Mast cells proteases and some allergens possess protease activity and can directly process IL-33 by cleaving within the protease-sensor domain to generate a more potent cytokine domain, which will directly activate local and infiltrating basophils, mast cells, group 2 innate lymphoid cells (ILC2), T cells, and eosinophils to induce allergic inflammation.

Th2 immune response could be significantly neutralized by the intraperitoneal injection with recombinant soluble IL-33 decoy receptor in sensitization phase (79). This suppressive effect was even more significant in mice of young age than that of adult. Therefore, IL-33-ST2 axis is crucial for asthma development at childhood and intervention of such allergic axis is beneficial for the prevention of the later development of allergic asthma (79). Similarly, IL-33 concentration was found to be increased in the airways after exposure to *Staphylococcus aureus*–derived serine protease–like protein D (82).

Recent Study of IL-33 and AD

Severe pruritus and skin inflammation are the main manifestations of poison ivy-induced allergic contact dermatitis (ACD). In a murine study, the central role of IL-33/ST2 signaling in pruritus and skin inflammation of this ACD has been illustrated (83), and the pruritic mechanism is associated with the interaction of IL-33/ST2 signaling with primary sensory neurons. Therefore, blockage of IL-33/ST2 signaling may represent a therapeutic target to relieve pruritus and skin inflammation of IL-33/ST2 signaling-related dermatitis (83). Apart from pruritic conditions, it has been shown that IL-33 are involved in boosting pain in a formalin-induced inflammatory pain mice model (84).

IL-33 and Inflammatory Bowel Diseases (IBD)

Inflammatory bowel disease (IBD) is highly complex immune mediated sickness and mainly involved two disorders: Crohn's disease and ulcerative colitis with unclear pathophysiology. However, there are some clinical and pathophysiological similarities between IBD with asthma and non-pulmonary allergic diseases such as mast cell activity and the involvement of IgE (85). Moreover, upregulation of IL-33 and ST2 has been repeatedly demonstrated in the inflamed intestinal mucosa of IBD (86–90). Elevated IL-33 serum level of IBD patient has been shown to be reversed after anti-TNF-α treatment (87, 90). Different from asthma in which the AAM polarization is pro-inflammatory, IL-33 could prime macrophages into AAM in murine TNBS-induced colitis for inhibiting disease activity and the release of inflammatory mediators (91).

IL-33 AS THE NEW THERAPEUTIC TARGET FOR ALLERGIC DISEASES

Production of pro-inflammatory cytokines induced by IL-33 from ST2-expressing structural cells and hematopoietic cells including ILC2s, mast cells, Th2 cells, eosinophils, basophils, dendritic cells, and alternatively activated macrophages (AAM)

is crucial to provoke atopic diseases such as allergic asthma and AD (83, 92, 93). *In vivo* murine studies with IL-33- and ST2-deficient transgenic mice, together with the analysis of patient samples further support the crucial role of the IL-33/ST2 axis in those allergic conditions (7, 33, 94, 95). Therefore, IL-33-blocking agents may be a novel therapeutic modality to treat allergic diseases and some promising compounds have recently been developed. Conventionally, glucocorticoids suppress the mRNA expression of pro-inflammatory mediators and exert broadly suppressive activities on inflammatory reactions via binding to the glucocorticoid receptors. IL-33-mediated pulmonary inflammation can be glucocorticoid resistant because other cytokines such as TSLP and IL-17 synergistically expressed at local inflammatory sites (50, 96). For example, allergic airway IL-33 production in house dust mite-induced murine asthmatic model was found to be corticosteroid-resistant (96). Compared with healthy controls, serum levels of IL-33 were significantly increased in psoriasis, psoriatic arthritis, and pustular psoriasis patients, and related to TNF-α. Anti-TNF-α therapy may also be effective against IL-33-related diseases (97). As the production of IL-33 is regulated by the upstream activation of ERK1/2, ERK1/2 inhibitors have also been shown to suppress the IL-33 production (98). The activation of β2-receptors and protein kinase A (PKA) could promote the IL-33 mRNA expression in dendritic cells, thereby suggesting that β-receptor blockers and PKA inhibitors may also be the candidates for IL-33–blocking agents (99, 100). Using mice model, butyrate has recently been found to inhibit proliferation and function of ILC2s by inhibiting intracellular GATA3 activity to suppress IL-33-mediated airway hyperresponsiveness and airway inflammation (101). Similar observations were found in human ILC2s, both *in vivo* and *in vitro* (101).

Proteases play important role in IL-33-mediated allergic diseases. Mast cell proteases are capable to cleave full length IL-33 to a more active IL-33 domain. It becomes a potential therapeutic target for IL-33 mediated allergic diseases. Endogenous protease inhibitors (cystatin A and SPINK5) have been shown to protect the airway epithelium from exogenous protease of patients with eosinophilic chronic rhinosinusitis (102). The development of protease inhibitor may exert therapeutic benefit in eosinophilic airway diseases.

The prolyl cis-trans isomerase proteinase inhibitor I (PIN1) is known to abnormally induce cytokines for eosinophil survival and activation by stabilizing cytokine mRNAs (103). Interleukin receptor associated kinase M (IRAK-M) is a PIN1 target critical for IL-33 signaling in allergic asthma (104). Nuclear magnetic resonance analysis with docking simulations suggests that PIN1 might regulate IRAK-M conformation and function in IL-33 signaling. The IL-33/ST2 signaling pathway recruits adapter protein MyD88 to transduce intracellular signaling (105, 106). MyD88 forms a complex with IL-R–associated kinases (IRAKs), IRAK4, and IRAK2, called the myddosome (MyD88–IRAK4–IRAK2). The myddosome subsequently activates downstream NF-kB, p38 MAPK, and JNK. A small synthetic molecule mimetics of α-helical domain of IRAK2 called compound 7004, which can inhibit the IL-33–induced NF-kB activity, disrupt myddosome formation, and attenuate the pro-inflammatory effects in an asthma-like animal model (105).

Traditional Chinese Medicines (TCM) Targeting IL-33/ST2 Axis Against Allergic Inflammatory Diseases

Apart from the small molecules with specific target in the IL-33/ST2 axis as mentioned above, blocking IL-33 and its receptor by monoclonal antibodies is the major therapeutic approach in targeting IL-33/ST2 axis of allergic inflammatory diseases, and serval clinical trials are in progress (105, 107–111). The main side effect of monoclonal antibody administration is the risk of immune reactions such as serum sickness and acute anaphylaxis which may be fatal (112, 113). TCM and natural products may provide a great source of blockings agents against IL-33 activities. Some TCM formulae have been shown to be effective in attenuating IL-33 activities in both *in vitro* and *in vivo* studies (**Table 1**). Most of the component herbs in those formulae have been traditionally used to treat allergic and inflammatory diseases (39, 43, 127, 128).

Besides TCM formulae, some natural compounds include flavonoids and alkaloids have been shown to be active against targeting IL-33/ST2 axis. Calycosin, a flavonoid, is a major component in Radix Astragli (117) that has been used in the treatment of allergy-related symptoms. When AD mice were treated with calycosin (0.4–10 mg/kg), the protein levels of TSLP and IL-33 were significantly suppressed (118). The inhibitory mechanism was associated to TLR4-mediated NF-κB signaling, with the significant inhibition of the expression of MyD88, toll/interleukin-1 receptor domain-containing adapter protein (TIRAP), and transforming growth factor beta-activated kinase 1 (TAK1) (118).

Cimifugin is a bioactive and major component of Radix Saposhnikoviae, a TCM has been used for treating allergy. Using FITC sensitized and challenged AD mice, cimifugin can significantly inhibit TSLP and IL-33 production in the initial stage of AD model. Moreover, cimifugin could reduce the separated gap among the epithelial cells and increase the expression of tight junctions (TJs). Similar effects on TSLP/IL-33 and TJs were obtained using keratinocyte HaCaT cells. Using siRNA blockage, cimifugin was found to inhibit initiative cytokines through restoring TJs. In addition, cimifugin administered alone in the initial stage obviously attenuated the ultimate allergic inflammation, thereby indicating the sufficient impact of cimifugin in the initial stage on TSLP/IL-33 and TJs for suppressing allergic inflammation. This study therefore implies the possibility of key cytokines such as IL-33 and TJs can be the therapeutic targets for AD (119).

Eupatilin (5,7-dihydroxy-30,40,6-trimethoxyflavone) is the major lipophilic flavonoid isolated from the Artemisia species (120). Eupatilin has been shown to promote the transcriptional activity and expression of peroxisome proliferator-activated receptor α (PPARα) in keratinocyte HaCaT cells (121) and acts as an agonist of PPARα to ameliorate atopic dermatitis (AD) and restore the skin barrier function. Eupatilin (20 ml of 1.5% or 3.0%) improved AD-like symptoms in an oxazolone-induced

TABLE 1 | TCM formulae and natural compounds those are active against the IL-33/ST2 axis.

Name	Uses, treatment or activities	Disease evaluated and animal model used/ human trials	Effect	References
Soshiho-tang: Radix Bupleuri, Radix Scutellariae, Radix Ginseng, Tuber Pinelliae, Radix et Rhizoma Glycyrrhizae, Rhizoma zingiberis crudae, and Fructus Zizyphi	Pulmonary disorders such as the common cold and pneumonitis	Asthma,OVA-induced asthmatic mice model	Reduce leukocyte and eosinophilic counts, downregulate the production of IL-33 and other Th2-type cytokines, decrease mucus hypersecretion and IgE serum levels	(114, 115)
Qingre-Qushi Recipe(QRQS): Herba Hedyotis Diffusae, Radix Sophorae flavescentis, Herba Taraxaci, and Fructus Xanthii	Skin inflammation and itching	Atopic dermatitis (AD), OVA-induced atopic dermatitis mice model	Suppress both epidermal and dermal thickness, alleviating dermatitis and reducing IL-33 and ST2 positive cell numbers in OVA-induced AD mice Suppress the concentration of specific IgE, IgG, IgG1, and IgG2a antibodies in serum and the expression of IL-33, ST2, IL-1RAcP, IL-4, and IL-13 mRNA in the skin Down-regulate TNF-α and IFN-γ-induced IL-33 mRNA and protein expression in human keratinocyte HaCaT cells	(116)
Calycosin: A flavonoid, is a major component in Radix Astragli	Allergy related symptoms	AD, AD mice	Supress TSLP and IL-33 associated toll-liked receptor (TLR)4-mediated NF-κB signaling, the protein expression of MyD88, TIRAP, and TAK1	(117, 118)
Cimifugin: Bioactive and major component of Radix Saposhnikoviae	Allergy	AD, FITC sensitized, and challenged AD mice	Inhibit TSLP and IL-33 in the initial stage of AD to reduce the separated gap among the epithelial cells and increase the expression of tight junctions (TJs)	(119)
Eupatilin: Main lipophilic flavonoid obtained from the Artemisia species	Anti-oxidative, anti-inflammatory, and anti-apoptotic activities	AD, oxazolone-induced AD-like mouse model	An agonist of PPARα to ameliorate AD and restores the skin barrier function	(120–122)
Protostemonine: An alkaloid from Radix Stemonae	Anti-inflammatory activities	Asthma, DRA (dust mites, ragweed, and aspergillus)-induced murine asthma model	Inhibit pulmonary eosinophil infiltration, goblet cell hyperplasia, mucus secretion, IgE, and Th2 cytokine (IL-4, IL-5, IL-13, and IL-33) production Attenuate the expression of Arginase-1 (Arg-1), Ym-1, and Fizz-1, markers of AAM (alternatively activated macrophage) polarization,in lung tissues	(123)
Tetramethoxyluteolin: The structural analog of luteolin, a common flavonoids present in edible plants (e.g., carrots)	Suppressing mast cell activation	AD, Clinical trials	Inhibit mast cell activation stimulated by IL-33, substance P, or their combination Reduce skin inflammation in patients with AD in clinical studies	(124–126)

AD-like mouse model by suppressing the serum levels of IgE, IL-4, and AD-related cytokines including TNFα, IFN-γ, IL-1β, TSLP, IL-25, and IL-33 (122).

Protostemonine (PSN), an alkaloid isolated from Radix Stemonae was found to suppress inflammatory conditions, IL-33 production and polarization of macrophage into AAM phenotype in the lung tissues of a dust mites, ragweed and aspergillus-induced murine asthma model (123).

Tetramethoxyluteolin (methlut), a natural flavonoid, has been shown to inhibit mast cells stimulated by IL-33, substance P, or their combination. This has been further validated in a clinical trial in which a skin lotion containing tetramethoxyluteolin that can reduce skin inflammation in AD patients. In experimental study, methlut has also been shown to be effective in psoriasis conditions (124, 125).

Apart from Chinese herbs and natural products, acupuncture seems to be effective in attenuating the IL-33 associated airway inflammation in an OVA-induced mouse model by reducing the serum concentrations of IL-33, sST2, and other inflammatory cytokines (129). In summary, these TCM formulae and natural compounds could lower the IL-33 production and other inflammatory cytokines from the tested targets, thereby ameliorating the allergic symptoms. Some of them could target on the specific IL-33 associated immune cells type such as IL-33-mediated mast cell activation and macrophage polarization into AAM phenotypes (**Table 1**). In view of limited clinical evidence and laboratory studies on the action mechanism, further investigations on these two aspects are essential for the future development of TCM in IL-33-related diseases (126).

POTENTIAL FUTURE DEVELOPMENTS AND CONCLUDING REMARKS

With ample experimental evidences, the multiple roles of IL-33 in allergic and inflammatory diseases are not only restricted as an alarmin, but also as a cytokine for additional stimulatory signals: (i) to increase IL-33 expression in the nucleus or cytoplasm, and (ii) to induce IL-33 production into the extracellular space without cell death (29). Those stimulatory signals provide an amplification system for IL-33–mediated inflammatory responses. IL-33–blocking agents which target precisely at different molecular levels (both signaling and amplification pathways) could be potential therapeutic drugs for treatment of allergic and inflammatory diseases. For instance, an important mechanism for the direct activation of IL-33 by proteases from environmental allergens has been recently discovered (30). Targeting the "sensor" domain to prevent the cleavage and activation of IL-33FL, as well as the mast cell protease inhibitors might represent a new approach for reducing allergic responses in asthma and other allergic diseases. Apart from the pathological role in allergic diseases, IL-33 participates in diverse immune regulatory events. Therefore, to optimize the therapeutic outcomes, further evaluations, are essential to manipulate the IL-33/ST2 axis in diseases state and regulatory/physiological roles (130). As with other immunomodulating therapies, investigations on the effect of attenuating IL-33/ST2 axis on immune defense against infection and other immune responses are essential before further therapeutic development (131).

Since most of previous studies on IL-33 blocking agents are at the stage of *in vitro* and animal testing, pharmacological evaluations to develop IL-33–blocking agents are still on-going (132) and some are in phase I–II clinical trials for asthma and chronic obstructive pulmonary disease (133). The combination of IL-33 blocking agents may also be the synergistic intervention in IL-33-associated allergic and inflammatory diseases. For the future translational elucidation of IL-33, human studies are essential such as large scale clinical trials. Furthermore, the IL-33/ST2 axis is participating in both Th2/IL-31 and Th17 immune response during the progression of allergic airway diseases (92). Natural products and herbal medicines with the pluripotent activities to inhibit the production and actions of IL-33 are also promising candidates for further pharmacological evaluation for the treatment of allergic diseases. TCM and natural products, especially flavonoids with proven *in vitro* and *in vivo* activities to target the IL-33/ST2 axis, are potential candidates and warrant further development for the lead compounds as adjuvant anti-allergic and anti-inflammatory agents.

AUTHOR CONTRIBUTIONS

CW and BC wrote the manuscript and prepared the figure. L-ST, CL, and CW drafted sections, structured, and edited the manuscript.

FUNDING

This study was supported by funding of the State Key Laboratory of Research on Bioactivities and Clinical Applications of Medicinal Plants (The Chinese University of Hong Kong/CUHK) from Innovation and Technology Commission and CUHK.

REFERENCES

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
2. Moussion C, Ortega N, Girard JP. 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:e3331. doi: 10.1371/journal.pone.0003331
3. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, 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 USA.* (2007) 104:282–7. doi: 10.1073/pnas.0606854104
4. Haraldsen G, Balogh J, Pollheimer J, Sponheim J, Kuchler AM. Interleukin-33 - cytokine of dual function or novel alarmin? *Trends Immunol.* (2009) 30:227–33. doi: 10.1016/j.it.2009.03.003
5. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci USA.* (2009) 106:9021–6. doi: 10.1073/pnas.0812690106
6. Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* (2014) 31:31–7. doi: 10.1016/j.coi.2014.09.004
7. Cayrol C, Girard JP. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. *Immunol Rev.* (2018) 281:154–68. doi: 10.1111/imr.12619
8. Xu D, Chan WL, Leung BP, Huang F, Wheeler R, Piedrafita 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) 187:787–94.
9. Bonilla WV, Frohlich A, Senn K, Kallert S, Fernandez M, Johnson S, et al. The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses. *Science.* (2012) 335:984–9. doi: 10.1126/science.1215418
10. Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J Immunol.* (1998) 161:4866–74.
11. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature.* (2010) 463:540–4. doi: 10.1038/nature08636
12. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature.* (2010) 464:1367–70. doi: 10.1038/nature08900
13. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci USA.* (2010) 107:11489–94. doi: 10.1073/pnas.1003988107
14. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol.* (2009) 123:1047–54. doi: 10.1016/j.jaci.2009.02.026
15. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood.* (2009) 113:1526–34. doi: 10.1182/blood-2008-05-157818

16. Bourgeois E, Van LP, Samson M, Diem S, Barra A, Roga S, et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol.* (2009) 39:1046–55. doi: 10.1002/eji.200838575
17. Nabekura T, Girard JP, Lanier LL. IL-33 receptor ST2 amplifies the expansion of NK cells and enhances host defense during mouse cytomegalovirus infection. *J Immunol.* (2015) 194:5948–52. doi: 10.4049/jimmunol.1500424
18. Smith DE. The biological paths of IL-1 family members IL-18 and IL-33. *J Leukoc Biol.* (2011) 89:383–92. doi: 10.1189/jlb.0810470
19. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. *Immunol Rev.* (2008) 223:20–38. doi: 10.1111/j.1600-065X.2008.00624.x
20. Pushparaj PN, Tay HK, H'Ng S C, Pitman N, Xu D, McKenzie A, et al. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci USA.* (2009) 106:9773–8. doi: 10.1073/pnas.0901206106
21. Smith DE. IL-33: a tissue derived cytokine pathway involved in allergic inflammation and asthma. *Clin Exp Allergy.* (2010) 40:200–8. doi: 10.1111/j.1365-2222.2009.03384.x
22. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol.* (2007) 37:2779–86. doi: 10.1002/eji.200737547
23. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy Clin Immunol.* (2014) 134:170–7. doi: 10.1016/j.jaci.2013.12.1080
24. Makrinioti H, Toussaint M, Jackson DJ, Walton RP, Johnston SL. Role of interleukin 33 in respiratory allergy and asthma. *Lancet Respir Med.* (2014) 2:226–37. doi: 10.1016/S2213-2600(13)70261-3
25. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med.* (2010) 363:1211–21. doi: 10.1056/NEJMoa0906312
26. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet.* (2011) 43:887–92. doi: 10.1038/ng.888
27. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity.* (2015) 42:1005–19. doi: 10.1016/j.immuni.2015.06.006
28. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun.* (2015) 6:8327. doi: 10.1038/ncomms9327
29. Morita H, Nakae S, Saito H, Matsumoto K. IL-33 in clinical practice: size matters? *J Allergy Clin Immunol.* (2017) 140:381–3. doi: 10.1016/j.jaci.2017.03.042
30. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat Immunol.* (2018) 19:375–85. doi: 10.1038/s41590-018-0067-5
31. Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity.* (2009) 31:84–98. doi: 10.1016/j.immuni.2009.05.007
32. Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N, et al. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int Immunol.* (2008) 20:791–800. doi: 10.1093/intimm/dxn037
33. Mitchell PD, Salter BM, Oliveria JP, El-Gammal A, Tworek D, Smith SG, et al. IL-33 and its receptor ST2 after inhaled allergen challenge in allergic asthmatics. *Int Arch Allergy Immunol.* (2018) 176:133–42. doi: 10.1159/000488015
34. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol.* (2008) 121:1484–90. doi: 10.1016/j.jaci.2008.04.005
35. Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H, et al. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest.* (2008) 88:1245–53. doi: 10.1038/labinvest.2008.82
36. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet.* (2009) 41:342–7. doi: 10.1038/ng.323
37. Wong CK, Cheung PF, Lam CW. Leptin-mediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation. *Eur J Immunol.* (2007) 37:2337–48. doi: 10.1002/eji.200636866
38. Ip WK, Wong CK, Li ML, Li PW, Cheung PF, Lam CW. Interleukin-31 induces cytokine and chemokine production from human bronchial epithelial cells through activation of mitogen-activated protein kinase signalling pathways: implications for the allergic response. *Immunology.* (2007) 122:532–41. doi: 10.1111/j.1365-2567.2007.02668.x
39. Leung TF, Wong KY, Wong CK, Fung KP, Lam CW, Fok TF, et al. *In vitro* and clinical immunomodulatory effects of a novel Pentaherbs concoction for atopic dermatitis. *Br J Dermatol.* (2008) 158:1216–23. doi: 10.1111/j.1365-2133.2008.08502.x
40. Wong CK, Ng SS, Lun SW, Cao J, Lam CW. Signalling mechanisms regulating the activation of human eosinophils by mast-cell-derived chymase: implications for mast cell-eosinophil interaction in allergic inflammation. *Immunology.* (2009) 126:579–87. doi: 10.1111/j.1365-2567.2008.02916.x
41. Chow JY, Wong CK, Cheung PF, Lam CW. Intracellular signaling mechanisms regulating the activation of human eosinophils by the novel Th2 cytokine IL-33: implications for allergic inflammation. *Cell Mol Immunol.* (2010) 7:26–34. doi: 10.1038/cmi.2009.106
42. Wong CK, Leung KM, Qiu HN, Chow JY, Choi AO, Lam CW. Activation of eosinophils interacting with dermal fibroblasts by pruritogenic cytokine IL-31 and alarmin IL-33: implications in atopic dermatitis. *PLoS ONE.* (2012) 7:e29815. doi: 10.1371/journal.pone.0029815
43. Liu KY, Hu S, Chan BC, Wat EC, Lau CB, Hon KL, et al. Anti-inflammatory and anti-allergic activities of Pentaherb formula, Moutan Cortex (Danpi) and gallic acid. *Molecules.* (2013) 18:2483–500. doi: 10.3390/molecules18032483
44. Jiao D, Wong CK, Qiu HN, Dong J, Cai Z, Chu M, et al. NOD2 and TLR2 ligands trigger the activation of basophils and eosinophils by interacting with dermal fibroblasts in atopic dermatitis-like skin inflammation. *Cell Mol Immunol.* (2016) 13:535–50. doi: 10.1038/cmi.2015.77
45. Cheung PF, Wong CK, Ho AW, Hu S, Chen DP, Lam CW. Activation of human eosinophils and epidermal keratinocytes by Th2 cytokine IL-31: implication for the immunopathogenesis of atopic dermatitis. *Int Immunol.* (2010) 22:453–67. doi: 10.1093/intimm/dxq027
46. Chen CC, Kobayashi T, Iijima K, Hsu FC, Kita H. IL-33 dysregulates regulatory T cells and impairs established immunologic tolerance in the lungs. *J Allergy Clin Immunol.* (2017) 140:1351–63.e7. doi: 10.1016/j.jaci.2017.01.015
47. Baumann C, Bonilla WV, Frohlich A, Helmstetter C, Peine M, Hegazy AN, et al. T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell responses. *Proc Natl Acad Sci USA.* (2015) 112:4056–61. doi: 10.1073/pnas.1418549112
48. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity.* (2014) 40:425–35. doi: 10.1016/j.immuni.2014.01.011
49. Oliphant CJ, Hwang YY, Walker JA, Salimi M, Wong SH, Brewer JM, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity.* (2014) 41:283–95. doi: 10.1016/j.immuni.2014.06.016
50. Hirahara K, Mato N, Hagiwara K, Nakayama T. The pathogenicity of IL-33 on steroid-resistant eosinophilic inflammation via the activation of memory-type ST2(+) CD4(+) T cells. *J Leukoc Biol.* (2018) 104:895–901. doi: 10.1002/JLB.MR1117-456R
51. Drake LY, Kita H. Group 2 innate lymphoid cells in the lung. *Adv Immunol.* (2014) 124:1–16. doi: 10.1016/B978-0-12-800147-9.00001-7
52. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature.* (2015) 519:242–6. doi: 10.1038/nature14115
53. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol.* (2013) 132:205–13. doi: 10.1016/j.jaci.2013.03.048

54. Liu T, Barrett NA, Kanaoka Y, Yoshimoto E, Garofalo D, Cirka H, et al. Type 2 Cysteinyl leukotriene receptors drive IL-33-dependent type 2 immunopathology and aspirin sensitivity. *J Immunol.* (2018) 200:915–27. doi: 10.4049/jimmunol.1700603
55. Yazdani R, Sharifi M, Shirvan AS, Azizi G, Ganjalikhani-Hakemi M. Characteristics of innate lymphoid cells (ILCs) and their role in immunological disorders (an update). *Cell Immunol.* (2015) 298:66–76. doi: 10.1016/j.cellimm.2015.09.006
56. Lund S, Walford HH, Doherty TA. Type 2 innate lymphoid cells in allergic disease. *Curr Immunol Rev.* (2013) 9:214–21. doi: 10.2174/15733955106661403042359l6
57. Johansson K, Malmhall C, Ramos-Ramirez P, Radinger M. Bone marrow type 2 innate lymphoid cells: a local source of interleukin-5 in interleukin-33-driven eosinophilia. *Immunology.* (2018) 153:268–78. doi: 10.1111/imm.12842
58. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. *Eur J Pharmacol.* (2016) 778:33–43. doi: 10.1016/j.ejphar.2015.07.017
59. Jang TY, Kim YH. Interleukin-33 and mast cells bridge innate and adaptive immunity: from the allergologist's perspective. *Int Neurourol J.* (2015) 19:142–50. doi: 10.5213/inj.2015.19.3.142
60. Mukai K, Tsai M, Saito H, Galli SJ. Mast cells as sources of cytokines, chemokines, and growth factors. *Immunol Rev.* (2018) 282:121–50. doi: 10.1111/imr.12634
61. Caughey GH. Mast cell proteases as pharmacological targets. *Eur J Pharmacol.* (2016) 778:44–55. doi: 10.1016/j.ejphar.2015.04.045
62. Roy A, Ganesh G, Sippola H, Bolin S, Sawesi O, Dagalv A, et al. Mast cell chymase degrades the alarmins heat shock protein 70, biglycan, HMGB1, and interleukin-33 (IL-33) and limits danger-induced inflammation. *J Biol Chem.* (2014) 289:237–50. doi: 10.1074/jbc.M112.435156
63. Galand C, Leyva-Castillo JM, Yoon J, Han A, Lee MS, McKenzie ANJ, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J Allergy Clin Immunol.* (2016) 138:1356–66. doi: 10.1016/j.jaci.2016.03.056
64. Zoltowska AM, Lei Y, Fuchs B, Rask C, Adner M, 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) 46:479–90. doi: 10.1111/cea.12683
65. Liu T, Kanaoka Y, Barrett NA, Feng C, Garofalo D, Lai J, et al. Aspirin-exacerbated respiratory disease involves a Cysteinyl leukotriene-driven IL-33-mediated mast cell activation pathway. *J Immunol.* (2015) 195:3537–45. doi: 10.4049/jimmunol.1500905
66. Olivera A, Beaven MA, Metcalfe DD. Mast cells signal their importance in health and disease. *J Allergy Clin Immunol.* (2018) 142:381–93 doi: 10.1016/j.jaci.2018.01.034
67. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. *Eur J Immunol.* (2015) 45:3034–44. doi: 10.1002/eji.201545501
68. Wang JX, Kaieda S, Ameri S, Fishgal N, Dwyer D, Dellinger A, et al. IL-33/ST2 axis promotes mast cell survival via BCLXL. *Proc Natl Acad Sci USA.* (2014) 111:10281–6. doi: 10.1073/pnas.1404182111
69. Gordon ED, Simpson LJ, Rios CL, Ringel L, Lachowicz-Scroggins ME, Peters MC, et al. Alternative splicing of interleukin-33 and type 2 inflammation in asthma. *Proc Natl Acad Sci USA.* (2016) 113:8765–70. doi: 10.1073/pnas.1601914113
70. Fu Z, Thorpe M, Alemayehu R, Roy A, Kervinen J, de Garavilla L, et al. Highly Selective cleavage of cytokines and chemokines by the human mast cell chymase and neutrophil cathepsin G. *J Immunol.* (2017) 198:1474–83. doi: 10.4049/jimmunol.1601223
71. Waern I, Jonasson S, Hjoberg J, Bucht A, Abrink M, Pejler G, et al. Mouse mast cell protease 4 is the major chymase in murine airways and has a protective role in allergic airway inflammation. *J Immunol.* (2009) 183:6369–76. doi: 10.4049/jimmunol.0900180
72. Jogie-Brahim S, Min HK, Fukuoka Y, Xia HZ, Schwartz LB. Expression of alpha-tryptase and beta-tryptase by human basophils. *J Allergy Clin Immunol.* (2004) 113:1086–92. doi: 10.1016/j.jaci.2004.02.032
73. Kroeger KM, Sullivan BM, Locksley RM. IL-18 and IL-33 elicit Th2 cytokines from basophils via a MyD88- and p38alpha-dependent pathway. *J Leukoc Biol.* (2009) 86:769–78. doi: 10.1189/jlb.0708452
74. Locksley RM. Asthma and allergic inflammation. *Cell.* (2010) 140:777–83. doi: 10.1016/j.cell.2010.03.004
75. Melgert BN, ten Hacken NH, Rutgers B, Timens W, Postma DS, Hylkema MN. More alternative activation of macrophages in lungs of asthmatic patients. *J Allergy Clin Immunol.* (2011) 127:831–3. doi: 10.1016/j.jaci.2010.10.045
76. Lee YG, Jeong JJ, Nyenhuis S, Berdyshev E, Chung S, Ranjan R, et al. Recruited alveolar macrophages, in response to airway epithelial-derived monocyte chemoattractant protein 1/CCl2, regulate airway inflammation and remodeling in allergic asthma. *Am J Respir Cell Mol Biol.* (2015) 52:772–84. doi: 10.1165/rcmb.2014-0255OC
77. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol.* (2009) 183:6469–77. doi: 10.4049/jimmunol.0901575
78. Espinassous Q, Garcia-de-Paco E, Garcia-Verdugo I, Synguelakis M, von Aulock S, Sallenave JM, et al. IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating lipopolysaccharide receptor complex. *J Immunol.* (2009) 183:1446–55. doi: 10.4049/jimmunol.0803067
79. de Kleer IM, Kool M, de Bruijn MJ, Willart M, van Moorleghem J, Schuijs MJ, et al. Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung. *Immunity.* (2016) 45:1285–98. doi: 10.1016/j.immuni.2016.10.031
80. Saluzzo S, Gorki AD, Rana BMJ, Martins R, Scanlon S, Starkl P, et al. First-breath-induced type 2 pathways shape the lung immune environment. *Cell Rep.* (2017) 18:1893–905. doi: 10.1016/j.celrep.2017.01.071
81. Mindt BC, Fritz JH, Duerr CU. Group 2 innate lymphoid cells in pulmonary immunity and tissue homeostasis. *Front Immunol.* (2018) 9:840. doi: 10.3389/fimmu.2018.00840
82. Teufelberger AR, Nordengrun M, Braun H, Maes T, De Grove K, Holtappels G, et al. The IL-33/ST2 axis is crucial in type 2 airway responses induced by Staphylococcus aureus-derived serine protease-like protein D. *J Allergy Clin Immunol.* (2018) 141:549–59.e7. doi: 10.1016/j.jaci.2017.05.004
83. Liu B, Tai Y, Achanta S, Kaelberer MM, Caceres AI, Shao X, et al. IL-33/ST2 signaling excites sensory neurons and mediates itch response in a mouse model of poison ivy contact allergy. *Proc Natl Acad Sci USA.* (2016) 113:E7572–9. doi: 10.1073/pnas.1606608113
84. Han P, Zhao J, Liu SB, Yang CJ, Wang YQ, Wu GC, et al. Interleukin-33 mediates formalin-induced inflammatory pain in mice. *Neuroscience.* (2013) 241:59–66. doi: 10.1016/j.neuroscience.2013.03.019
85. Kotlyar DS, Shum M, Hsieh J, Blonski W, Greenwald DA. Non-pulmonary allergic diseases and inflammatory bowel disease: a qualitative review. *World J Gastroenterol.* (2014) 20:11023–32. doi: 10.3748/wjg.v20.i32.11023
86. Seidelin JB, Bjerrum JT, Coskun M, Widjaya B, Vainer B, Nielsen OH. IL-33 is upregulated in colonocytes of ulcerative colitis. *Immunol Lett.* (2010) 128:80–5. doi: 10.1016/j.imlet.2009.11.001
87. Beltran CJ, Nunez LE, Diaz-Jimenez D, Farfan N, Candia E, Heine C, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1097–107. doi: 10.1002/ibd.21175
88. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, 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 USA.* (2010) 107:8017–22. doi: 10.1073/pnas.0912678107
89. Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarstrom C, Loos T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am J Pathol.* (2010) 177:2804–15. doi: 10.2353/ajpath.2010.100378
90. Nunes T, Bernardazzi C, de Souza HS. Interleukin-33 and inflammatory bowel diseases: lessons from human studies. *Mediators Inflamm.* (2014) 2014:423957. doi: 10.1155/2014/423957

91. Tu L, Chen J, Xu D, Xie Z, Yu B, Tao Y, et al. IL-33-induced alternatively activated macrophage attenuates the development of TNBS-induced colitis. *Oncotarget.* (2017) 8:27704–14. doi: 10.18632/oncotarget.15984
92. Vocca L, Di Sano C, Uasuf CG, Sala A, Riccobono L, Gangemi S, et al. IL-33/ST2 axis controls Th2/IL-31 and Th17 immune response in allergic airway diseases. *Immunobiology.* (2015) 220:954–63. doi: 10.1016/j.imbio.2015.02.005
93. Fattori V, Borghi SM, Verri WA Jr. IL-33/ST2 signaling boosts inflammation and pain. *Proc Natl Acad Sci USA.* (2017) 114:E10034–5. doi: 10.1073/pnas.1716120114
94. Khaitov MR, Gaisina AR, Shilovskiy IP, Smirnov VV, Ramenskaia GV, Nikonova AA, et al. The role of interleukin-33 in pathogenesis of bronchial asthma. New experimental data. *Biochemistry.* (2018) 83:13–25. doi: 10.1134/S0006297918010029
95. Johnston LK, Hsu CL, Krier-Burris RA, Chhiba KD, Chien KB, McKenzie A, et al. IL-33 precedes IL-5 in regulating eosinophil commitment and is required for eosinophil homeostasis. *J Immunol.* (2016) 197:3445–53. doi: 10.4049/jimmunol.1600611
96. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol.* (2013) 132:676–85.e13. doi: 10.1016/j.jaci.2013.04.012
97. Balato A, Di Caprio R, Canta L, Mattii M, Lembo S, Raimondo A, et al. IL-33 is regulated by TNF-alpha in normal and psoriatic skin. *Arch Dermatol Res.* (2014) 306:299–304. doi: 10.1007/s00403-014-1447-9
98. Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. *J Gastroenterol.* (2010) 45:999–1007. doi: 10.1007/s00535-010-0245-1
99. Yanagawa Y, Matsumoto M, Togashi H. Adrenoceptor-mediated enhancement of interleukin-33 production by dendritic cells. *Brain Behav Immun.* (2011) 25:1427–33. doi: 10.1016/j.bbi.2011.04.012
100. Nabe T. Interleukin (IL)-33: new therapeutic target for atopic diseases. *J Pharmacol Sci.* (2014) 126:85–91. doi: 10.1254/jphs.14R12CP
101. Thio CL, Chi PY, Lai AC, Chang YJ. Regulation of type 2 innate lymphoid cell-dependent airway hyperreactivity by butyrate. *J Allergy Clin Immunol.* (2018) 142:1867–83.e12. doi: 10.1016/j.jaci.2018.02.032
102. Kouzaki H, Matsumoto K, Kikuoka H, Kato T, Tojima I, Shimizu S, et al. Endogenous protease inhibitors in airway epithelial cells contribute to eosinophilic chronic rhinosinusitis. *Am J Respir Crit Care Med.* (2017) 195:737–47. doi: 10.1164/rccm.201603-0529OC
103. Shen ZJ, Esnault S, Malter JS. The peptidyl-prolyl isomerase Pin1 regulates the stability of granulocyte-macrophage colony-stimulating factor mRNA in activated eosinophils. *Nat Immunol.* (2005) 6:1280–7. doi: 10.1038/ni1266
104. Nechama M, Kwon J, Wei S, Kyi AT, Welner RS, Ben-Dov IZ, et al. The IL-33-PIN1-IRAK-M axis is critical for type 2 immunity in IL-33-induced allergic airway inflammation. *Nat Commun.* (2018) 9:1603. doi: 10.1038/s41467-018-03886-6
105. Li J, Saruta K, Dumouchel JP, Magat JM, Thomas JL, Ajami D, et al. Small molecule mimetics of alpha-helical domain of IRAK2 attenuate the proinflammatory effects of IL-33 in asthma-like mouse models. *J Immunol.* (2018) 200:4036–43. doi: 10.4049/jimmunol.1700693
106. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. *Nature.* (2010) 465:885–90. doi: 10.1038/nature09121
107. Park CS, Jang TY, Heo MJ, Jung AY, Kim YH. Antiallergic effects of anti-interleukin-33 are associated with suppression of immunoglobulin light chain and inducible nitric oxide synthase. *Am J Rhinol Allergy.* (2016) 30:17–22. doi: 10.2500/ajra.2015.29.4251
108. Fursov N, Lu J, Healy C, Wu SJ, Lacy E, Filer A, et al. Monoclonal antibodies targeting ST2L Domain 1 or Domain 3 differentially modulate IL-33-induced cytokine release by human mast cell and basophilic cell lines. *Mol Immunol.* (2016) 75:178–87. doi: 10.1016/j.molimm.2016.05.019
109. Ohta K, Nagase H, Suzukawa M, Ohta S. Antibody therapy for the management of severe asthma with eosinophilic inflammation. *Int Immunol.* (2017) 29:337–43. doi: 10.1093/intimm/dxx045
110. Katial RK, Bensch GW, Busse WW, Chipps BE, Denson JL, Gerber AN, et al. Changing paradigms in the treatment of severe asthma: the role of biologic therapies. *J Allergy Clin Immunol Pract.* (2017) 5:S1–14. doi: 10.1016/j.jaip.2016.11.029
111. Hodzic Z, Schill EM, Bolock AM, Good M. IL-33 and the intestine: the good, the bad, and the inflammatory. *Cytokine.* (2017) 100:1–10. doi: 10.1016/j.cyto.2017.06.017
112. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. *Nat Rev Drug Discov.* (2010) 9:325–38. doi: 10.1038/nrd3003
113. Liu L, Li Y. The unexpected side effects and safety of therapeutic monoclonal antibodies. *Drugs Today.* (2014) 50:33–50. doi: 10.1358/dot.2014.50.1.2076506
114. Jeon WY, Shin HK, Shin IS, Kim SK, Lee MY. Soshiho-tang water extract inhibits ovalbumin-induced airway inflammation via the regulation of heme oxygenase-1. *BMC Complement Altern Med.* (2015) 15:329. doi: 10.1186/s12906-015-0857-3
115. Ohtake N, Nakai Y, Yamamoto M, Ishige A, Sasaki H, Fukuda K, et al. The herbal medicine Shosaiko-to exerts different modulating effects on lung local immune responses among mouse strains. *Int Immunopharmacol.* (2002) 2:357–66. doi: 10.1016/S1567-5769(01)00161-8
116. Chen M, Ding P, Yang L, He X, Gao C, Yang G, et al. Evaluation of anti-inflammatory activities of Qingre-Qushi Recipe (QRQS) against atopic dermatitis: potential mechanism of inhibition of IL-33/ST2 signal transduction. *Evid Based Complement Alternat Med.* (2017) 2017:2489842. doi: 10.1155/2017/2489842
117. Qiu R, Ma G, Zheng C, Qiu X, Li X, Li X, et al. Antineoplastic effect of calycosin on osteosarcoma through inducing apoptosis showing *in vitro* and *in vivo* investigations. *Exp Mol Pathol.* (2014) 97:17–22. doi: 10.1016/j.yexmp.2014.04.014
118. Tao Y, Wang Y, Wang X, Wang C, Bao K, Ji L, et al. Calycosin suppresses epithelial derived initiative key factors and maintains epithelial barrier in allergic inflammation via TLR4 mediated NF-kappaB pathway. *Cell Physiol Biochem.* (2017) 44:1106–19. doi: 10.1159/000485416
119. Wang X, Jiang X, Yu X, Liu H, Tao Y, Jiang G, et al. Cimifugin suppresses allergic inflammation by reducing epithelial derived initiative key factors via regulating tight junctions. *J Cell Mol Med.* (2017) 21:2926–36. doi: 10.1111/jcmm.13204
120. Giangaspero A, Ponti C, Pollastro F, Del Favero G, Della Loggia R, Tubaro A, et al. Topical anti-inflammatory activity of Eupatilin, a lipophilic flavonoid from mountain wormwood (Artemisia umbelliformis Lam.). *J Agric Food Chem.* (2009) 57:7726–30. doi: 10.1021/jf901725p
121. Jung Y, Kim JC, Choi Y, Lee S, Kang KS, Kim YK, et al. Eupatilin with PPARalpha agonistic effects inhibits TNFalpha-induced MMP signaling in HaCaT cells. *Biochem Biophys Res Commun.* (2017) 493:220–6. doi: 10.1016/j.bbrc.2017.09.043
122. Jung Y, Kim JC, Park NJ, Bong SK, Lee S, Jegal H, et al. Eupatilin, an activator of PPARalpha, inhibits the development of oxazolone-induced atopic dermatitis symptoms in Balb/c mice. *Biochem Biophys Res Commun.* (2018) 496:508–14. doi: 10.1016/j.bbrc.2018.01.098
123. Song Y, Wu Y, Li X, Shen Y, Ding Y, Zhu H, et al. Protostemonine attenuates alternatively activated macrophage and DRA-induced asthmatic inflammation. *Biochem Pharmacol.* (2018) 155:198–206. doi: 10.1016/j.bcp.2018.07.003
124. Weng Z, Patel AB, Panagiotidou S, Theoharides TC. The novel flavone tetramethoxyluteolin is a potent inhibitor of human mast cells. *J Allergy Clin Immunol.* (2015) 135:1044–52.e5. doi: 10.1016/j.jaci.2014.10.032
125. Theoharides TC, Stewart JM, Tsilioni I. Tolerability and benefit of a tetramethoxyluteolin-containing skin lotion. *Int J Immunopathol Pharmacol.* (2017) 30:146–51. doi: 10.1177/0394632017707610
126. Patel AB, Tsilioni I, Weng Z, Theoharides TC. TNF stimulates IL-6, CXCL8 and VEGF secretion from human keratinocytes via activation of mTOR, inhibited by tetramethoxyluteolin. *Exp Dermatol.* (2018) 27:135–43. doi: 10.1111/exd.13461
127. Tsang MS, Jiao D, Chan BC, Hon KL, Leung PC, Lau CB, et al. Anti-inflammatory activities of pentaherbs formula, berberine, gallic acid and chlorogenic acid in atopic dermatitis-like skin

inflammation. *Molecules.* (2016) 21:519. doi: 10.3390/molecules21040519
128. Chan BC, Li LF, Hu SQ, Wat E, Wong EC, Zhang VX, et al. Gallic acid is the major active component of cortex moutan in inhibiting immune maturation of human monocyte-derived dendritic cells. *Molecules.* (2015) 20:16388–403. doi: 10.3390/molecules200916388
129. Dong M, Ma C, Wang WQ, Chen J, Wei Y. Regulation of the IL-33/ST2 pathway contributes to the anti-inflammatory effect of acupuncture in the ovalbumin-induced murine asthma model. *Acupunct Med.* (2018) 36:319–26. doi: 10.1136/acupmed-2017-011377
130. Chen WY, Tsai TH, Yang JL, Li LC. Therapeutic strategies for targeting IL-33/ST2 signalling for the treatment of inflammatory diseases. *Cell Physiol Biochem.* (2018) 49:349–58. doi: 10.1159/000492885
131. Salas A. The IL-33/ST2 axis: yet another therapeutic target in inflammatory bowel disease? *Gut.* (2013) 62:1392–3. doi: 10.1136/gutjnl-2012-303920
132. Alves MF, da Fonseca DV, de Melo SAL, Scotti MT, Scotti L, Dos Santos SG, et al. New therapeutic targets and drugs for the treatment of asthma. *Mini Rev Med Chem.* (2018) 18:684–96. doi: 10.2174/1389557517666170927145011
133. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol.* (2017) 8:475. doi: 10.3389/fimmu.2017.00475

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Chan, Lam, Tam and Wong. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

SYSTEMATIC REVIEW
published: 20 March 2019
doi: 10.3389/fimmu.2019.00522

Pleiotropic Effects of IL-33 on CD4$^+$ T Cell Differentiation and Effector Functions

Fernando Alvarez[1,2,3], *Jörg H. Fritz*[1,3,4] *and Ciriaco A. Piccirillo*[1,2,3,4]*

[1] Department of Microbiology and Immunology, McGill University, Montréal, QC, Canada, [2] Program in Infectious Diseases and Immunology in Global Health, Centre for Translational Biology, The Research Institute of the McGill University Health Center, Montréal, QC, Canada, [3] Centre of Excellence in Translational Immunology, Montréal, QC, Canada, [4] McGill University Research Center on Complex Traits, McGill University, Montréal, QC, Canada

IL-33, a member of the IL-1 family of cytokines, was originally described in 2005 as a promoter of type 2 immune responses. However, recent evidence reveals a more complex picture. This cytokine is released locally as an alarmin upon cellular damage where innate cell types respond to IL-33 by modulating their differentiation and influencing the polarizing signals they provide to T cells at the time of antigen presentation. Moreover, the prominent expression of the IL-33 receptor, ST2, on GATA3$^+$ T helper 2 cells (T_H2) demonstrated that IL-33 could have a direct impact on T cells. Recent observations reveal that T-bet$^+$ T_H1 cells and Foxp3$^+$ regulatory T (T_{REG}) cells can also express the ST2 receptor, either transiently or permanently. As such, IL-33 can have a direct effect on the dynamics of T cell populations. As IL-33 release was shown to play both an inflammatory and a suppressive role, understanding the complex effect of this cytokine on T cell homeostasis is paramount. In this review, we will focus on the factors that modulate ST2 expression on T cells, the effect of IL-33 on helper T cell responses and the role of IL-33 on T_{REG} cell function.

Keywords: T cell differentiation, Th17 and Tregs cells, th1/th2 balance, infection, immunoregulation, IL-33, ST2

OPEN ACCESS

Edited by:
Jose Carlos Alves-Filho, University of São Paulo, Brazil

Reviewed by:
Mariola Stefania Kurowska-Stolarska, University of Glasgow, United Kingdom
Miodrag L. Lukic, University of Kragujevac, Serbia

***Correspondence:**
Ciriaco A. Piccirillo
Ciro.piccirillo@mcgill.ca

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *23 November 2018*
Accepted: *26 February 2019*
Published: *20 March 2019*

Citation:
Alvarez F, Fritz JH and Piccirillo CA (2019) Pleiotropic Effects of IL-33 on CD4$^+$ T Cell Differentiation and Effector Functions. Front. Immunol. 10:522. doi: 10.3389/fimmu.2019.00522

MULTI-FACETED FUNCTIONS OF IL-33

Barrier sites are exposed to varying levels of danger at every moment, which requires the constant involvement of the local immune system to maintain epithelial function and immune homeostasis. As such, many foreign and self-derived warning signals dictate the response of these immune cells. The molecules that provide these signals are classified as pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). However, some specialized endogenous molecules, released upon cellular damage, were improperly organized using these definitions. Thus, a new concept was introduced during the *EMBO Workshop on Innate Danger Signals and HMGB1* in February 2006, which would separate PAMPs from self-signals. Joost Oppenheim introduced at that meeting what he coined "alarmins," self-molecules released upon cellular damage that play a role in modulating the immune response (1, 2). The proposed description classifies "alarmins" as molecules that (1) are released upon non-programmed cells death; (2) can be produced by immune cells without dying; (3) can recruit and activate receptor-expressing immune cells; and (4) can contribute to the restoration of immune homeostasis and epithelial repair mechanisms (1). In recent years, several examples of dysregulated expression or

activity of alarmins were associated with immune-related pathologies in many diseases. Thus, alarmins can play pro-inflammatory or regulatory roles at the site of inflammation (3).

Of the many members of alarmins, the IL-1 family, comprised of 11 members, was introduced early in this classification (4). IL-1 family members include IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, and IL-37 which possess agonist properties and IL-1Ra, IL-36Ra, and IL-38, which possess antagonist properties on their respective receptors (5). A unique feature of this family, with the exception of IL-1Ra, is their capacity to accumulate as pro-cytokines and possess enzymatic cleavage sites in their sequence (6). However, cleavage is not always required for these pro-cytokines to bind and activate their respective receptors. For example, as caspase 1 and caspase 8 are required for the activation of IL-1β and IL-18, pro-IL-33 does not require enzymatic processing to exert its biological activity (6). However, processing by neutrophils proteases, notably cathepsin G and elastase, and proteases brought by airway allergens were shown to enhance IL-33 activity (6, 7). This peculiarity reveals that IL-33, as opposed to IL-1β or IL-18, exerts most of its effect in a caspase-independent manner (6). Thus, IL-33 possesses intrinsic biomolecular peculiarities that dictate its role at mucosal sites and its effect on the innate and adaptive immune system.

Expression of ST2 was first described in $CD4^+$ T_H2 cells (8). However, a wide range of immune cells has been described to respond to IL-33 directly. A functional ST2 receptor was notably described in eosinophils (9), basophils (10), natural killer (NK), and NK-T cells (11, 12), as well as group 2 innate lymphoid cells (ILC2s) (13). In eosinophils, IL-33 was shown to directly facilitate their maturation through enhanced survival, activation and adhesion (14). Similarly, IL-33 potentiates adhesion and histamine release in basophils (15). IL-33 is also known to facilitate the maturation, migration from the bone marrow and local functions of ILC2s in the lungs (13, 16). Furthermore, dendritic cells (DCs) can respond to IL-33 directly to polarize naïve T cells into T_H2 or facilitate T_{REG} proliferation (17, 18). Interestingly, although the effect of IL-33 was originally thought to be a determinant of type 2 immune responses, it was shown to also favor the expansion of NK and NK T cells during viral infections (11, 12). Thus, IL-33 has pleiotropic functions in directing the innate immune response, a feature that is also found in its effect on adaptive immunity, most notably in the function and differentiation of $CD4^+$ T cells.

In mammals, T cells are critical members of the immune system and play a pivotal role in all aspects of immune responses from the effective clearance of pathogens to the establishment of a memory response and the quick return to immune homeostasis. $CD4^+$ T cells are characterized by their ability to recognize antigens through their T cell specific receptor (TCR), upon which they undergo rapid clonal expansion and differentiate into functionally distinct T_H subsets. These subsets then migrate and orchestrate the immune response at inflammatory sites. It is of no surprise that the distinct subsets of helper $CD4^+$ T cells, T_H1, T_H2, and T_H17 cells, respond to alarmins of the IL-1 family in order to proliferate and function locally (19). However, the categorization of T cells by their master transcription factors like T-bet, GATA3, RORγT or Foxp3, does not reflect the high level of T cell plasticity observed *in vivo*. For example, T cells expressing both GATA3 and T-bet were observed in the lung during infection with parasites (20). Similarly, although ST2 is strongly associated with the function of T_H2 T cells (8), T_H1 cells can also transiently express it (21). Moreover, it was shown that $Foxp3^+$ T_{REG} cells and T_H17 cells signal through IL-33 to modulate their respective functions (22, 23).

In this review, we will focus on the effects of IL-33 on $CD4^+$ T cell responses. We will highlight recent advances in our understanding of the IL-33 pathway and its impact on T cell differentiation and effector functions, including the modulatory role of IL-33 on $Foxp3^+$ T_{REG} cells, in both autoimmune and infectious diseases.

REGULATION OF IL-33 EXPRESSION AND SECRETION

IL-33 is constitutively expressed as a nuclear protein in epithelial and endothelial cells. Body-wide analysis through immunohistochemistry, mRNA transcripts and a unique *il-33-LacZ* reporter mouse line revealed that IL-33 is constitutively expressed in secondary lymphoid tissues, but more prominently found at mucosal sites like the gut and lungs, as well as in the brain and adipose tissues (24). However, although humans and mice share most of the constitutive expression of IL-33, species-specific differences exist. For example, it was shown that murine keratinocytes express IL-33 constitutively whereas human keratinocytes required prior IFNγ stimulation (25). Thus, conclusions derived from mouse models must be corroborated with human samples.

Many biological mechanisms regulate the half-life and activity of IL-33. On one hand, pro-IL-33, a 31 kDa protein, does not require enzymatic cleavage to exert its biological functions, although these can be potentiated by the action of self and non-self proteases and elastases that cut it down to a more potent 20 kDa protein (6, 7, 26). On the other hand, the activity of IL-33 is known to be reduced by: (1) cleavage of IL-33 after Asp178 by caspases 3 and 7 (27); (2) upregulation of the LMP2 proteasome by IFNγ during type 1 immune responses (28); (3) extracellular cysteine oxidation that cause the formation of two disulfide bridges on IL-33 and disrupts its binding to ST2 (29), and (4) the extracellular release of the soluble ST2 (sST2), that acts as a decoy receptor for IL-33 (30, 31). Furthermore, IL-33 lacks a conventional signal sequence or any non-canonical export pathway and thus requires either cellular death by necrosis or necroptosis of endothelial and epithelial cells or a still unknown excretory mechanism by innate immune cells to be released in the extracellular milieu (5, 30). In fact, the full-length IL-33 was shown to bind to chromatin causing it to be 10 times slower than IL-1α (32). This novel post-translational mechanism of cytokine release, along with the many enzymatic and environmental processes described, reveals the fine control of the activity of IL-33 at mucosal surfaces and illustrates the evolutionary control of these immunomodulatory signals.

IL-33 SIGNALING

ST2 was first described as an orphan receptor until the discovery of IL-33 (31). A member of the Toll-like/Interleukine-1 receptor superfamily, it was shown that it forms a heterodimer with the ubiquitous IL1R accessory protein (IL1RAcp) at the membrane surface in order to bind IL-33. Interestingly, all the members of the IL-1 family share a common intracellular Toll/IL-1 receptor (TIR) domain. However, four distinct isoforms of ST2 were described: (1) the membrane-bound ST2 (ST2L or ST2), which provides the activation pathway; (2) the soluble ST2 (sST2)–that originates from another promoter region of the *il1rl1* gene and lacks the transmembrane and cytoplasmic domains of ST2–acts as a decoy for IL-33, and is notably used as a biological marker of cardiac injury (31, 33); the latter two forms are splice variants identified in a tumor cells line 3) ST2V (34), which possesses a hydrophobic tail at the C-terminal; and 4) in chicken, ST2LV (35), which lacks the transmembrane domain of ST2 and whose function remains to be elucidated.

IL-33 binds specifically to ST2, which in turn associates to the IL1RAcP to form a heterodimeric receptor that leads to the dimerization of the TIR domain with the TIR domain of cytosolic adaptor protein myeloid differentiation factor 88 (MyD88). In turn, the N-terminal death domain (28) of MyD88 recruits the IL-1-associated kinase 1 (36) and 4 (37). The IRAK1/4 complex can then activate the downstream mitogen-activated protein kinase (MAPK) through the TNF receptor-associated factor 6 (TRAF6). TRAF6 does not possess enzymatic activity but plays a critical role through its ubiquitin E3 ligase (38). TRAF6 is thus required for the induction of several kinase cascades such as NF-kB, JNK, p38, and PI3K. Interestingly, IL-33 can activate ERK even in TRAF6-deficient cells, indicating a parallel activation cascade upon signaling (38). In fact, IL-33 could still induce the expression of ST2L in TRAF6-deficient embryonic fibroblasts (38), indicating the presence of distinct pathways in the IL-33 cascade. However, most of these analyses were conducted using non-T cell lines, and studies in primary immune cells are warranted (39, 40).

TRAF6 Activation in T Cells

In T cells, TRAF6 is known to regulate TCR signaling via ubiquitination at Lys(88) of the LAT adapter and phosphorylation of the IKK/NEMO complex (41). Interestingly, TRAF6 deficiency leads to a hyperactivation of the PI3K-AKT pathway in T cells and to T_H2 polarization in mice (42). Furthermore, TRAF6 is essential for the survival and proliferation of T_{REG} cells that suppress T_H2 type autoimmunity (43, 44). As such, TRAF6 is required for the maintenance of peripheral tolerance and control of T cell hyper-reactivity. The downstream targets of TRAF6 include the phosphorylation of JNK1/2 (38). JNK1/2 activation is required for T cell differentiation, but not activation, as the lack of JNK leads to a decrease in inflammatory cytokine production, but not proliferation or IL-2 production (45). In fact, the p38-MAPK pathway plays a non-redundant role on memory $ST2^+$ T_H2 cells, since selective inhibition of p38, but not JNK, PI3K or ERK, leads to a decrease in IL-5 production in these cells upon IL-33 stimulation (46). Thus, although TRAF6 deficiency leads to increased T_H2 differentiation and a lack of T_{REG}-mediated suppression, IL-33 signaling is required for T_H2 function, illustrating the complexity of this signal in T cells.

ERK Activation in T Cells

Biochemical dissection of the IL-33/ST2 pathway in mammalian cell lines was performed using data mined through an extensive survey of the literature (40). This model includes the phosphorylation and activation of ERK1/2, JNK1/2, p38, and PI3K/AKT downstream of IL-33. However, the underlying processes affected by these changes remain unknown. This is likely due to the large heterogeneity of the recipient cells and their varied epigenetic status. In T cells, ERK activity is notably linked to a reduction in the TCR activation threshold, as it delays the binding of the inhibitory protein SHP-1 to the complex, leading to the activation of T cells under suboptimal stimulation (47). ERK1 is particularly required for T_H2 but not T_H1 proliferation and function and plays a major role in a model of experimental asthma (48). On the other hand, lack of ERK2 inhibits T_H1 and T_H17 T cell differentiation and function (49, 50). This was shown to occur notably through the control of the master transcription factors of these subsets, as ERK2 suppresses the transcription of Foxp3 (T_{REG}) and GATA3 (T_H2) and favors the expression of T-bet (T_H1) (49). Interestingly, although the lack of either ERK2 or ERK1 does not hinder the suppressive ability of T_{REG} cells (49), it favors the TGFβ-mediated induction of Foxp3 (50). Thus, ERK1/2 activation is a major pathway involved in the control of the function of T_H1, T_H17, T_H2, and T_{REG} cells at mucosal sites. Further investigation into the T cell-intrinsic modulation of ERK1 and ERK2 by IL-33 might reveal how the distinct T cell subsets respond to this alarmin.

On the other hand, p38, composed of four known members (α, β, γ, δ), plays key roles in T cell activation and proliferation. Constitutive activation of p38α and p38β ($p38\alpha\beta^{Y323F}$) was shown to skew T cell differentiation toward T_H1 and T_H17 cells (51), whereas knock-down of p38 α/β led to increased T_{REG} cells (52). Interestingly, the IL-33-p38 pathway was shown to be directly linked to the function of $ST2^+$ T_H2 cells, as inhibition of p38-MAPK, but not JNK or PI3K, resulted in a lack of IL-5 production by T_H2 cells upon IL-33 stimulation (46). Finally, although we know little about the role of JNK activation by IL-33 on T cells, JNK1/2 was shown to play a critical role in T cell function but not activation (45).

While some signaling pathways downstream of IL-33 are known, the transcriptional targets downstream of IL-33 depend largely on the state of the recipient T cell and the environmental context. Thus, in order to fully understand the role of IL-33 on T cells, assessing the effects of IL-33 on the functions of T_H cell subsets is required.

EFFECT OF IL-33 ON T_H CELL RESPONSES

Regulation of ST2 Expression

In an early study, inflammatory factors such as tumor necrosis factor (TNF), IL-1α, IL-1β or Phorbol 12-myristate 13-acetate

were shown to be required for the upregulation of the membrane-bound ST2 on responding cells (53). T_H2 cells were the first to be shown to express ST2 (8). T_H2 polarizing conditions, involving both STAT5 (IL-2) and STAT6 (IL-4) activation, were shown to induce ST2 on T cells *in vitro*, although multiple rounds of polarization were required (54). In fact, the transcription factor GATA3, associated with the development and function of T_H2 cells, was necessary for the selective upregulation of ST2 *in vitro*, as genetic deficiency of GATA3 abrogated ST2 expression in T_H2 cells (55). GATA3 binds an enhancer region situated 12kb upstream of the transcription start site of *il1rl1* (ST2) (55, 56), a finding confirmed through genome-wide mapping of GATA3 binding (57). Under the same conditions, the expression of ST2 was also dependent on the binding of STAT5 to the intron 7 of *il1rl1* (55) which also leads to the production of IL-13 and IL-5, but not IL-4, *in vitro*, suggesting that STAT5-activating signals, such as IL-2, IL-7 or TSLP are required for the upregulation of ST2 in T_H2 cells (**Figure 1**).

Interestingly, the expression of ST2 is particularly enhanced by the provision of exogenous IL-33 in $CD4^+$ T cell cultures, illustrating that IL-33, in a positive feedback loop, is directly involved in the up-regulation of its own receptor (55). It has been suggested that IL-33 potentiates STAT5 signaling in T cells since *in vitro* polarized T_H2 cells show increased STAT5 phosphorylation when exposed to IL-33 (55). On the other hand, a consensus site for NF-κB was found in the *Il1rl1* promoter region (58), revealing a potential mechanism by which IL-33 could regulate its own expression. Nonetheless, further investigations are required in order to understand why and how IL-33 is required for the expression of its own receptor. Thus, both a STAT5 signal (IL-2, IL-7 or TSLP) and IL-33 are sufficient to upregulate ST2 on T_H2 cells (55) and T_{REG} cells (23) (**Figure 1**).

The involvement of a STAT6 signal in the development of $ST2^+$ T_H2 cells remains to be understood. In early experiments, IL-4 was required for the polarization of T_H2 cells, and thus was involved, amid indirectly, in the cells' responsiveness to IL-33. On a molecular level, STAT6 is not known to bind the promoter region of ST2 but does bind to the distal promoter of *gata3* (59). Yet, STAT6, but not GATA3, is necessary for binding the locus control region (LCR) inside the T_H2 cytokine gene cluster of *il4, il5*, and *il13* (60). As such, STAT6 remodels the LCR, whereas GATA3 acts as a local promoter of these genes. Nonetheless, forced expression of a constitutively activated form of STAT5A (STAT5A1*6) through retroviral transduction in T cells revealed that a STAT6 signal was not essential to differentiate T_H2 cells (61). The binding sites of STAT5 on the gene cluster differs from STAT6 and could illustrate a parallel evolutionary mechanism in the polarization of T_H2 cells (61). Interestingly, even in these conditions, co-expression of a constitutive GATA3 potentiated the effect of STAT5 in T_H2 cell development (61). Thus, although STAT5 plays a significant role, a co-stimulatory STAT6 signal is required to potentiate GATA3 expression and leads to the full differentiation of T_H2 cells.

Apart from GATA3, other transcription factors were shown to bind to the distal promoter site of *il1rl1* (ST2). Four GATA1 binding sites were identified within 1,001 bp of the distal promoter region of *il1rl1* in human and murine cells lines (62, 63). GATA2 and PU.1 were further identified to exert key roles in the expression of ST2 in mast cells and basophils as they bind the distal promoter region of the *il1rl1* gene (64, 65). Interestingly, while GATA1 acted as a repressor, GATA2 provided a transactivation signal for the expression of *il1rl1* (64). Little is known as to the role of GATA1 and GATA2 in the later stages of T cell polarization and function. A report demonstrated that GATA1 possesses a degree of redundancy with GATA3 in T cells, as it suppresses T_H1 differentiation and functions in a similar, yet less efficient, manner as GATA3 (66). More recent evidence points to a possible role of PU.1 in the regulation of GATA3 expression in T cell differentiation. PU.1 is required for the development of T cells in the thymus (67), and is expressed in T_H9 and not in T_H1 cells (68). Interestingly, PU.1 can alter GATA3 promoter regions in dendritic and T cells and was found to facilitate the expression of *il5* and *il13*, but not *il4* (68, 69). Although yet unknown, the role of PU.1 in $ST2^+$ T_H2 might reveal why these cells respond to IL-33 by expressing IL-5 and IL-13, but not IL-4 (55). A recent report identifies the transcription factors IRF4 and BATF as binding the *il1rl1* loci in T_{REG} cells (70) (**Figure 1**). In fact, a reduced expression of BATF lead to a decrease in ST2 expression in T_{REG} cells (71). Thus, T_{REG} cells may possess distinct mechanisms to control the transcription of *il1rl1*.

Surprisingly, ST2 can be transiently expressed by T_H1 cells (21). In these reports, the upregulation of ST2 was significantly lower and short lived when compared to $ST2^+$ T_H2 cells and was dependent on the expression of the transcription factor T-bet and the IL-12-dependent STAT4 signal (21, 72). Interestingly, co-stimulation with IL-33 was also required for the expression of ST2. Although these observations were corroborated *in vivo* with $STAT4^{-/-}$ and $Tbet^{-/-}$ mice during the course of a lymphocytic choriomeningitis virus (LCMV) infection (21), it was suggested that these cells might represent a hybrid $T\text{-}bet^+GATA3^+$ cell subset as low levels of GATA3 were found to be upregulated in a subset of $Tbet^+$ cells (20, 56). Nonetheless, further investigations are required to understand the transcriptional mechanisms by which T_H1 cells express ST2.

Finally, T_H17 cell, expressing the transcription factor RORγT and producing IL-17A, could, under strong TCR stimuli, express ST2 in the small intestine (22). The process by which T_H17 cells upregulate ST2 remains unclear. However, it is well-known that STAT3 signaling plays a major role in the development and cytokine expression of T_H17 T cells (73). Recently, it was show that STAT3, along with ERK, had the potential to upregulate the proximal promoter region of ST2 in both human and murine fibroblastic cells lines (74). Although the proximal region of ST2 results in the truncated soluble form of ST2 (sST2), an analog mechanism involving the distal promoter might be found in $ST2^+$ T_H17 cells.

T_H2 Cell Development and Function

Since the discovery of IL-33, progress has been made to identify its multifunctional roles. Initially, IL-33 was described for its role in promoting type 2 immunity in infectious and allergic diseases (75). Polymorphisms in the *il1rl1* or *il33* genes are found in

patients suffering from exacerbated type 2 immune responses, notably severe atopic dermatitis and asthma, illustrating the important role of these genes in the susceptibility to allergic diseases (36, 76). IL-33 administration in the airways of mice enhances T_H2-associated cytokine production in the lungs, increases mucus production and causes a severe type 2 airway hyper-reactivity that mimics the pathophysiology of asthma (37). These reports highlight the role of IL-33 in the differentiation, and function of T_H2 cells (77–79).

Naïve T cell express little to no ST2 on their surface. ST2 is expressed *in vitro* when T cells receive TCR activation in combination with cytokine polarization that drives T_H2 T cell differentiation (55, 80). Thus, unique to T cells, TCR engagement is, along with STAT5 and IL-33, a critical signal for naïve T cells to upregulate the ST2 receptor. Conversely, differentiated T_H2 cells maintain the ability, long after TCR stimuli, to respond to IL-33 and produce IL-5 and IL-13 (55). Thus, T cells must undergo a round of activation to upregulate the receptor but, once activated remain capable of responding to IL-33. Human $CD4^+$ T cells also express the ST2 receptor *in vitro* upon T_H2, but not T_H1, differentiation, although IL-4, a STAT6 inducer, was used in these assays (81).

In vitro, IL-33 enhances IL-5 and IL-13 production, but not IL-4, in T_H2 polarized cells (55, 80). This phenotype is unusual, as IL-4 expression was long thought to be the hallmark of differentiated T_H2 cells (82) and hinted that *in vitro* IL-33-responding T_H2 cells may have undergone further epigenetic modifications (83). In contrast, IL-33 administration leads to the accumulation of IL-4^+ T_H2 cells in the lungs and lymph nodes of treated mice (37, 84). This discrepancy could be due to the effect of IL-33 on APCs, directly involved in T cell differentiation. In fact, IL-33 modulates the differentiation and maturation of DCs as they polarize naïve T cells into $ST2^+$ T_H2 cells (18). Similarly, IL-33, in conjunction with TGFβ, can facilitate IL-9 production in both mouse and human T cells (85, 86). Thus, the effect of IL-33 signaling on the cytokine production of T cells is highly dependent on the cytokine microenvironment (**Figure 2**).

IL-33 plays an important role in the pathology of asthma and the T_H2 cell differentiation *in vivo*. Immunization of mice to a single dose of ovalbumin (OVA) (5) together with IL-33 induces long-lasting memory T_H2 cells that leads to severe asthma-like pathology in the lungs. These IL-33-induced OVA-specific T_H2 cells produce particularly high levels of IL-5 and IL-13 upon re-stimulation with OVA, a phenomenon not seen in memory T_H2 cells of mice were immunized with OVA alone (84). Furthermore, when mice are exposed to airway antigens, $ST2^{-/-}$ T_H2 cells produce less IL-13, while $ST2^{-/-}$ ILC2 functions remain unaffected (87). Concomitantly, memory IL-5–secreting $ST2^+$ T_H2 cells have been isolated from patients suffering from eosinophilic chronic rhinosinusitis, a common allergic condition (46).

However, once $ST2^+$ T_H2 cells are developed, unexpected outcomes have been observed in response to IL-33. When *in vitro* polarized OVA-specific, ST2-deficient (OVA-Tg/$ST2^{-/-}$) or WT (OVA-Tg) TH2 cells are donor $ST2^{-/-}$ T_H2, not WT T_H2, cells expressed higher levels of IL-5 production concomitant with a more severe cellular infiltrations in the lungs (88). Similarly, when $ST2^{-/-}$ mice were exposed to extracts containing ragweed, dust mite and *Aspergillus fumigatus*, a more severe form of airway hyper-reactivity was observed compared to WT mice (87); an observation that correlated with a reduction of T_{REG} cells in the lungs of $ST2^{-/-}$ mice. Thus, although IL-33 enhances T_H2 responses, it is not essential for the development of airway hyper-reactivity but seems to play a prominent role in T_{REG} cell homeostasis. Overall, these data are in contrast to the known *in vitro* effects of IL-33 and illustrates the multifaceted roles of IL-33 in both enhancing or dampening T_H2 cell responses in a context-dependent manner.

T_H1 and T_H17 Cell Differentiation and Function

Recent experimental evidence revealed that IL-33 plays a role in the development and maintenance of type 1 immune responses. When studying the T cell response to a systemic LCMV infection, Baumann et al. identified a prominent subset of T-bet^+ $ST2^+$ T cells within the antigen-specific memory T cell pool (21). In contrast to $ST2^+$ T_H2 cells, T_H1 cells expressed ST2 transiently. Interestingly, after injecting LCMV-TCR specific T cells in infected mice, WT, but not Tbet- or STAT4- deficient T cells were able to express ST2, demonstrating that during strong type 1 immunity, T_H1 cells can upregulate ST2. Furthermore, $ST2^{-/-}$ T cells failed to expand and produce high levels of IFNγ, TNFα or IL-2 after transfer in LCMV-infected mice (21), suggesting that T_H1 cells require ST2 in order to optimally expand and function during the course of LCMV. Interestingly, a similar observation was revealed upon influenza infection, where the rapid release of IL-33 correlated with enhanced IFNγ and TNFα production (89). In fact, IL-33 was shown to potentiate *in vitro* the action of IL-12, a STAT4 inducer, in T_H1 cells, resulting in increased production of IFNγ (72). Similarly, $CD8^+$ T cells were also shown to transiently express the ST2 receptor. IL-33 enhanced the clonal expansion of activated $CD8^+$ T cells and was necessary for the effective control of LCMV infection (90). These observations demonstrate a role of IL-33 to enhance IFNγ through the action of IL-12 without affecting T_H1 polarization (**Figure 2**).

Finally, a recent account suggests a possible role of IL-33 in T_H17 cell differentiation (22). These cells express the transcription factor RORγT and release IL-17A and IL-17F. Upon anti-CD3 treatment *in vivo,* ST2 surface-expression was observed by IL-17-producing T cells in the gut (22). However, IL-33 inhibited the proliferation and pro-inflammatory cytokine production of T_H17 cells both *in vivo* and *in vitro*. Here, contrarily to T_H2 and T_H1 cells, IL-33 signaling controlled the exacerbated inflammatory response by T_H17 cells, although further work is required to understand the full extent of the role of IL-33 on these cells. In summary, many T cell subsets can respond to IL-33, making the modulation of T cells responses by IL-33 complex and context-dependent.

IL-33-Mediated Regulation of T Cells in Infection

A way to dissect the distinct roles of IL-33 on T cells is to study its effect in distinct infectious diseases. Little is known about the role of IL-33 in human diseases, as there is currently a lack of tools to identify and follow human ST2$^+$ T cells, yet important progress has been made in the field through rodent models of infectious disease. IL-33 most likely plays a key role in human disease, as evidenced by increased levels of the cytokine or its decoy receptor sST2 during both viral (91–93) and bacterial (94) infections. In rodent models, IL-33 was shown to play both protective and deleterious roles during the course of infection(95).

This is seen in models of viral infections, where IL-33 plays ambiguous roles on the T cell response. In certain cases, viral virulence is linked to enhanced IL-33 release, as observed upon infection with respiratory syncytial virus (RSV) in both human and mice (96). When mice are infected with RSV, IL-33 is rapidly released in the early phases of viral infection in the lung (97). Antibody-mediated blockade of ST2 leads to a decrease in IL-13 production and eosinophil recruitment but does not affect viral growth or clearance of RSV by type 1 immune responses (97). Concomitantly, anti-IL-33 therapy was shown to mitigate the establishment of the deleterious type 2 memory response during a Rhinovirus infection that promotes airway hyper-reactivity (98). On the other hand, IL-33 was also shown to contribute to the clearance of LCMV and Coxsackievirus-B5 systemic viral infections through enhanced T_H1 and CD8$^+$ T cell responses (90, 99).

IL-33 can also play an important role in the control and clearance of parasites. In a model of intestinal infection with *Nippostrongylus brasiliensis*, a mouse-pathogenic hookworm, clearance of the parasite and the establishment of a T cell memory response required IL-33 (100). Interestingly, IL-4$^+$ T_H2 cells–as well as high levels of IgE, basophils and mast cells responses—were readily detected in infected mice lacking ST2 (ST2$^{-/-}$) yet insufficient IL-13$^+$ T_H2 cells and ILC2s lead to a failure to clear the parasite (100). Similarly, mice infected with *Trichuris muris or Strongyloides venezuelensis* require IL-33 signaling for the effective control of the parasite (101, 102). On the other hand, in a model of visceral *Leishmania donovani* infection, IL-33 was shown to be deleterious to the host, as it inhibited the T_H1 response necessary for the clearance of this parasite (103). This was attributed to a skewed ST2$^+$ T_H2 immune response, as these cells accumulated in the chronic lesion of *Leishmania* (104). Similarly, lack of ST2 in mice infected with the protozoa *Toxoplasma gondii*, lead to a more severe form of encephalitis, characterized by increased levels of TNFα and IFNγ (105). Finally, lack of ST2 signaling leads to a better control of the fungus *Cryptococcus neoformans*, characterized by a significant reduction in IL-5 and IL-13 production by T_H2 cells, but no difference in the level of expression of IFNγ and IL-17A (106). Importantly, the effect of IL-33 on the skewing of T cell responses may play a major role in predisposing to virus-induced asthma through the differentiation of pathogenic T_H2 cells over anti-viral T cells (98). These experiments provide further evidence that IL-33 influences the function of T cells in disease and this effect is highly dependent on the target tissue of infection and type of pathogen. Furthermore, IL-33 modulates important functions in other compartments of the immune system, notably the innate immune response, which was not addressed here but contributes to the overall response against pathogens (107).

REGULATORY T CELLS

T_{REG} cells are an important immunosuppressive subset of CD4$^+$ T cells characterized by the expression of the transcription factor Foxp3, the key master regulator that enforces the transcriptional program global phenotype and function of T_{REG} cells (108). However, T_{REG} cells can also undergo distinct epigenetic modifications and co-express transcription factors in order to acquire effector functions enabling them to migrate, survive and suppress in inflammatory sites, particularly at mucosal surfaces (109, 110). This particular ability enables them to adapt to specific environmental conditions. IL-33 was recently identified as one of the signals involved in the maintenance of Foxp3$^+$ T_{REG} cell homeostasis at mucosal sites. At the steady-state, ST2$^+$ T_{REG} cells represent the majority of ST2-expressing CD4$^+$ T cells and are notably found in the gut (23) and lungs (111).

Phenotypic Characteristics of ST2$^+$ T_{REG}

CD4$^+$ T_{REG} cells, including those found at mucosal surfaces, originate from the thymus (thymic-derived tT_{REG}) or develop *de novo* from polarizing signals in the periphery (peripherally-induced pT_{REG}). Both tT_{REG} and pT_{REG} cells effectively suppress innate and adaptive responses including a variety of effector T cell functions (112). Interestingly, tT_{REG} and pT_{REG} were shown to play non-redundant functions in the suppression of the adaptive immune response, as both of these subsets are required to maintain immune homeostasis in the mucosa. Although surface markers capable of distinguishing them remain poorly defined, tT_{REG} generally have a fully demethylated T_{REG}-specific demethylated region (TSDR), located in the *foxp3* locus, compared to pT_{REG} cells (113, 114). Helios, a transcription factor that is prominently expressed in tTreg cells, is frequently regarded as a marker of T_{REG} cells of thymic origin (115) but is also contested (116). Both Helios$^+$ and Helios$^-$ T_{REG} cells isolated from the lamina propria of the gut express ST2 (23), while the vast majority of Helios$^+$ T_{REG} cells express ST2 in secondary lymphoid organs and in the lungs (17), all-the-while expressing high levels of other proposed markers of tT_{REG}, such as Neuropilin 1 and TIGIT (*unpublished observations*). Interestingly, the expression of Helios was recently associated with distinct T_{REG} cell functions in the periphery (117) as well as the stability of *foxp3* expression on T_{REG} cells (118). Similarly, IL-33 signaling on T_{REG} cells was shown to play an important role in enhancing the stability of Foxp3 in T_{REG} cells and is notably necessary for these cells to prevent T cell-mediated colitis (23). However, the molecular relationship between IL-33 signaling and Helios expression in T_{REG} cells remains to be understood.

On the other hand, ST2$^+$ T_{REG} cells also express the transcription factor GATA3. Upon IL-33 stimulation, GATA3 is rapidly phosphorylated in T_{REG} cells (23), in turn enhancing the expression of its own receptor. Expression of GATA3, like ST2, was identified in T_{REG} cells in the gut (119) where it plays

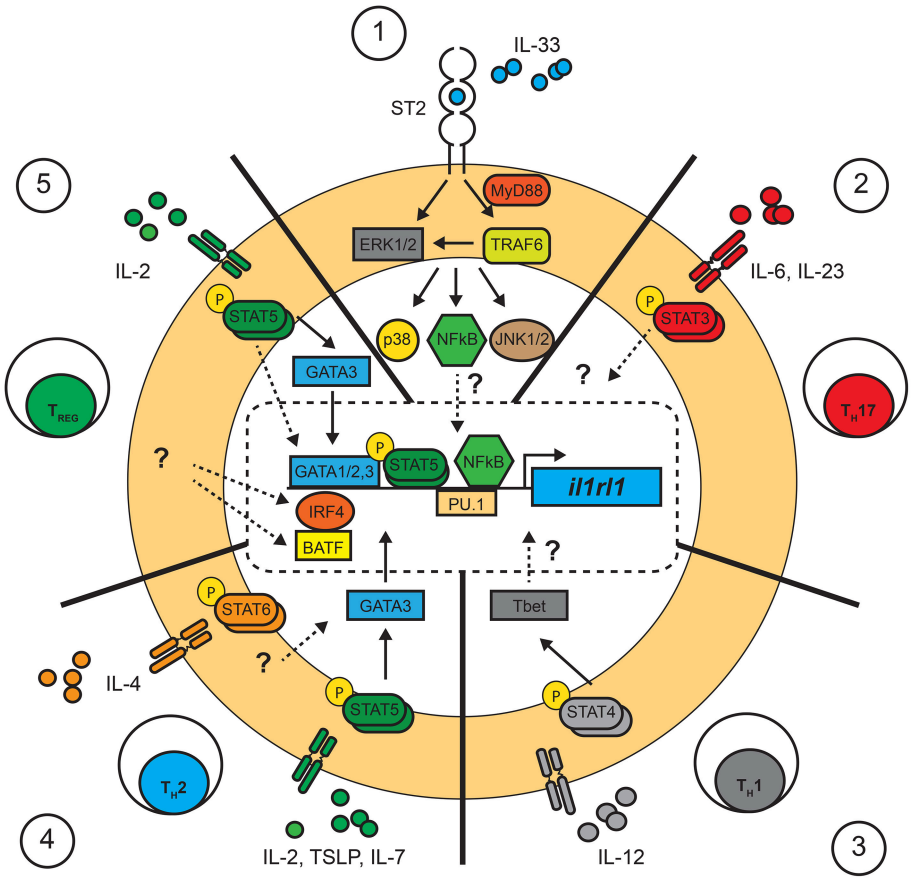


FIGURE 1 | Pathways involved in the control of *il1rl1* (ST2) transcription in T cells. Summary of the transcription factors confirmed or suggested to interact with the promoter region of *il1rl1* (ST2) in T cells. Confirmed (full lines), suggested (dotted lines) and unknown (?) pathways involved in the control of *il1rl1* in T cells. **(1)** IL-33 is required for the maintained expression of ST2 in T cells in T_H2, T_{REG}, and T_H1 through unknown mechanisms. This process has been suggested to require the involvement of NF-κB translocation and its binding to a consensus sequence in the promoter region of *il1rl1*. **(2)** T_H17 cells: The molecular pathways involved in the expression of ST2 in T_H17 T cells remains largely unknown, although the transcription factor STAT3 was shown to bind the promoter region of *il1rl1* in fibroblast cell lines. **(3)** T_H1 cells: Expression of ST2 in T_H1 cells was shown to be dependent on a STAT4 signal leading to T-bet expression, although the molecular interaction with *il1rl1* remains unknown. **(4)** T_H2 cells: It has been suggested that expression of ST2 requires STAT5 signals through the upregulation of GATA3 in conjunction with IL-33 stimulation. Although a STAT6 signal is not necessary, little is known about its role in the maintenance of ST2. **(5)** T_{REG} cells: Expression of ST2 on T_{REG} cells follows a similar pathway as in T_H2 cells, requiring a STAT5 signal and IL-33 activation for the upregulation of GATA3 and ST2. The transcription factors IRF4 and BAFT were also shown to promote expression of ST2 by T_{REG} cells although little is known about the upstream signals involved.

a central role in (1) the maintenance of immune homeostasis (120), (2) in the stability of *foxp3* and (3) is critical for T_{REG} cells to prevent T cell mediated colitis (119). Thus, ST2 and GATA3 follow a similar pattern of expression and play similar functional roles in T_{REG} cells, indicating a strong inter-relationship between the two in orchestrating T_{REG} adaptation in the mucosa.

Finally, the STAT5 signaling pathway can be triggered by IL-2, IL-7, IL-15, or TSLP. T_{REG} cells constitutively express high levels of the IL-2 receptor α chain (CD25), as they are highly dependent on exogeneous IL-2 for survival, function and proliferation (121, 122). In contrast, T_{REG} cells express little IL-7R outside of the thymus in human and mice (123), yet IL-7 could play a role on the expansion of T_{REG} cells at mucosal sites (124). Although there is little information on the role of IL-15 on T_{REG} cells, a recent account reveals that gut-resident T cells depend on IL-15 to enhance Foxp3 over RORγT expression and block a Th17-driven inflammatory bowel disease (125). Finally, T_{REG} cells in the lungs were recently shown to express the TSLP receptor (126). So far, however, only IL-2, in the presence of IL-33, was shown to facilitate the expression of the ST2 receptor on T_{REG} cells (23). Thus, further investigation into the role of the cytokines involved in STAT5 signaling is required.

Role of IL-33 on T_{REG} Function

IL-33 can support many aspects of T_{REG} cell functions. IL-33 facilitates the selective expansion of T_{REG} cells *in vitro* in a MyD88-dependent manner (127, 128). Moreover, $ST2^+$ T_{REG} cells show increased suppressive capacity *in vitro* and *in vivo* in the presence of IL-33 (127, 129, 130), although this was recently contested (131). However, the techniques used by these groups

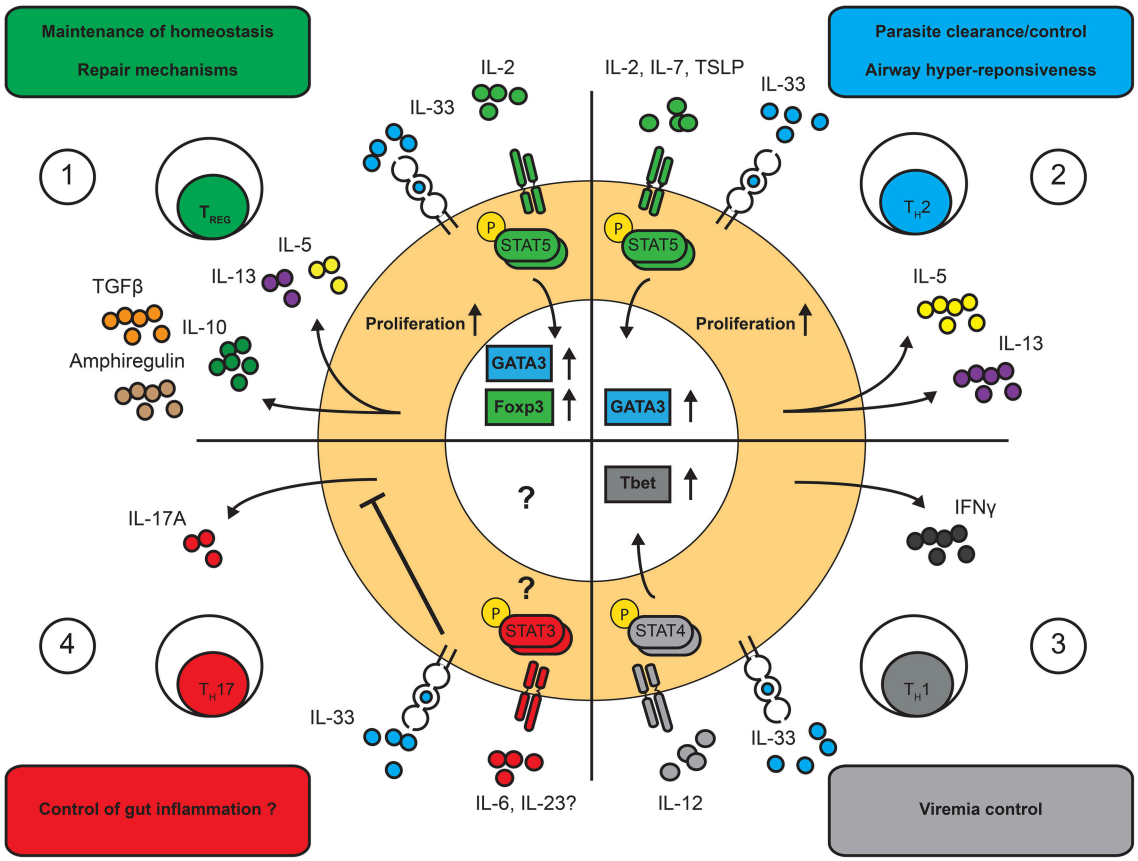


FIGURE 2 | Effects of IL-33 on T cell functions. IL-33 is a multi-faceted cytokine regulating distinct T cell functions and in a highly context-dependent manner. Known functional outcomes of IL-33 on T cell driven immune responses. **(1)** T_{REG} cells: IL-33 increases proliferation of T_{REG} cells and facilitates the production of amphiregulin, IL-10 and TGFβ as well as low levels of IL-5 and IL-13 in a STAT5-dependent manner. **(2)** T_H2 cells: IL-33 enhances the proliferation and the expression of IL-5 and IL-13 in T_H2 cells in a STAT5-dependent manner. **(3)** T_H1 cells: IL-33 was shown to enhance IFNγ production in T_H1 cells in a STAT4-dependent manner. **(4)** T_H17 cells: IL-33 was shown to inhibit IL-17 production in T_H17 cells. The effects on other T cell functions remains to be assessed.

differed and this might provide insight into the modulation of the suppressive ability of $ST2^+$ T_{REG} cells.

Moreover, *in vivo*, the increased fitness and suppressive function of $ST2^+$ T_{REG} cells is also highlighted by the effect of IL-33 on the maintenance of *foxp3* expression in the gut and their ability to suppress T-cell mediated colitis (23). Concomitantly, $ST2^+$ T_{REG} cells readily expand in the mucosa during the course of distinct infectious diseases (111, 129), where they resist the expression of pro-inflammatory cytokines like IFNγ, even strong polarizing conditions (129). IL-33-responsive T_{REG} cells are also endowed with unique cytokine production potential. For example, $ST2^+$ T_{REG} cells were found to produce high levels of IL-10, TGFβ and amphiregulin, which favor a tolerogenic environment and the establishment of tissue repair mechanisms (111, 129) (**Figure 2**). On the other hand, $ST2^+$ T_{REG} cells can also express type 2 cytokines, like IL-5 and IL-13, when stimulated *in vitro* in the presence of IL-33 (129). Similarly, in mice exposed to airway allergens in combination with IL-33, WT, but not $ST2^{-/-}$, T_{REG} cells express high levels of IL-5 and IL-13 (131). Thus, there are reports of both highly suppressive and pro-inflammatory $ST2^+$ T_{REG} cells. To answer this disparity, it was proposed that IL-33 could facilitate the transition from suppressive to dysregulated T_{REG} cells in a dose-dependent manner, although more investigations are required (56). On the other hand, we know little about the potential effect of secondary signals on $ST2^+$ T_{REG} cells, as these cells could have acquired the ability to respond to other environmental cues.

$ST2^+$ T_{REG} in Disease

We do not know the full extent of the role of $ST2^+$ T_{REG} cells in infectious diseases. Nonetheless, $ST2^+$ T_{REG} cells were shown to (1) promote the establishment of memory T cells, (2) control the expansion of inflammatory T_H1 and T_H17 cells, and (3) promote epithelial cell repair (23, 111, 129). The role of IL-33 on T_{REG} cells has been studied in several infectious and non-infectious inflammatory models. In models that elicit prominent T_H1 or T_H17 responses, the role of IL-33 on T_{REG} cells was shown to be protective. For example, during Influenza infection, $ST2^+$ T_{REG} cells accumulate in the lung where they produce amphiregulin, a cytokine involved in tissue repair (111). Throughout infection, $ST2^+$ T_{REG} cells are refractory to inflammatory signals and resist the production of inflammatory cytokines. Moreover, in

a mouse model of T-cell induced colitis, ST2 expression by T_{REG} cells was shown to be critical to prevent the onset of disease in the gut (23). Moreover, $ST2^+$ T_{REG} cells are induced upon cytomegalovirus (CMV) infection in mice where they play a critical role in dampening liver damage (132). Finally, we recently observed that in chronic infection with *Cryptococcus neoformans*, which leads to a prominent T_H17 response, $ST2^+$ T_{REG} cells resist the up-regulation of RORγT and the production of IL-17 (133). However, this suppressive function of T_{REG} cells could have a negative impact, as it was shown that in helminth infections $ST2^+$ T_{REG} cells, but not $ST2^-$, suppress T_H2 cells and facilitate helminth fecundity (134). Similarly, a recent account revealed that the tumor-specific release of IL-33 can promote the accumulation of T_{REG} cells at the site where they contribute to tumor growth and immune evasion (135). Thus, the effect of IL-33 was suggested to be generally protective and promote immune regulation, notably through an enhanced suppressive ability of T_{REG} cells. However, this effect seems to be context-dependent, as recent evidence reveals that IL-33 can also fuel inflammatory responses (131, 136).

Role of IL-33 in Autoimmune Diseases

IL-33 was shown to play important roles in either driving or dampening dysregulated T cells responses in autoimmune diseases. Polymorphisms in the *Il33* gene are detected in patients with Alzheimer's disease (137) and Inflammatory Bowel disease (IBD) (138) suggesting that a complete or partial loss of function leads to exacerbated disease (139). In addition, increased levels of IL-33 are detected in patients with multiple sclerosis (MS) (140), systemic lupus erythematous (SLE) (141), type 1 diabetes (T1D) (142) and rheumatoid arthritis (RA) (143). At the steady-state, high levels of IL-33 are produced in the central nervous system (CNS), where it favors the release of IL-1β and IL-10 (144). Expectedly, IL-33 is a major component of the global inflammatory process within the CNS. In experimental autoimmune encephalitis (EAE), a mouse model for multiple sclerosis (MS), IL-33 plays a protective role by dampening the generation of inflammatory astrocytes and the expansion of effector T cells, while enhancing T_{REG} and T_H2 responses (145). IL-33 directly attenuates the production of IL-17 and IFNγ by pathogenic T_H17 or T_H1 cells (146). Moreover, adoptive transfer of MOG-specific T cells from $ST2^{-/-}$ but not $ST2^{+/+}$ mice fail to prevent EAE onset in BALB/c mice, a strain that is naturally resistant to the disease (147). On the other hand, administration of recombinant IL-33 (rIL-33) is shown to exacerbate EAE in C57BL/6 mice while anti-IL-33 therapy attenuates IL-17 and IFNγ production *in situ* (148). This strain-specific difference may to be due to a time or context-dependent effect of IL-33, as signaling during the onset of disease is most likely protective, while IL-33 activity in the later stages likely exacerbates T_H1 and T_H17 responses (149).

A similarly complex role of IL-33 is found in rheumatoid arthritis (RA). While IL-33 is produced at high levels in joints during both RA in human and in experimental arthritis in mice, anti-ST2 therapy significantly attenuates the progression of disease (150). However, while $ST2^{-/-}$ mice show reduced disease severity, $IL\text{-}33^{-/-}$ mice do not (151), although the reasons for this difference remain unknown. Similarly, the attenuating effect of IL-33 in the onset of disease was also shown in mouse models of uveitis (152) and T1D (153), although this observation is yet to be described in human disease.

Finally, IL-33 is closely associated to asthma, since it is increased in asthmatic patients (154) and was shown to potentiate airway hyper-reactivity (136). Notably, IL-33 was shown to directly impair T_{REG} cell function during antigen-driven type 2 airway hyper-reactivity (131) and enhance T_H2 differentiation through enhanced OX40 ligand interaction (155). Interestingly, this unexpected effect of IL-33 on T_{REG} cells differed from prior reports showing that IL-33 facilitated the suppressive function of T_{REG} cells. Future experiments will have to address these controversial observations.

Clinical Implications of IL-33 and Related Therapeutics

The immunomodulatory functions of IL-33 are being exploited to develop novel therapeutic avenues. The IL-33/ST2 axis is currently being targeted in pre-clinical studies [reviewed by Chen et colleagues (156)]. Among the latest strategies developed to inhibit IL-33 signaling in exacerbated type 2 immune responses are monoclonal antibodies against IL-33 that mimic the capturing effect of the sST2, as they bind the biologically active IL-33 and prevent its association with the membrane receptor (157). Similarly, the use of IL-33 traps, using the extracellular domains of ST2 and IL1RAcP, and blocking the membrane-bound ST2 are strategies currently being investigated with drugs in Phase I or II clinical trials (156).

Although the rationale for the use of inhibitory drugs is mostly based on the effects of IL-33 on innate immune responses, the use of drugs or biologics that enhance the IL-33 signaling pathway generally aims to target the adaptive immune response. One notable exception is the use of IL-33 blockade in tumor microenvironments. For example, it was recently shown that monoclonal antibody blockade of IL-33 in mice xenografted with human non-small-cell lung carcinoma (NSCLC) decreased the accumulation of T_{REG} cells and reduced macrophage M2 polarization, leading to the efficient inhibition of tumor growth (158). Similarly, neutralization of IL-33 inhibits the development of colorectal cancer in mice (135), as IL-33 promotes T_{REG} cell accumulation. On the other hand, an engineered IL-2-IL-33 fusion protein was developed to reduce renal injury in mice by targeting and enhancing T_{REG} cells homeostasis and proliferation *in situ* (159). Moreover, administration of IL-33 during the recovery phase of DSS-induced colitis in mice was shown to enhance recovery, by skewing the accumulation of T_H2 and T_{REG} cells over T_H1/T_H17 responses in the gut (160). Finally, it was recently suggested to use IL-33 to potentiate highly suppressive T_{REG} cells *ex vivo*, as adoptive transfer of these cells attenuates disease progression in a model of type 1 diabetes (130). However, care must be taken when considering the use of drugs that influence the IL-33 axis. For example, local IL-33 production in a mouse model of hepatocellular carcinoma was shown to enhance $CD4^+$ and $CD8^+$ anti-tumor activity (135), warranting a re-evaluation of the use of IL-33-neutralizing drugs in tumor models.

CONCLUSION

T cell function at mucosal sites is intimately linked to the processes of antigen presentation, polarizing cytokine signaling, migration to inflamed sites and the subsequent adaptation to local conditions. The role of "alarmins" in the modulation of mucosal T cell function is yet to be fully understood. Nonetheless, IL-33 was shown to play a major role in this process, illustrating the potential for other, less studied, alarmins to play similar roles.

We focused this review on the recent advances in IL-33 and T cells, but the complexity of the relationship between the adaptive and the innate immune response dictate further investigation into the effect of IL-33 on APC-T cell activation. Notably, little is known about the effect of IL-33 on the modulation of Notch signaling, a key component of T cell differentiation.

In T cells, IL-33 plays a major role in cytokine production, cell proliferation and immune regulation. However, many aspects of T cell responses to IL-33 remain to be elucidated. Notably, many reports have shown that GATA3 and STAT5 play clear roles in promoting the transcription of *il1rl1*, yet the role of T-bet, STAT4, and STAT3 remain obscure. Thus, we need more insights into the factors that influence IL-33 signaling, from the transcription of the receptor to its effect on the function of T cells. The discovery that IL-33 could directly impact distinct T cell subset differentiation and effector functions is of particular interest, as favoring a given type of response might alter the proper course of immune control and cause irreparable damage to the host. Further investigation into co-stimulatory factors might reveal how distinct alarmins influence each other. Multiple factors may compete, synergize or otherwise influence each other in the inflammatory "soup" to which T cells are exposed to. Finally, a thorough understanding of the kinetics of each alarmin might reveal the intrinsic mechanism by which competing alarmins orchestrate the balance between inflammation and tolerance.

In summary, IL-33 can play both inflammatory and regulatory roles during the evolution of an immune response. A deeper understanding of the effects of IL-33 will undoubtedly open the door toward the generation of unique therapeutic approaches. In fact, the use of a chimeric IL2/IL33 protein was shown to be protective in renal injury (159) and monoclonal anti-IL-33 antibodies where shown to excert promising effects in the control of atopic dermatitis. (161). However, when considering a therapeutic modulation of IL-33 signaling, care must be observed in light of the multifaceted roles of IL-33.

DATA AVAILABILITY

No datasets were generated in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

Financial support came from the Fonds de partenariat pour un Québec innovant et en santé (FPQIS) (CP), Canadian Institutes of Health Research (CIHR) operating grant (PJT-148821 to CP), and the Canada Research Chair program (CP). CP is supported by the Anna Maria Solinas Laroche Career Award in Immunology. Financial support also came from a CIHR Foundation grant (#354133 to JF) and a Leaders Opportunity Fund infrastructure grant from the Canadian Foundation of Innovation (CFI) (JF). JF is further supported by a Junior 1 and 2 Investigator Award by the Fonds de recherche santé (FRQS).

REFERENCES

1. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol.* (2007) 81:1–5. doi: 10.1189/jlb.0306164
2. Oppenheim JJ, Yang D. Alarmins: chemotactic activators of immune responses. *Curr Opin Immunol.* (2005) 17:359–65. doi: 10.1016/j.coi.2005.06.002
3. Matta BM, Reichenbach DK, Blazar BR, Turnquist HR. Alarmins and their receptors as modulators and indicators of alloimmune responses. *Am J Transplant.* (2017) 17:320–7. doi: 10.1111/ajt.13887
4. Kim B, Lee Y, Kim E, Kwak A, Ryoo S, Bae SH, et al. The interleukin-1alpha precursor is biologically active and is likely a key alarmin in the IL-1 family of cytokines. *Front Immunol.* (2013) 4:391. doi: 10.3389/fimmu.2013.00391
5. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family:back to the future. *Immunity.* (2013) 39:1003–18. doi: 10.1016/j.immuni.2013.11.010
6. Afonina IS, Muller C, Martin SJ, Beyaert R. Proteolytic processing of interleukin-1 family cytokines:variations on a common theme. *Immunity.* (2015) 42:991–1004. doi: 10.1016/j.immuni.2015.06.003
7. Scott IC, Majithiya JB, Sanden C, Thornton P, Sanders PN, Moore T, et al. Interleukin-33 is activated by allergen- and necrosis-associated proteolytic activities to regulate its alarmin activity during epithelial damage. *Sci Rep.* (2018) 8:3363. doi: 10.1038/s41598-018-21589-2
8. Lohning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, Gutierrez-Ramos JC, et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc Natl Acad Sci USA.* (1998) 95:6930–5. doi: 10.1073/pnas.95.12.6930
9. Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H, et al. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest.* (2008) 88:1245–53. doi: 10.1038/labinvest.2008.82
10. Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A, et al. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol.* (2008) 181:5981–9. doi: 10.4049/jimmunol.181.9.5981
11. Nabekura T, Girard JP, Lanier LL. IL-33 receptor ST2 amplifies the expansion of NK cells and enhances host defense during mouse cytomegalovirus infection. *J Immunol.* (2015) 194:5948–52. doi: 10.4049/jimmunol.1500424
12. Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int Immunol.* (2008) 20:1019–30. doi: 10.1093/intimm/dxn060
13. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol.* (2011) 12:1045–54. doi: 10.1038/ni.2131
14. Johnston LK, Bryce PJ. Understanding interleukin 33 and its roles in eosinophil development. *Front Med.* (2017) 4:51. doi: 10.3389/fmed.2017.00051

15. Valent P. Interleukin-33:a regulator of basophils. *Blood.* (2009) 113:1396–7. doi: 10.1182/blood-2008-11-189811
16. Stier MT, Zhang J, Goleniewska K, Cephus JY, Rusznak M, Wu L, et al. IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow. *J Exp Med.* (2018) 215:263–81. doi: 10.1084/jem.20170449
17. Matta BM, Lott JM, Mathews LR, Liu Q, Rosborough BR, Blazar BR, 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) 193:4010–20. doi: 10.4049/jimmunol.1400481
18. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol.* (2009) 123:1047–54. doi: 10.1016/j.jaci.2009.02.026
19. Sims JE, Smith DE. The IL-1 family:regulators of immunity. *Nat Rev Immunol.* (2010) 10:89–102. doi: 10.1038/nri2691
20. Peine M, Rausch S, Helmstetter C, Frohlich A, Hegazy AN, Kuhl AA, et al. Stable T-bet(+)GATA-3(+) Th1/Th2 hybrid cells arise *in vivo*, can develop directly from naive precursors, and limit immunopathologic inflammation. *PLoS Biol.* (2013) 11:e1001633. doi: 10.1371/journal.pbio.1001633
21. Baumann C, Bonilla WV, Frohlich A, Helmstetter C, Peine M, Hegazy AN, et al. T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell responses. *Proc Natl Acad Sci USA.* (2015) 112:4056–61. doi: 10.1073/pnas.1418549112
22. Pascual-Reguant A, Bayat Sarmadi J, Baumann C, Noster R, Cirera-Salinas D, Curato C, et al. TH17 cells express ST2 and are controlled by the alarmin IL-33 in the small intestine. *Mucosal Immunol.* (2017) 10:1431–42. doi: 10.1038/mi.2017.5
23. Schiering C, Krausgruber T, Chomka A, Frohlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature.* (2014) 513:564–8. doi: 10.1038/nature13577
24. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, 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) 188:3488–95. doi: 10.4049/jimmunol.1101977
25. Sundnes O, Pietka W, Loos T, Sponheim J, Rankin AL, Pflanz S, et al. Epidermal expression and regulation of interleukin-33 during homeostasis and Inflammation: Strong species differences. *J Invest Dermatol.* (2015) 135:1771–80. doi: 10.1038/jid.2015.85
26. Lefrancais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, Girard JP, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc Natl Acad Sci USA.* (2012) 109:1673–8. doi: 10.1073/pnas.1115884109
27. Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity.* (2009) 31:84–98. doi: 10.1016/j.immuni.2009.05.007
28. Kopach P, Lockatell V, Pickering EM, Haskell RE, Anderson RD, Hasday JD, et al. IFN-gamma directly controls IL-33 protein level through a STAT1- and LMP2-dependent mechanism. *J Biol Chem.* (2014) 289:11829–43. doi: 10.1074/jbc.M113.534396
29. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, et al. Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat Commun.* (2015) 6:8327. doi: 10.1038/ncomms9327
30. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. *Eur J Immunol.* (2015) 45:3034–44. doi: 10.1002/eji.201545501
31. Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS Lett.* (1989) 258:301–4. doi: 10.1016/0014-5793(89)81679-5
32. Travers J, Rochman M, Miracle CE, Habel JE, Brusilovsky M, Caldwell JM, et al. Chromatin regulates IL-33 release and extracellular cytokine activity. *Nat Commun.* (2018) 9:3244. doi: 10.1038/s41467-018-05485-x
33. Rehman SU, Mueller T, Januzzi JL Jr. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. *J Am Coll Cardiol.* (2008) 52:1458–65. doi: 10.1016/j.jacc.2008.07.042
34. Tominaga S, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, 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) 264:14–8. doi: 10.1006/bbrc.1999.1469
35. Iwahana H, Hayakawa M, Kuroiwa K, Tago K, Yanagisawa K, Noji S, et al. Molecular cloning of the chicken ST2 gene and a novel variant form of the ST2 gene product, ST2LV. *Biochim Biophys Acta.* (2004) 1681:1–14. doi: 10.1016/j.bbaexp.2004.08.013
36. Shimizu M, Matsuda A, Yanagisawa K, Hirota T, Akahoshi M, Inomata N, et al. Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. *Hum Mol Genet.* (2005) 14:2919–27. doi: 10.1093/hmg/ddi323
37. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
38. Funakoshi-Tago M, Tago K, Hayakawa M, Tominaga S, Ohshio T, Sonoda Y, et al. TRAF6 is a critical signal transducer in IL-33 signaling pathway. *Cell Signal.* (2008) 20:1679–86. doi: 10.1016/j.cellsig.2008.05.013
39. Pinto SM, Nirujogi RS, Rojas PL, Patil AH, Manda SS, Subbannayya Y, et al. Quantitative phosphoproteomic analysis of IL-33-mediated signaling. *Proteomics.* (2015) 15:532–44. doi: 10.1002/pmic.201400303
40. Pinto SM, Subbannayya Y, Rex DAB, Raju R, Chatterjee O, Advani J, et al. A network map of IL-33 signaling pathway. *J Cell Commun Signal.* (2018) 12:615–24. doi: 10.1007/s12079-018-0464-4
41. Xie JJ, Liang JQ, Diao LH, Altman A, Li Y. TNFR-associated factor 6 regulates TCR signaling via interaction with and modification of LAT adapter. *J Immunol.* (2013) 190:4027–36. doi: 10.4049/jimmunol.1202742
42. King CG, Kobayashi T, Cejas PJ, Kim T, Yoon K, Kim GK, et al. TRAF6 is a T cell-intrinsic negative regulator required for the maintenance of immune homeostasis. *Nat Med.* (2006) 12:1088–92. doi: 10.1038/nm1449
43. Chiffoleau E, Kobayashi T, Walsh MC, King CG, Walsh PT, Hancock WW, et al. TNF receptor-associated factor 6 deficiency during hemopoiesis induces Th2-polarized inflammatory disease. *J Immunol.* (2003) 171:5751–9. doi: 10.4049/jimmunol.171.11.5751
44. Muto G, Kotani H, Kondo T, Morita R, Tsuruta S, Kobayashi T, et al. TRAF6 is essential for maintenance of regulatory T cells that suppress Th2 type autoimmunity. *PLoS ONE.* (2013) 8:e74639. doi: 10.1371/journal.pone.0074639
45. Dong C, Yang DD, Tournier C, Whitmarsh AJ, Xu J, Davis RJ, et al. JNK is required for effector T-cell function but not for T-cell activation. *Nature.* (2000) 405:91–4. doi: 10.1038/35011091
46. Endo Y, Hirahara K, Iinuma T, Shinoda K, Tumes DJ, Asou HK, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity.* (2015) 42:294–308. doi: 10.1016/j.immuni.2015.01.016
47. Singh K, Deshpande P, Pryshchep S, Colmegna I, Liarski V, Weyand CM, et al. ERK-dependent T cell receptor threshold calibration in rheumatoid arthritis. *J Immunol.* (2009) 183:8258–67. doi: 10.4049/jimmunol.0901784
48. Goplen N, Karim Z, Guo L, Zhuang Y, Huang H, Gorska MM, et al. ERK1 is important for Th2 differentiation and development of experimental asthma. *FASEB J.* (2012) 26:1934–45. doi: 10.1096/fj.11-196477
49. Chang CF, D'Souza WN, Ch'en IL, Pages G, Pouyssegur J, Hedrick SM. Polar opposites:Erk direction of CD4 T cell subsets. *J Immunol.* (2012) 189:721–31. doi: 10.4049/jimmunol.1103015
50. Liu H, Yao S, Dann SM, Qin H, Elson CO, Cong Y. ERK differentially regulates Th17- and Treg-cell development and contributes to the pathogenesis of colitis. *Eur J Immunol.* (2013) 43:1716–26. doi: 10.1002/eji.201242889
51. Jirmanova L, Giardino Torchia ML, Sarma ND, Mittelstadt PR, Ashwell JD. Lack of the T cell-specific alternative p38 activation pathway reduces autoimmunity and inflammation. *Blood.* (2011) 118:3280–9. doi: 10.1182/blood-2011-01-333039
52. Hayakawa M, Hayakawa H, Petrova T, Ritprajak P, Sutavani RV, Jimenez-Andrade GY, et al. Loss of functionally redundant p38 isoforms in T cells enhances regulatory T cell induction. *J Biol Chem.* (2017) 292:1762–72. doi: 10.1074/jbc.M116.764548
53. Kumar S, Tzimas MN, Griswold DE, Young PR. Expression of ST2, an interleukin-1 receptor homologue, is induced by

proinflammatory stimuli. *Biochem Biophys Res Commun.* (1997) 235:474–8. doi: 10.1006/bbrc.1997.6810
54. Meisel C, Bonhagen K, Lohning M, Coyle AJ, Gutierrez-Ramos JC, Radbruch A, et al. Regulation and function of T1/ST2 expression on CD4+ T cells: induction of type 2 cytokine production by T1/ST2 cross-linking. *J Immunol.* (2001) 166:3143–50. doi: 10.4049/jimmunol.166.5.3143
55. Guo L, Wei G, Zhu J, Liao W, Leonard WJ, Zhao K, et al. IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc Natl Acad Sci USA.* (2009) 106:13463–8. doi: 10.1073/pnas.0906988106
56. Peine M, Marek RM, Lohning M. IL-33 in T cell differentiation, function, and immune homeostasis. *Trends Immunol.* (2016) 37:321–33. doi: 10.1016/j.it.2016.03.007
57. Wei G, Abraham BJ, Yagi R, Jothi R, Cui K, Sharma S, et al. Genome-wide analyses of transcription factor GATA3-mediated gene regulation in distinct T cell types. *Immunity.* (2011) 35:299–311. doi: 10.1016/j.immuni.2011.08.007
58. Weinberg EO, Shimpo M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation.* (2002) 106:2961–6. doi: 10.1161/01.CIR.0000038705.69871.D9
59. Scheinman EJ, Avni O. Transcriptional regulation of GATA3 in T helper cells by the integrated activities of transcription factors downstream of the interleukin-4 receptor and T cell receptor. *J Biol Chem.* (2009) 284:3037–48. doi: 10.1074/jbc.M807302200
60. Lee DU, Rao A. Molecular analysis of a locus control region in the T helper 2 cytokine gene cluster: a target for STAT6 but not GATA3. *Proc Natl Acad Sci USA.* (2004) 101:16010–5. doi: 10.1073/pnas.0407031101
61. Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. *Immunity.* (2003) 19:739–48. doi: 10.1016/S1074-7613(03)00292-9
62. Gachter T, Werenskiold AK, Klemenz R. Transcription of the interleukin-1 receptor-related T1 gene is initiated at different promoters in mast cells and fibroblasts. *J Biol Chem.* (1996) 271:124–9. doi: 10.1074/jbc.271.1.124
63. Iwahana H, Yanagisawa K, Ito-Kosaka A, Kuroiwa K, Tago K, Komatsu N, et al. Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. *Eur J Biochem.* (1999) 264:397–406. doi: 10.1046/j.1432-1327.1999.00615.x
64. Baba Y, Maeda K, Yashiro T, Inage E, Kasakura K, Suzuki R, et al. GATA2 is a critical transactivator for the human IL1RL1/ST2 promoter in mast cells/basophils: opposing roles for GATA2 and GATA1 in human IL1RL1/ST2 gene expression. *J Biol Chem.* (2012) 287:32689–96. doi: 10.1074/jbc.M112.374876
65. Baba Y, Maeda K, Yashiro T, Inage E, Niyonsaba F, Hara M, et al. Involvement of PU.1 in mast cell/basophil-specific function of the human IL1RL1/ST2 promoter. *Allergol Int.* (2012) 61:461–7. doi: 10.2332/allergolint.12-OA-0424
66. Sundrud MS, Vancompernolle SE, Eger KA, Bruno TC, Subramaniam A, Mummidi S, et al. Transcription factor GATA-1 potently represses the expression of the HIV-1 coreceptor CCR5 in human T cells and dendritic cells. *Blood.* (2005) 106:3440–8. doi: 10.1182/blood-2005-03-0857
67. Spain LM, Guerriero A, Kunjibettu S, Scott EW. T cell development in PU.1-deficient mice. *J Immunol.* (1999) 163:2681–7.
68. Chang HC, Zhang S, Thieu VT, Slee RB, Bruns HA, Laribee RN, et al. PU.1 expression delineates heterogeneity in primary Th2 cells. *Immunity.* (2005) 22:693–703. doi: 10.1016/j.immuni.2005.03.016
69. Yashiro T, Kubo M, Ogawa H, Okumura K, Nishiyama C. PU.1 Suppresses Th2 cytokine expression via silencing of GATA3 transcription in dendritic cells. *PLoS ONE.* (2015) 10:e0137699. doi: 10.1371/journal.pone.0137699
70. Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, Kawamoto S, et al. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. *Nat Immunol.* (2015) 16:276–85. doi: 10.1038/ni.3085
71. Hayatsu N, Miyao T, Tachibana M, Murakami R, Kimura A, Kato T, et al. Analyses of a mutant Foxp3 allele reveal BATF as a critical transcription factor in the differentiation and accumulation of tissue regulatory T cells. *Immunity.* (2017) 47:268–283e9. doi: 10.1016/j.immuni.2017.07.008
72. Komai-Koma M, Wang E, Kurowska-Stolarska M, Li D, McSharry C, Xu D. Interleukin-33 promoting Th1 lymphocyte differentiation dependents on IL-12. *Immunobiology.* (2016) 221:412–7. doi: 10.1016/j.imbio.2015.11.013
73. Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem.* (2007) 282:9358–63. doi: 10.1074/jbc.C600321200
74. Tago K, Ohta S, Funakoshi-Tago M, Aoki-Ohmura C, Matsugi J, Tominaga SI, et al. STAT3 and ERK pathways are involved in cell growth stimulation of the ST2/IL1RL1 promoter. *FEBS Open Bio.* (2017) 7:293–302. doi: 10.1002/2211-5463.12192
75. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
76. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med.* (2010) 363:1211–21. doi: 10.1056/NEJMoa0906312
77. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. *Respir Res.* (2001) 2:71–9. doi: 10.1186/rr41
78. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med.* (1992) 326:298–304. doi: 10.1056/NEJM199201303260504
79. Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4:its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. *Respir Res.* (2001) 2:66–70. doi: 10.1186/rr40
80. Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol.* (2008) 181:4780–90. doi: 10.4049/jimmunol.181.7.4780
81. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol.* (2007) 37:2779–86. doi: 10.1002/eji.200737547
82. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol.* (2010) 10:225–35. doi: 10.1038/nri2735
83. Zhu J. Transcriptional regulation of Th2 cell differentiation. *Immunol Cell Biol.* (2010) 88:244–9. doi: 10.1038/icb.2009.114
84. Kobayashi T, Iijima K, Checkel JL, Kita H. IL-1 family cytokines drive Th2 and Th17 cells to innocuous airborne antigens. *Am J Respir Cell Mol Biol.* (2013) 49:989–98. doi: 10.1165/rcmb.2012-0444OC
85. Blom L, Poulsen BC, Jensen BM, Hansen A, Poulsen LK. IL-33 induces IL-9 production in human CD4+ T cells and basophils. *PLoS ONE.* (2011) 6:e21695. doi: 10.1371/journal.pone.0021695
86. Ramadan A, Griesenauer B, Adom D, Kapur R, Hanenberg H, Liu C, et al. Specifically differentiated T cell subset promotes tumor immunity over fatal immunity. *J Exp Med.* (2017) 214:3577–96. doi: 10.1084/jem.20170041
87. Verma M, Liu S, Michalec L, Sripada A, Gorska MM, Alam R. Experimental asthma persists in IL-33 receptor knockout mice because of the emergence of thymic stromal lymphopoietin-driven IL-9(+) and IL-13(+) type 2 innate lymphoid cell subpopulations. *J Allergy Clin Immunol.* (2018) 142:793–803e8. doi: 10.1016/j.jaci.2017.10.020
88. Mangan NE, Dasvarma A, McKenzie AN, Fallon PG. T1/ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. *Eur J Immunol.* (2007) 37:1302–12. doi: 10.1002/eji.200636520
89. Le Goffic R, Arshad MI, Rauch M, L'Helgoualc'h A, Delmas B, Piquet-Pellorce C, et al. Infection with influenza virus induces IL-33 in murine lungs. *Am J Respir Cell Mol Biol.* (2011) 45:1125–32. doi: 10.1165/rcmb.2010-0516OC
90. Bonilla WV, Frohlich A, Senn K, Kallert S, Fernandez M, Johnson S, et al. The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses. *Science.* (2012) 335:984–9. doi: 10.1126/science.1215418
91. Becerra A, Warke RV, de Bosch N, Rothman AL, Bosch I. Elevated levels of soluble ST2 protein in dengue virus infected patients. *Cytokine.* (2008) 41:114–20. doi: 10.1016/j.cyto.2007.11.001
92. Wu X, Li Y, Song CB, Chen YL, Fu YJ, Jiang YJ, et al. Increased expression of sST2 in early HIV infected patients attenuated the IL-33 induced T cell responses. *Front Immunol.* (2018) 9:2850. doi: 10.3389/fimmu.2018.02850
93. Wang J, Cai Y, Ji H, Feng J, Ayana DA, Niu J, et al. Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis B. *J Interferon Cytokine Res.* (2012) 32:248–53. doi: 10.1089/jir.2011.0109

94. Lv R, Zhao J, Lei M, Xiao D, Yu Y, Xie J. IL-33 Attenuates sepsis by inhibiting IL-17 receptor signaling through upregulation of SOCS3. *Cell Physiol Biochem.* (2017) 42:1961–72. doi: 10.1159/000479836
95. Rostan O, Arshad MI, Piquet-Pellorce C, Robert-Gangneux F, Gangneux JP, Samson M. Crucial and diverse role of the interleukin-33/ST2 axis in infectious diseases. *Infect Immun.* (2015) 83:1738–48. doi: 10.1128/IAI.02908-14
96. Becker Y. Respiratory syncytial virus (RSV) evades the human adaptive immune system by skewing the Th1/Th2 cytokine balance toward increased levels of Th2 cytokines and IgE, markers of allergy–a review. *Virus Genes.* (2006) 33:235–52. doi: 10.1007/s11262-006-0064-x
97. Zeng S, Wu J, Liu J, Qi F, Liu B. IL-33 receptor (ST2) signalling is important for regulation of Th2-mediated airway inflammation in a murine model of acute Respiratory syncytial virus infection. *Scand J Immunol.* (2015) 81:494–501. doi: 10.1111/sji.12284
98. Werder RB, Zhang V, Lynch JP, Snape N, Upham JW, Spann K, et al. Chronic IL-33 expression predisposes to virus-induced asthma exacerbations by increasing type 2 inflammation and dampening antiviral immunity. *J Allergy Clin Immunol.* (2018) 141:1607–19e9. doi: 10.1016/j.jaci.2017.07.051
99. Sesti-Costa R, Silva GK, Proenca-Modena JL, Carlos D, Silva ML, Alves-Filho JC, et al. The IL-33/ST2 pathway controls coxsackievirus B5-induced experimental pancreatitis. *J Immunol.* (2013) 191:283–92. doi: 10.4049/jimmunol.1202806
100. Hung LY, Lewkowich IP, Dawson LA, Downey J, Yang Y, Smith DE, et al. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proc Natl Acad Sci USA.* (2013) 110:282–7. doi: 10.1073/pnas.1206587110
101. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol.* (2008) 180:2443–9. doi: 10.4049/jimmunol.180.4.2443
102. Yasuda K, Muto T, Kawagoe T, Matsumoto M, Sasaki Y, Matsushita 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 USA.* (2012) 109:3451–6. doi: 10.1073/pnas.1201042109
103. Rostan O, Gangneux JP, Piquet-Pellorce C, Manuel C, McKenzie AN, Guiguen C, et al. The IL-33/ST2 axis is associated with human visceral leishmaniasis and suppresses Th1 responses in the livers of BALB/c mice infected with Leishmania donovani. *MBio.* (2013) 4:e00383–13. doi: 10.1128/mBio.00383-13
104. Kropf P, Bickle Q, Herath S, Klemenz R, Muller I. Organ-specific distribution of CD4+ T1/ST2+ Th2 cells in Leishmania major infection. *Eur J Immunol.* (2002) 32:2450–2459. doi: 10.1002/1521-4141(200209)32:9<2450::AID-IMMU2450>3.0.CO;2-O
105. Jones LA, Roberts F, Nickdel MB, Brombacher F, McKenzie AN, Henriquez FL, et al. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with Toxoplasma gondii. *Eur J Immunol.* (2010) 40:426–36. doi: 10.1002/eji.200939705
106. Flaczyk A, Duerr CU, Shourian M, Lafferty EI, Fritz JH, Qureshi ST. IL-33 signaling regulates innate and adaptive immunity to Cryptococcus neoformans. *J Immunol.* (2013) 191:2503–13. doi: 10.4049/jimmunol.1300426
107. Cayrol C, Girard JP. IL-33:an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol.* (2014) 31:31–7. doi: 10.1016/j.coi.2014.09.004
108. Bin Dhuban K, Kornete M, Mason SE, Piccirillo CA. Functional dynamics of Foxp3(+) regulatory T cells in mice and humans. *Immunol Rev.* (2014) 259:140–58. doi: 10.1111/imr.12168
109. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol.* (2012) 30:531–64. doi: 10.1146/annurev.immunol.25.022106.141623
110. Rothstein DM, Camirand G. New insights into the mechanisms of Treg function. *Curr Opin Organ Transplant.* (2015) 20:376–84. doi: 10.1097/MOT.0000000000000212
111. Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, Yuan S, et al. A distinct function of regulatory T cells in tissue protection. *Cell.* (2015) 162:1078–89. doi: 10.1016/j.cell.2015.08.021
112. Schmitt EG, Williams CB. Generation and function of induced regulatory T cells. *Front Immunol.* (2013) 4:152. doi: 10.3389/fimmu.2013.00152
113. Toker A, Engelbert D, Garg G, Polansky JK, Floess S, Miyao T, et al. Active demethylation of the Foxp3 locus leads to the generation of stable regulatory T cells within the thymus. *J Immunol.* (2013) 190:3180–8. doi: 10.4049/jimmunol.1203473
114. Hori S. Lineage stability and phenotypic plasticity of Foxp3(+) regulatory T cells. *Immunol Rev.* (2014) 259:159–72. doi: 10.1111/imr.12175
115. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *J Immunol.* (2010) 184:3433–41. doi: 10.4049/jimmunol.0904028
116. Szurek E, Cebula A, Wojciech L, Pietrzak M, Rempala G, Kisielow P, et al. Differences in expression level of helios and neuropilin-1 do not distinguish thymus-derived from extrathymically-induced CD4+Foxp3+ regulatory T cells. *PLoS ONE.* (2015) 10:e0141161. doi: 10.1371/journal.pone.0141161
117. Sebastian M, Lopez-Ocasio M, Metidji A, Rieder SA, Shevach EM, Thornton AM. Helios controls a limited subset of regulatory T cell functions. *J Immunol.* (2016) 196:144–55. doi: 10.4049/jimmunol.1501704
118. Nakagawa H, Sido JM, Reyes EE, Kiers V, Cantor H, Kim HJ. Instability of Helios-deficient Tregs is associated with conversion to a T-effector phenotype and enhanced antitumor immunity. *Proc Natl Acad Sci USA.* (2016) 113:6248–53. doi: 10.1073/pnas.1604765113
119. Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, Ribeiro CH, et al. GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. *J Clin Invest.* (2011) 121:4503–15. doi: 10.1172/JCI57456
120. Wang Y, Su MA, Wan YY. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity.* (2011) 35:337–48. doi: 10.1016/j.immuni.2011.08.012
121. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in Treg cell function. *Nat Immunol.* (2016) 17:1322–33. doi: 10.1038/ni.3540
122. Mahmud SA, Manlove LS, Farrar MA. Interleukin-2 and STAT5 in regulatory T cell development and function. *JAKSTAT.* (2013) 2:e23154. doi: 10.4161/jkst.23154
123. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *J Exp Med.* (2006) 203:1701–11. doi: 10.1084/jem.20060772
124. Simonetta F, Gestermann N, Bloquet S, Bourgeois C. Interleukin-7 optimizes FOXP3+CD4+ regulatory T cells reactivity to interleukin-2 by modulating CD25 expression. *PLoS ONE.* (2014) 9:e113314. doi: 10.1371/journal.pone.0113314
125. Tosiek MJ, Fiette L, El Daker S, Eberl G, Freitas AA. IL-15-dependent balance between Foxp3 and RORgammat expression impacts inflammatory bowel disease. *Nat Commun.* (2016) 7:10888. doi: 10.1038/ncomms10888
126. Nguyen KD, Vanichsarn C, Nadeau KC. TSLP directly impairs pulmonary Treg function: association with aberrant tolerogenic immunity in asthmatic airway. *Allergy Asthma Clin Immunol.* (2010) 6:4. doi: 10.1186/1710-1492-6-4
127. Matta BM, Turnquist HR. Expansion of regulatory T cells *in vitro* and *in vivo* by IL-33. *Methods Mol Biol.* (2016) 1371:29–41. doi: 10.1007/978-1-4939-3139-2_3
128. Xu L, Li W, Wang X, Zhang L, Qi Q, Dong L, et al. The IL-33-ST2-MyD88 axis promotes regulatory T cell proliferation in the murine liver. *Eur J Immunol.* (2018) 48:1302–7. doi: 10.1002/eji.201747402
129. Siede J, Frohlich A, Datsi A, Hegazy AN, Varga DV, Holecska V, et al. IL-33 receptor-expressing regulatory T cells are highly activated, Th2 biased and suppress CD4 T cell proliferation through IL-10 and TGFbeta Release. *PLoS ONE.* (2016) 11:e0161507. doi: 10.1371/journal.pone.0161507
130. Ryba-Stanislawowska M, Buksa L, Brandt A, Juhas U, Mysliwiec M. IL-33 improves the suppressive potential of regulatory T cells in patients with type 1 diabetes. *Diabetes Res Clin Pract.* (2017) 128:67–73. doi: 10.1016/j.diabres.2017.04.011

131. Chen CC, Kobayashi T, Iijima K, Hsu FC, Kita H. IL-33 dysregulates regulatory T cells and impairs established immunologic tolerance in the lungs. *J Allergy Clin Immunol.* (2017) 140:1351–1363.e7. doi: 10.1016/j.jaci.2017.01.015
132. Popovic B, Golemac M, Podlech J, Zeleznjak J, Bilic-Zulle L, Lukic ML, et al. IL-33/ST2 pathway drives regulatory T cell dependent suppression of liver damage upon cytomegalovirus infection. *PLoS Pathog.* (2017) 13:e1006345. doi: 10.1371/journal.ppat.1006345
133. Alvarez F, Istomine R, Shourian M, Pavey N, Al-Aubodah T, Qureshi S, et al. The alarmins IL-1 and IL-33 differentially regulate the functional specialization of Foxp3+ regulatory T cells during mucosal inflammation. *Mucosal Immunol.* (2019). doi: 10.1038/s41385-019-0153-5. [Epub ahead of print].
134. Obata-Ninomiya K, Ishiwata K, Nakano H, Endo Y, Ichikawa T, Onodera A, et al. CXCR6(+)ST2(+) memory T helper 2 cells induced the expression of major basic protein in eosinophils to reduce the fecundity of helminth. *Proc Natl Acad Sci USA.* (2018) 115:E9849–58. doi: 10.1073/pnas.1714731115
135. Zhou Y, Ji Y, Wang H, Zhang H, Zhou H. IL-33 Promotes the development of colorectal cancer through inducing tumor-infiltrating ST2L(+) regulatory T cells in mice. *Technol Cancer Res Treat.* (2018) 17:1533033818780091. doi: 10.1177/1533033818780091
136. Sjoberg LC, Nilsson AZ, Lei Y, Gregory JA, Adner M, Nilsson GP. Interleukin 33 exacerbates antigen driven airway hyperresponsiveness, inflammation and remodeling in a mouse model of asthma. *Sci Rep.* (2017) 7:4219. doi: 10.1038/s41598-017-03674-0
137. Chapuis J, Hot D, Hansmannel F, Kerdraon O, Ferreira S, Hubans C, et al. Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. *Mol Psychiatry.* (2009) 14:1004–16. doi: 10.1038/mp.2009.10
138. Latiano A, Palmieri O, Pastorelli L, Vecchi M, Pizarro TT, Bossa F, et al. Associations between genetic polymorphisms in IL-33, IL1R1 and risk for inflammatory bowel disease. *PLoS ONE.* (2013) 8:e62144. doi: 10.1371/journal.pone.0062144
139. Seo DH, Che X, Kwak MS, Kim S, Kim JH, Ma HW, et al. Interleukin-33 regulates intestinal inflammation by modulating macrophages in inflammatory bowel disease. *Sci Rep.* (2017) 7:851. doi: 10.1038/s41598-017-00840-2
140. Christophi GP, Gruber RC, Panos M, Christophi RL, Jubelt B, Massa PT. Interleukin-33 upregulation in peripheral leukocytes and CNS of multiple sclerosis patients. *Clin Immunol.* (2012) 142:308–19. doi: 10.1016/j.clim.2011.11.007
141. Yang Z, Liang Y, Xi W, Li C, Zhong R. Association of increased serum IL-33 levels with clinical and laboratory characteristics of systemic lupus erythematosus in Chinese population. *Clin Exp Med.* (2011) 11:75–80. doi: 10.1007/s10238-010-0115-4
142. Shruthi S, Mohan V, Amutha A, Aravindhan V. Increased serum levels of novel T cell cytokines IL-33, IL-9 and IL-17 in subjects with type-1 diabetes. *Cytokine.* (2016) 86:6–9. doi: 10.1016/j.cyto.2016.07.007
143. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci USA.* (2008) 105:10913–8. doi: 10.1073/pnas.0801898105
144. Yasuoka S, Kawanokuchi J, Parajuli B, Jin S, Doi Y, Noda M, et al. Production and functions of IL-33 in the central nervous system. *Brain Res.* (2011) 1385:8–17. doi: 10.1016/j.brainres.2011.02.045
145. Xiao Y, Lai L, Chen H, Shi J, Zeng F, Li J, et al. Interleukin-33 deficiency exacerbated experimental autoimmune encephalomyelitis with an influence on immune cells and glia cells. *Mol Immunol.* (2018) 101:550–63. doi: 10.1016/j.molimm.2018.08.026
146. Jiang HR, Milovanovic M, Allan D, Niedbala W, Besnard AG, Fukada SY, et al. IL-33 attenuates EAE by suppressing IL-17 and IFN-gamma production and inducing alternatively activated macrophages. *Eur J Immunol.* (2012) 42:1804–14. doi: 10.1002/eji.201141947
147. Milovanovic M, Volarevic V, Ljujic B, Radosavljevic G, Jovanovic I, Arsenijevic N, et al. Deletion of IL-33R (ST2)abrogates resistance to EAE in BALB/C mice by enhancing polarization of APC to inflammatory phenotype. *PLoS ONE.* (2012) 7:e45225. doi: 10.1371/journal.pone.0045225
148. Li M, Li Y, Liu X, Gao X, Wang Y. IL-33 blockade suppresses the development of experimental autoimmune encephalomyelitis in C57BL/6 mice. *J Neuroimmunol.* (2012) 247:25–31. doi: 10.1016/j.jneuroim.2012.03.016
149. Pei C, Barbour M, Fairlie-Clarke KJ, Allan D, Mu R, Jiang HR. Emerging role of interleukin-33 in autoimmune diseases. *Immunology.* (2014) 141:9–17. doi: 10.1111/imm.12174
150. Palmer G, Talabot-Ayer D, Lamacchia C, Toy D, Seemayer CA, Viatte S, et al. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. *Arthritis Rheum.* (2009) 60:738–49. doi: 10.1002/art.24305
151. Martin P, Talabot-Ayer D, Seemayer CA, Vigne S, Lamacchia C, Rodriguez E, et al. Disease severity in K/BxN serum transfer-induced arthritis is not affected by IL-33 deficiency. *Arthritis Res Ther.* (2013) 15:R13. doi: 10.1186/ar4143
152. Barbour M, Allan D, Xu H, Pei C, Chen M, Niedbala W, et al. IL-33 attenuates the development of experimental autoimmune uveitis. *Eur J Immunol.* (2014) 44:3320–9. doi: 10.1002/eji.201444671
153. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circ Res.* (2010) 107:650–8. doi: 10.1161/CIRCRESAHA.110.218867
154. Momen T, Ahanchian H, Reisi M, Shamsdin SA, Shahsanai A, Keivanfar M. Comparison of interleukin-33 serum levels in asthmatic patients with a control group and relation with the severity of the disease. *Int J Prev Med.* (2017) 8:65. doi: 10.4103/ijpvm.IJPVM_179_16
155. Murakami-Satsutani N, Ito T, Nakanishi T, Inagaki N, Tanaka A, Vien PT, et al. IL-33 promotes the induction and maintenance of Th2 immune responses by enhancing the function of OX40 ligand. *Allergol Int.* (2014) 63:443–55. doi: 10.2332/allergolint.13-OA-0672
156. Chen WY, Tsai TH, Yang JL, Li LC. Therapeutic strategies for targeting IL-33/ST2 signalling for the treatment of inflammatory diseases. *Cell Physiol Biochem.* (2018) 49:349–58. doi: 10.1159/000492885
157. Xi H, Katschke KJ, Jr., Li Y, Truong T, Lee WP, Diehl L, et al. IL-33 amplifies an innate immune response in the degenerating retina. *J Exp Med.* (2016) 213:189–207. doi: 10.1084/jem.20150894
158. Wang K, Shan S, Yang Z, Gu X, Wang Y, Wang C, et al. IL-33 blockade suppresses tumor growth of human lung cancer through direct and indirect pathways in a preclinical model. *Oncotarget.* (2017) 8:68571–82. doi: 10.18632/oncotarget.19786
159. Stremska ME, Jose S, Sabapathy V, Huang L, Bajwa A, Kinsey GR, et al. IL233, a novel IL-2 and IL-33 hybrid cytokine, ameliorates renal injury. *J Am Soc Nephrol.* (2017) 28:2681–93. doi: 10.1681/ASN.2016121272
160. Duan L, Chen J, Zhang H, Yang H, Zhu P, Xiong A, et al. Interleukin-33 ameliorates experimental colitis through promoting Th2/Foxp3(+) regulatory T-cell responses in mice. *Mol Med.* (2012) 18:753–61. doi: 10.2119/molmed.2011.00428
161. Peng G, Mu Z, Cui L, Liu P, Wang Y, Wu W, et al. Anti-IL-33 antibody has a therapeutic effect in an atopic dermatitis murine model induced by 2, 4-dinitrochlorobenzene. *Inflammation.* (2018) 41:154–63. doi: 10.1007/s10753-017-0673-7

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Alvarez, Fritz and Piccirillo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 18 April 2019
doi: 10.3389/fimmu.2019.00702

Blockade of IL-33R/ST2 Signaling Attenuates *Toxoplasma gondii* Ileitis Depending on IL-22 Expression

Bernhard Ryffel[1,2*], *Feng Huang*[1], *Pauline Robinet*[2], *Corine Panek*[2], *Isabelle Couillin*[2], *François Erard*[2], *Julie Piotet*[2], *Marc Le Bert*[2], *Claire Mackowiak*[2], *Marbel Torres Arias*[3], *Isabelle Dimier-Poisson*[4†] *and Song Guo Zheng*[5*†]

[1] Department of Clinical Immunology, Sun Yat-sen University Third Affiliated Hospital, Guangzhou, China, [2] INEM UMR 7355 CNRS and University of Orleans, Orléans, France, [3] Immunology and Virology Laboratory, Nanoscience and Nanotechnology Center, Universidad de las Fuerzas Armadas, ESPE, Sangolquí, Ecuador, [4] UMR 1282 Infectiologie Animale et Santé Publique, Université de Tours -INRA, Tours, France, [5] Department of Internal Medicine, Ohio State College of Medicine, Columbus, OH, United States

OPEN ACCESS

Edited by:
Fang-Ping Huang,
The University of Hong Kong,
Hong Kong

Reviewed by:
Xun Sun,
China Medical University, China
Anja Andrea Kühl,
Charité Medical University of Berlin,
Germany
JiYu Li,
Tongji University, China

***Correspondence:**
Bernhard Ryffel
bernhard.ryffel@cnrs-orleans.fr
Song Guo Zheng
SongGuo.Zheng@osumc.edu

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: 11 December 2018
Accepted: 14 March 2019
Published: 18 April 2019

Citation:
Ryffel B, Huang F, Robinet P, Panek C, Couillin I, Erard F, Piotet J, Le Bert M, Mackowiak C, Torres Arias M, Dimier-Poisson I and Zheng SG (2019) Blockade of IL-33R/ST2 Signaling Attenuates Toxoplasma gondii Ileitis Depending on IL-22 Expression. Front. Immunol. 10:702. doi: 10.3389/fimmu.2019.00702

Oral *T. gondii* infection (30 cysts of 76K strain) induces acute lethal ileitis in sensitive C57BL/6 (B6) mice with increased expression of IL-33 and its receptor ST2 in the ileum. Here we show that IL-33 is involved in ileitis, since absence of IL-33R/ST2 attenuated neutrophilic inflammation and Th1 cytokines upon *T. gondii* infection with enhanced survival. Blockade of ST2 by neutralizing ST2 antibody in B6 mice conferred partial protection, while rmIL-33 aggravated ileitis. Since IL-22 expression further increased in absence of ST2, we blocked IL-22 by neutralizing antibody, which abrogated protection from acute ileitis in ST2 deficient mice. In conclusion, severe lethal ileitis induced by oral *T. gondii* infection is attenuated by blockade of ST2 signaling and may be mediated in part by endogenous IL-22.

Keywords: *Toxoplasma gondii*, IL-33/ST2 receptor, neutralizing antibody, IL-22, parasite-induced ileitis, innate immunity

INTRODUCTION

Toxoplasma gondii is an opportunistic parasite with a worldwide distribution triggering an innate immune response. This response characterized by a rapid recruitment of neutrophils following the entry of infectious tachyzoites from the lumen into the intestinal mucosa eliciting a strong inflammatory Th1 response associated with the production of IFNγ, IL-12 and TNF-α. The parasite activates dendritic cells and macrophages to produce IL-12 leading to IFNγ expression (1). IL-17A is involved in neutrophil recruitment following infection, important for host defense and enhances a Th17 response via IL-17RA signaling (2). We found that IL-17RA deficient mice and B6 mice treated with neutralizing IL-17A antibody are more resistant to *T. gondii* induced acute ileitis as compared to infected B6 mice, suggesting that IL-17A contributes to the pathology of *T. gondii* inflammation (3).

IL-33, previously known as IL1F11 or nuclear factor from high endothelial venules (4), is a member of the IL-1 cytokine family (1, 5). IL-33 in the nucleus is associated with chromatin, but the role of nuclear IL-33 is not yet clarified (6). Upon cell stress or death, biologically active IL-33 is released and truncated by proteolytic cleavage (7). IL-33 may have a dual role in different inflammatory conditions, depending on the specific immune mechanisms underlying disease pathogenesis (5). IL-33R/ST2 is a stable cell marker on Th2 cells and innate immune cells (8). IL-33

induces the production of high amounts of the Th2 cytokines IL-5 and IL-13 by type-2 innate lymphoid cells in the intestine and the lung. The IL-33-IL-33R/ST2 axis is involved in inflammatory bowel diseases (IBD) (9, 10) and has a regulatory role in experimental mouse models of IBD. IL-33 controls intestinal permeability and negatively regulates wound healing in the colon (11), further supporting the notion that the IL-33-IL-33R/ST2 axis may represent an effective therapeutic target in IBD.

We showed before that oral infection with cysts of *T. gondii* (76K strain) caused upregulation of IL-1β and IL-17A in the ileum with acute lethal ileitis in sensitive B6 mice. Furthermore, both IL-1β and IL-17A are involved in acute inflammation of the proximal intestine caused by tachyzoites invasion of the mucosa (3, 12), while IL-22 confers protection (13).

Here we report a critical role of IL-33R/ST2 upon *T. gondii* infection (76K strain). Both ST2 and IL-33 are upregulated in the intestine and IL-33R/ST2 deficient mice have attenuated ileitis with increased IL-22 expression. Furthermore, the blockade of IL-22 by antibody neutralization reversed the protective effect found in IL-33R/ST2 deficient mice. Therefore, the data suggest that protection may be mediated by upregulation of the protective cytokine IL-22.

RESULTS

Increased IL-33 Expression in *T. gondii* Induced Acute Ileitis

Oral infection with 30 cysts of *T. gondii* (76K strain) causes a rapid upregulation of IL-33 and IL-33R/ST2 gene expression in the proximal ileum in C57BL/6 mice on day 7 (**Figures 1A,B**). Furthermore, IL-33 protein increases in the ileal mucosa (**Figure 1C**). To determine the source of IL-33 we performed immunostaining and found that IL-33 is expressed in the intestinal epithelium as well as myeloid and fibroblast like cells in the lamina propria as reported before (14). Therefore, we questioned whether IL-33 contributes to the inflammatory response in *T. gondii* infected mice.

Diminished Intestinal Cytokine Production in the Absence of IL-33R/ST2

Since IL-33 induces a proinflammatory response, we determined the cytokine profile in the mucosa of the ileum upon oral *T. gondii* infection, which in B6 mice has a Th1 signature. We confirm increased production of Th1 cytokines IFNγ, TNF, IL-12, IL-23, and IL-1β in the proximal ileum in B6 mice upon infection, while absence of IL-33R/ST2 attenuated the Th1 cytokine response (**Figure 2**). Moreover, an enhanced Th17 response with elevated IL-17A and IL-22 expression has been reported upon *T. gondii* infection with diminished ileitis in IL-17RA and IL-22 deficient mice (3, 13). Here we find augmented IL-17A tissue levels in infected B6 mice, which further increased in IL-33R/ST2 deficient mice (**Figure 2**). In conclusion, infection with *T. gondii* induces proinflammatory cytokine and chemokine responses, which are reduced in absence of IL-33R/ST2 signaling. Therefore, we asked whether blockade of this pathway would attenuate acute ileitis.

T. gondii Induced Ileitis Is Attenuated in IL-33R/ST2 Deficient Mice

Using IL-33R/ST2 deficient mice, we observe a reduced severity of *T. gondii* induced ileitis. Clinical signs of disease with loss of body weight and macroscopic inflammatory alterations of the ileum are diminished in IL-33R/ST2 deficient mice (**Figures 3D,E**). Increased CXCL1/KC levels are associated with enhanced MPO activity and neutrophil recruitment in the mucosa of the proximal ileum, which are significantly lower in the absence of IL-33R/ST2 (**Figures 3A–C**) and associated with enhanced survival as compared to B6 mice (**Figure 3F**). Microscopic analysis reveals reduced inflammation at day 7 of *T. gondii* infected IL-33R/ST2 deficient mice (**Figures 3G,H**). While infected B6 mice displayed severe acute inflammatory changes in the ileum, the severity of inflammation was attenuated in IL-33R/ST2 deficient mice (**Figures 3G,H**). Therefore, IL-33R/ST2 signaling mediates severe acute inflammation in the proximal ileum, which is fatal, but significantly reduced in the absence of IL-33R/ST2 signaling.

IL-33R/ST2 Antibody Blockade Dampens, While Exogenous IL-33 Enhances *T. gondii* Induced Ileitis

To ascertain that the protection observed is not a particularity of the IL-33R/ST2 deficient mice, we used neutralizing IL-33R/ST2 antibody in infected B6 mice and confirm a protective effect as observed in IL-33R/ST2 deficient mice. Neutrophil recruitment as measured by MPO activity and the chemokine CXCL1/KC are reduced in ileum comparable to that found in IL-33R/ST2 deficient mice (**Figures 4A,B**). Survival is significantly prolonged and severity acute ileitis reduced as shown for *T. gondii* infected IL-33R/ST2 deficient mice (**Figures 4C,D**).

To further confirm a critical role of IL-33, we investigated whether exogenous IL-33 enhances *T. gondii* induced inflammation. The injection of rmIL-33 (0.5 μg daily by i.p. route) in infected B6 mice augmented the inflammatory response with increased MPO activity and KC expression in the ileum and reduced survival due to enhanced severity of the ileitis confirmed by microscopic analysis (**Figures 4E-H**). Therefore, the data strongly suggests that IL-33 signaling via IL-33R/ST2 is critical for the inflammatory response in *T. gondii* induced ileitis.

Enhanced IL-22 Expression Contributes to Attenuate Ileitis in IL-33R/ST2 Deficient Mice

IL-22 has a protective effect as reported before in *T. gondii* induced acute ileitis (3, 13). We revisited IL-22 expression and found increased IL-22 expression in the parasitized ileum of IL-33R/ST2 deficient mice (**Figure 5A**). IL-33 tissue levels are decreased in IL-33R/ST2 deficient mice, but IL-22 antibody neutralization augmented local IL-33 levels (**Figure 5B**).

Therefore, we asked whether exogenous rmIL-22 contributes to the protective effect. First, we injected rmIL-22 in infected B6 mice, and found a significant reduction of MPO activity, KC and neutrophil recruitment (**Figure 5C**)

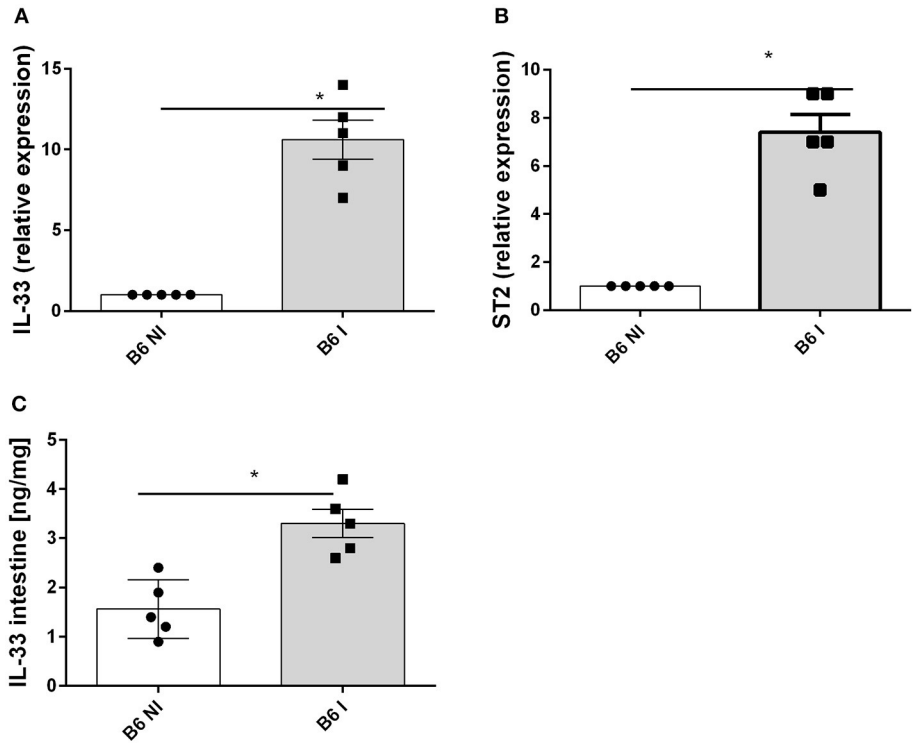


FIGURE 1 | *T. gondii* infection induces IL-33 and ST2 expression in the ileum. B6 mice were infected by gavage with 35 cysts of *T.gondii* (76K strain) and transcripts were measured by q-PCR in the homogenate of the proximal ileum (2 cm of jejunum) for IL−33 **(A)** and ST2 mRNA **(B)** and IL-33 protein by ELISA in intestinal homogenate **(C)** at day 7 post-infection. Values are representative of two independent experiments expressed as mean ± SEM. *, **, and *** refer to $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

and attenuated severity of ileitis (**Figure 5D**) with enhanced survival in B6 mice (data not show) consistent with our previous results (13).

In view of increased IL-22 expression and a protective effect of exogenous IL-22, we hypothesized that IL-22 blockade by neutralizing antibody may reduce protection from acute ileitis in IL-33R/ST2 deficient mice. Indeed, we showed that IL-22 antibody administration (100μg i.p. injection) largely abrogates protection from acute neutrophil recruitment and tissue inflammation in the infected ileum (**Figures 5E, F**). Therefore, rmIL-22 attenuates *T. gondii* induced inflammation. Furthermore, endogenous IL-22 contributes to the protection observed in the absence of IL-33R/ST2 signaling. The data suggests that IL-33 may suppress the protective action of endogenous IL-22 in *T. gondii* infection.

DISCUSSION

Here we used oral *T. gondii* infection (76K strain) induced severe ileitis in mice which may serve as a model of IBD (15). We reported that IL-33R/ST2 upon *T. gondii* infection induced ST2, IL-33 and IL-22 expression in the intestine. We discovered that IL-33R/ST2 deficient mice have attenuated ileitis associated with increased IL-22 expression. Since IL-22 antibody neutralization reversed the protective effect in IL-33R/ST2 deficient mice, we conclude that protection may be related to increased expression of the cytokine IL-22, known for its protective function (13).

IL-33 has an important regulatory roles in IBD as reviewed before (10) and we demonstrated enhanced healing in experimental IBD models (11). However, the role of IL-33 or IL-33R/ST2 upon *T. gondii* induced ileitis is unknown. Previous work demonstrated that neuroinflammation induced by *T. gondii* is IL-33-dependent, since IL-33R/ST2 deficient mice have increased parasite growth and severe cerebral toxoplasmosis, but the ileitis was not investigated (16). The transcription factor trefoil 2 (TFF2) has been shown to regulate IL-33 expression and Th2 differentiation (17), while in absence of TFF2, a Th1 response prevailed. Infected TFF2 deficient mice displayed low parasite replication and reduced intestinal inflammation upon *T. gondii* infection, whereas B6 mice experienced uncontrolled inflammation with lethal outcome (18).

The resistance to develop ileitis observed in IL-33R/ST2 deficient mice is replicated in infected B6 mice administered ST2 neutralizing antibody. Similar data of attenuated inflammation have been reported for other IL-33 dependent inflammatory conditions (1, 5). Further, rmIL-33 enhanced acute ileitis in B6 mice. Therefore, IL-33 appears to be critical for the control of *T. gondii* induced ileitis and we asked whether other inflammatory cytokine are involved.

We reported before that IL-22 has a protective effect, since IL-22 deficient mice develop acute ileitis (13) and showed

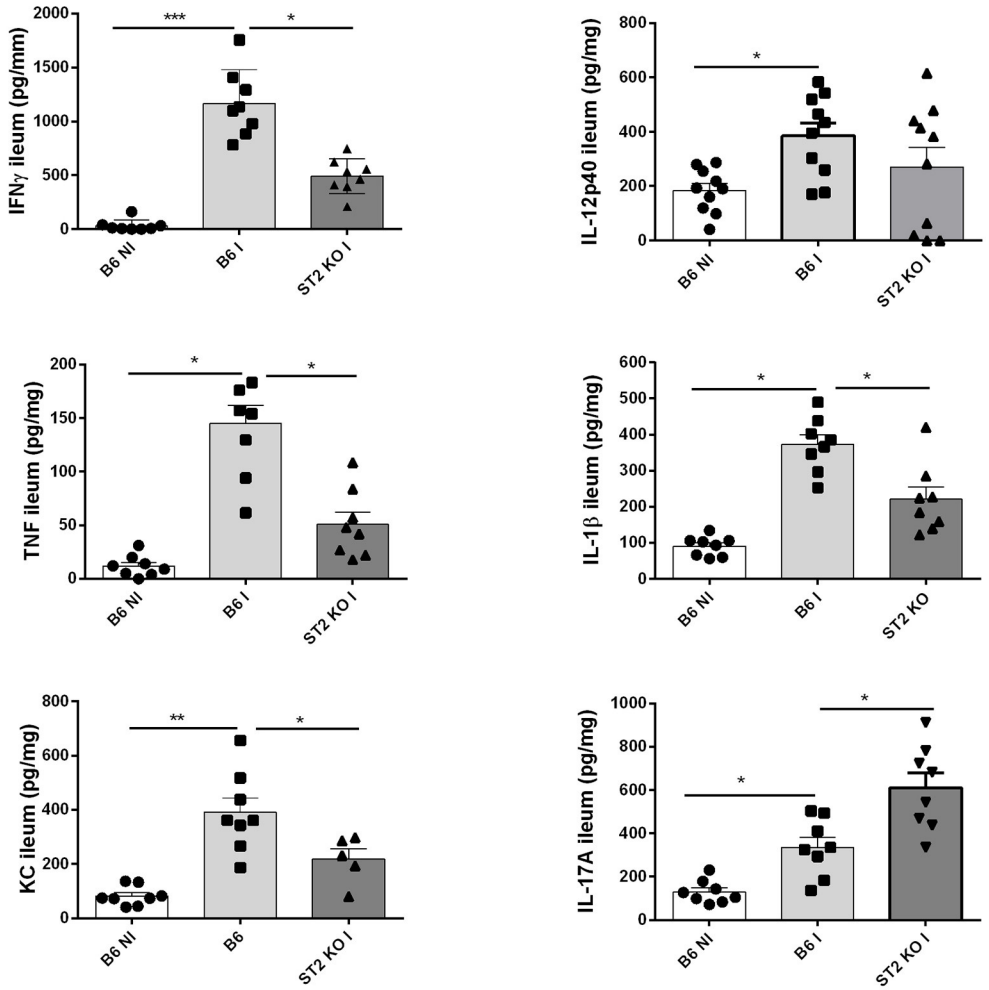


FIGURE 2 | Reduced cytokine and chemokine production in the absence of IL-33R/ST2. Levels of IFNγ, TNF, IL-12p40, IL-1β, CXCL1/KC, and IL-17A were determined by ELISA in homogenates of the proximal ileum at day 7 post-infection. Values are representative of two independent experiments expressed as mean ± SEM.

here that IL-22 administration reduced epithelial barrier injury and inflammation. By contrast, IL-17 another Th17 cytokine enhances *T. gondii* induced ileal inflammation, since IL-17RA deficient mice were protected (3). IL-10, another member of the broader IL-22/IL-10 cytokine family, plays a critical role in IBD as demonstrated by the spontaneous development of IBD in IL-10 deficient mice (19, 20). Interestingly, *T. gondii* infection in a model of IL-10 deficient intraepithelial lymphocyte transfer or NKT cell deficient (Jalpha281(-/-) mice had reduced ileitis with IL-10 expression (21, 22). Whether IL-10-dependent mechanisms contribute to the protective IL-33/ST2/IL-22 pathway has not been reported so far. Here we focused on the contribution of IL-22 in IBD, but investigations on the role of IL-10 in the protective IL-33/ST2/IL-22 axis deserves further investigations in the future.

The finding that IL-33R/ST2 signaling suppresses IL-22 is novel, but a recent study revealed IL-33 regulates IL-17A and IL-22 in fungal infection (23). IL-22 neutralizing antibody administration converted resistance to ileitis of IL-33R/ST2 deficient mice to an inflammatory Th1 phenotype, which may be due to enhanced prostaglandin E2 production (23). A role of increased expression of amphiregulin by IL-33 has been shown to contribute to control experimental colitis (14), which merits further investigations. Thus, alternative pathways may be considered such as the activation of the NLPR3 inflammasome complex (24), which is supported by reduced *T.gondii* induced ileitis in IL-1R1 deficient mice (12). The possibility that NLRP3/caspase-1 activation in *T.gondii* infection contributes to the down-modulation of IL-33 and the Th2 response should be considered as reported in allergic lung inflammation (25). Finally, in view of the critical role of neutrophils in *T.gondii* induced inflammation the polarization of neutrophils to express IL-17A may be of interest as previously shown in an ischemia reperfusion repair model (26).

In summary, IL-33R/ST2 signaling enhances *T. gondii* parasite-induced inflammation and IL-22 has an important

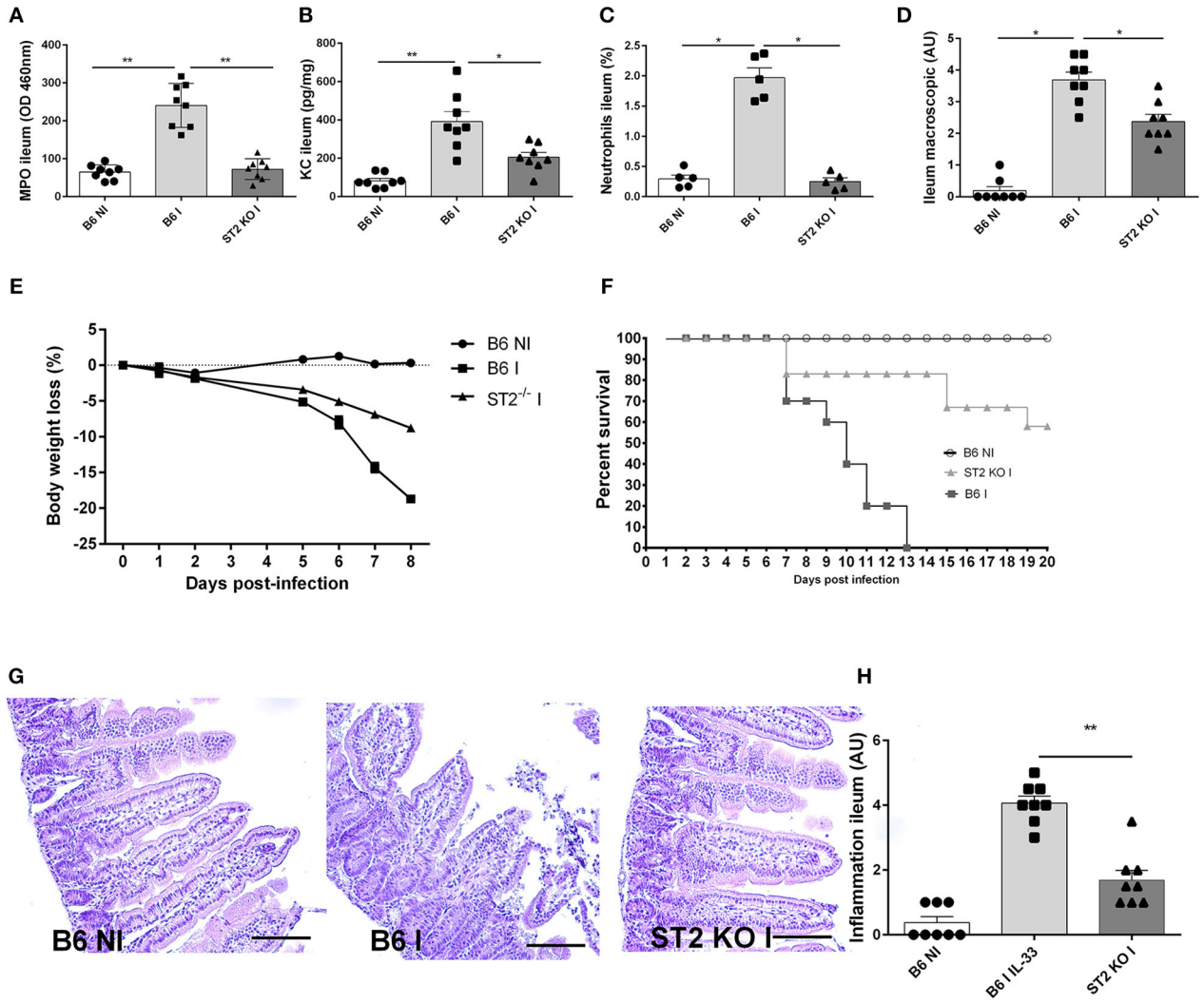


FIGURE 3 | *T. gondii* induced ileitis is attenuated in IL-33R/ST2 deficient mice. Reduced MPO activity, KC and neutrophils recruitment in the ileum of IL-33R KO mice **(A–C)**, macroscopic signs of colon inflammation **(D)** and body weight loss **(E)** with enhanced survival of IL-33R/ST2 mice **(F)** associated with reduced severity of microscopic inflammation in the proximal ileum in the absence of ST2 **(G)** and semi-quantitative score of inflammation **(H)**. Analysis at day 7 post-infection. Values are representative of two independent experiments expressed as mean ± SEM.

protective effect (13). Resistance in absence of IL-33R/ST2 appears to be mediated by endogenous IL-22. Therefore, the beneficial effect of IL-22 administration on toxoplasma-induced ileitis may be relevant for human IBD of different origins.

MATERIALS AND METHODS

Mice

IL-33R/ST2 T1/ST2-deficient mice (27) were back-crossed 8 times on C57BL/6J genetic background and bred with wild-type littermates in our animal facility at the Transgenose Institute (CNRS, TAAM, Orleans, France). All animal experimental protocols complied with the French ethical and animal experiments regulations (see Charte Nationale, Code Rural R 214-122, 214-124 and European Union Directive 86/609/EEC) and were approved by the "Ethics Committee for Animal Experimentation of CNRS Campus Orleans" (CCO), registered (N°3) by the French National Committee of Ethical Reflexion for Animal Experimentation (CLE CCO 2012-042).

Inoculation of *T. gondii* Cysts and Administration of IL-33, IL22, IL-33R, and IL-22 Neutralizing Antibodies

C57BL/6 (B6) and IL-33R/ST2 deficient mice were inoculated by gavage with 30 cysts of the 76K strain obtained as described before (13). Groups of 5 to 8 female mice of 8–12 weeks were used and the studies were repeated twice. Additional groups of mice were injected daily intraperitoneally with 0.5 μg of rm IL-33 (aa 109–266) or 5 μg rmIL-22 daily R&D system). Neutralizing

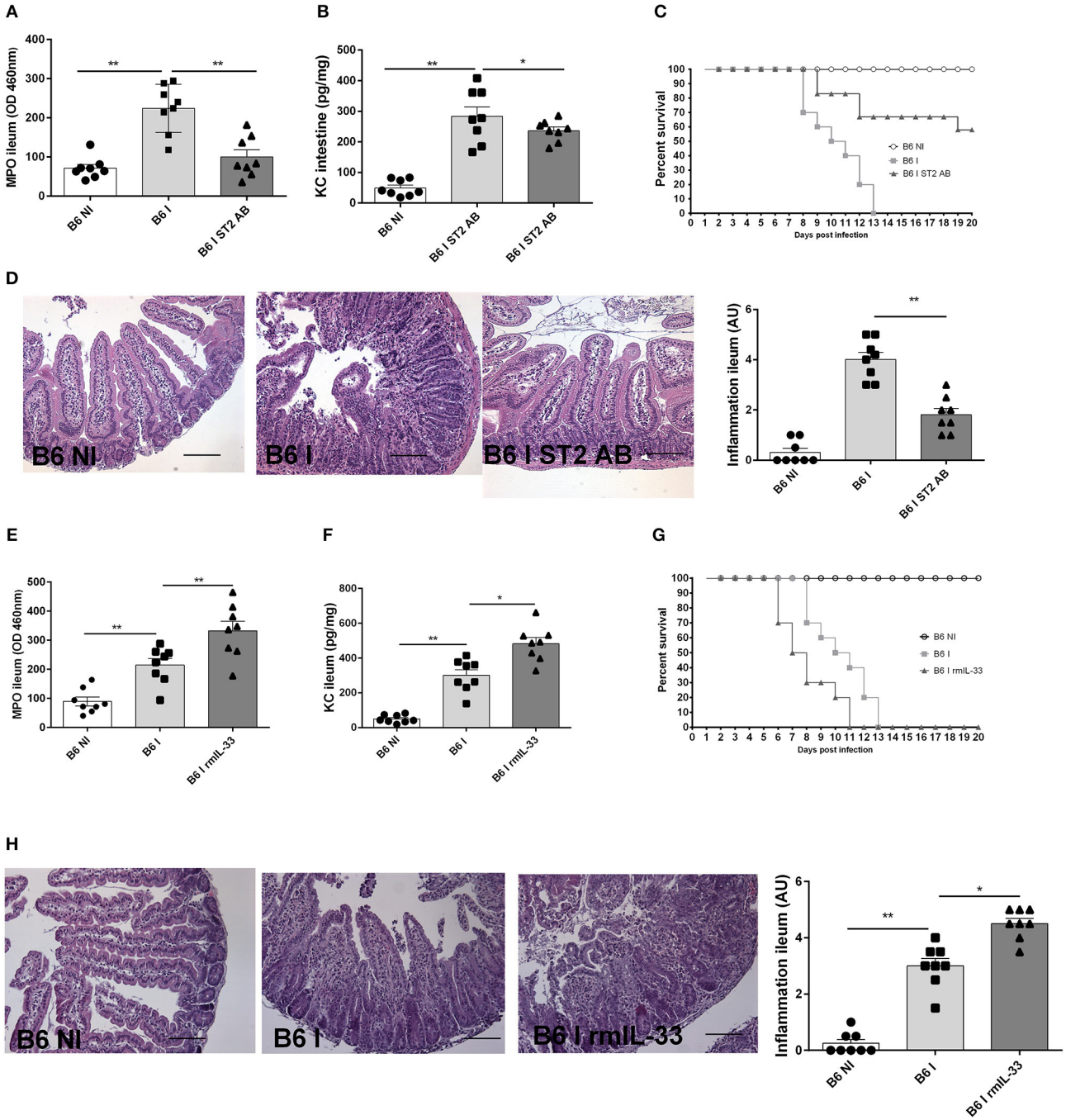


FIGURE 4 | Neutralization of IL-33 attenuates, while rmIL-33 aggravates ileitis in BL6 mice. Antibody IL-33R/ST2 neutralization reduces MPO activity **(A)** and CXCL1/KC **(B)**, and enhances survival **(C)** associated with attenuated microscopic inflammation **(D)**. By contrast, rmIL-33 augments MPO activity, CXCL1/KC, survival and severity of Inflammation **(E–H)**. Analysis at day 7 post-infection. Values are representative of two independent experiments.

rmIL-22 (R&D system) antibody and IL-33R/ST2 (gift from Dr. Dirk Smith, Amgen) antibody were injected at 50 μg per mouse or isotype control (rat IgG1, R&D system) on days 1, 3, and 5 after oral infection. The mice were analyzed at day 7 for neutrophil recruitment in the ileum and morphological alterations of the proximal ileum and additional groups were used for survival.

RNA Extraction and PCR in Ileum

Ileum from control and infected B6 mice was collected, snap-freezed in liquid nitrogen and kept at −80°C. Total RNA were isolated from 100 mg of intestinal tissue homogenized with 1 mL of TRI Reagent® (Sigma) using TRIzol/Chloroform extraction as described (13). RNA was then precipitated in

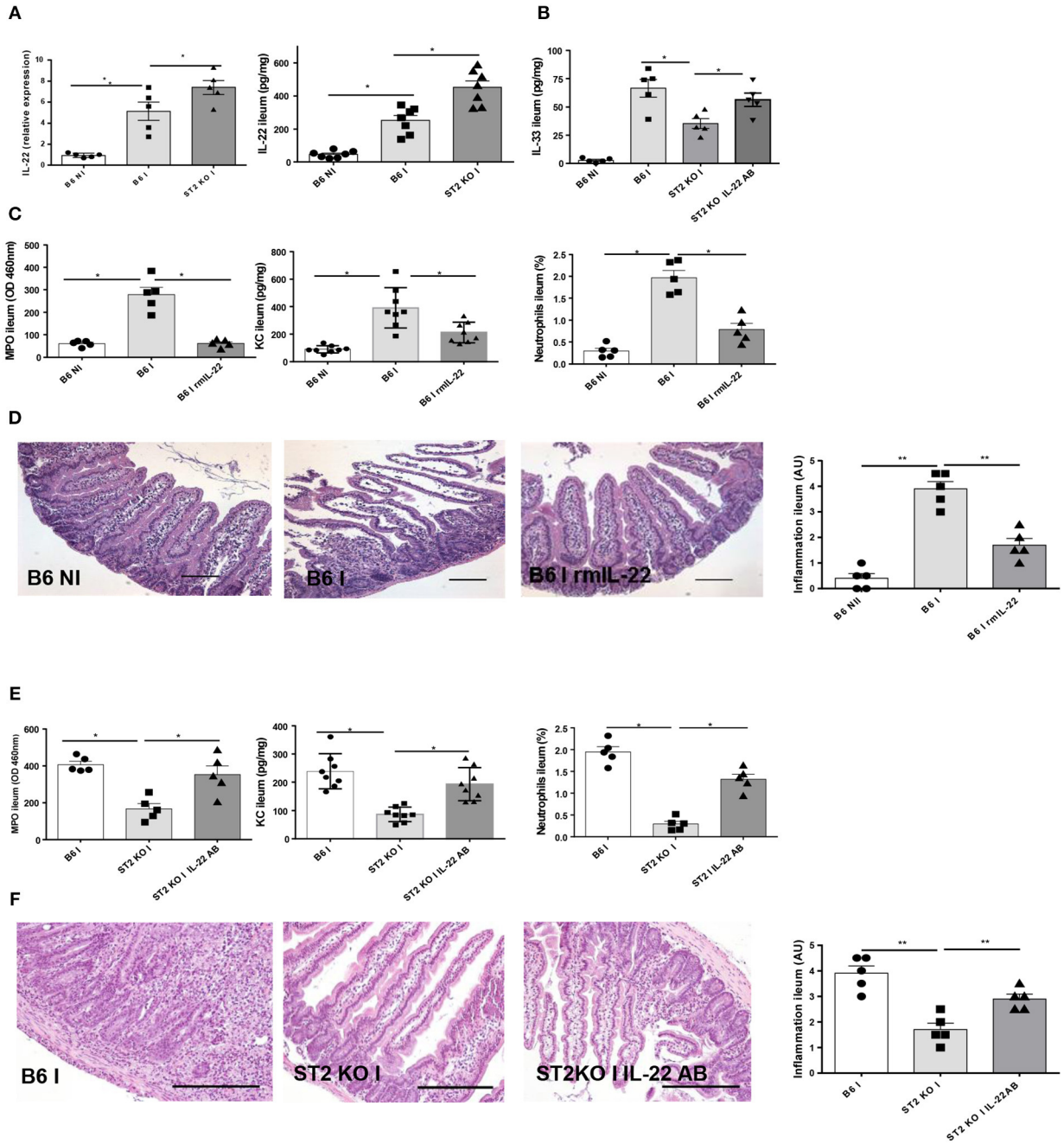


FIGURE 5 | IL-22 confers protection in IL-33R/ST2 deficient mice upon *T. gondii* infection Infection by *T. gondii* infection induces enhanced IL22 mRNA and protein expression in ST2 deficient mice **(A, B)** and increased IL-33 expression **(B)**. Administration of rmIL-22 reduces MPO activity, CXCL1/KC levels, neutrophil recruitment **(C)** and severity of ileitis in B6 mice **(D)**. By contrast, IL-22 antibody blockade in IL-33R/ST2 deficient mice increased MPO activity, CXCL1/KC levels and neutrophil recruitment **(E)** and enhanced severity of ileitis **(F)**. Analysis at day 7 post-infection. Values are representative of two independent experiments.

isopropanol, washed with 75% ethanol and resuspended in RNase-free water. Reverse transcription was performed on 1 μg of RNA using GoScript Reverse transcription system (Promega). Quantitative real-time PCR were realized on cDNA obtained using primers for Il22, Il33, and St2 (Qiagen), GoTaq® qPCR-Master Mix (Promega) and detected on a Stratagene Mx3005P (Agilent technologies). At the end of the PCR amplification, a DNA melting curve analysis was carried out to confirm the presence of a single amplicon. *Gapdh* expression was used for normalization of transcript levels. Relative mRNA levels were determined using ($2^{-\Delta\Delta Ct}$) method, determined by comparing (*i*) the PCR cycle thresholds (Ct) for the gene of interest

and *Gapdh* (ΔCt) and (*ii*) ΔCt values for treated and control groups (ΔΔCt).

Cytokine Measurement

Cytokine production for IFNγ, CXCL1/KC, IL-1β; IL-12p40, TNF-α, IL-17A, and IL-22 was evaluated in proximal ileum homogenate using commercial ELISA kits according to the manufacturer's instructions. Concentrations were normalized with organ weight and expressed in quantity per mg of tissue.

Myeloperoxidase Activity (MPO) in Ileum

MPO activity was evaluated in tissues of the small intestines as described. In brief, the right heart ventricle was perfused with saline to flush the vascular content and ileum was frozen at −20°C until use. Ileum was homogenized in PBS by Ultra Turrax, centrifuged and the supernatant was discarded. The pellets were resuspended in 1 ml PBS containing 0.5% hexadecyltrimethyl ammonium bromide (HTAB) and 5 mM ethylene-diamine tetra-acetic acid (EDTA). Following centrifugation, 150 μl of supernatants were placed in test tubes with 200 μl PBS-HTAB-EDTA, 1 ml Hanks' balanced salt solution (HBSS), 100 μl of o-dianisidine dihydrochloride (1.25 mg.ml^{-1}), and 100 μl H_2O_2 0.05%. After 15 min of incubation at 37°C in an agitator, the reaction was stopped with 100 μl NaN_3 1%. The MPO activity was determined as absorbance at 460 nm against medium.

Isolation of Lamina Propria Mononuclear Cells and Flow Cytometry

The small bowel was flushed with PBS and opened longitudinally, cut into 1 cm pieces and incubated in PBS/EDTA 3 mM during 20 min at 37°C under magnetic agitation. Pieces were then cut into 1 mm pieces and incubated in RPMI containing 0.5 mg/mL type IV collagenase (Life technologies), 1 ng/mL DNase (DN25, Sigma), 5% FCS (Perbio) and incubated 15 min at 37°C under magnetic agitation. Tissue debris and cell aggregates were removed by passage several times over a 10 mL syringe. Cells were filtered on 70 μm cells strainers and centrifuged 7 min at 1,700 rpm. Cells pellets were resuspended in 40% Percoll faction, overlayed on the top of a 80% Percoll fraction and centrifuged 20 min at 3,000 rpm without brake. LPMCs are collected in a white ring at the interphase of the two different percoll solutions and washed by RPMI 1640. Cells were then suspended in RPMI 1640 for experiments and 105 cells/mouse were stained by anti-CD11b PerCP Cy5.5 (Clone M1/70, BD Pharmingen) and anti-Ly6G PE-Cy7 (Clone RBL6-8C5, eBioscience) antibodies or by control isotypes in presence of Fc Block (anti-CD32/CD16) (Clone 24.G2, BD Pharmingen). FACS staining was assessed on a BD CANTO II cytometer and analyzed with FlowJo Software as described (13).

After 15 min of incubation at 37°C in an agitator, the reaction was stopped with 100 μl NaN_3 1%. The MPO activity was determined as absorbance at 460 nm against medium.

Macroscopic and Microscopic Investigations

Proximal jejunum was collected 7 days after the infection, macroscopically observed to identify major alterations, fixed in 4% buffered formaldehyde and processed under standard conditions. Tissue sections (3 μm) were stained with haematoxylin and eosin. The inflammatory cell infiltrate with epithelial lesion was assessed by a semi-quantitative score from 0 to 5 (with increasing extent) by two independent, blinded experts (BR and PR) as described before (13).

Statistical Analysis

Data were analyzed using Prism version 5 (Graphpad Software, San Diego, CA). The non-parametric Kruskal-Wallis test with Dunn's multiple comparison test or the parametric one-way ANOVA test with multiple Bonferroni's comparison test were used. Data were considered significant when $p < 0.05$ (*), 0.01 (**), 0.001 (***), or 0.0001 (****).

AUTHOR CONTRIBUTIONS

ID-P, SZ, FH, and BR conceived and designed the experiments. PR, CP, JP, MT, CM, and ML performed the experiments. PR, ID-P, CM, and BR analyzed the data. FE, IC, ML, and CM scientific advice. BR, PR, SZ, FE, and ID-P wrote the paper.

FUNDING

Centre National de la Recherche Scientifique, the University of Orléans, la Région Centre (2003-00085470) and European Regional Development Fund (FEDER n°2016-00110366).

ACKNOWLEDGMENTS

We thank Melody Thilloux, Nathalie Froux, Caroline Bertrand, Karine Jambou, Jérémy Paumier, Pascal Mauny, Tamara Durand, Ghislaine Chavaneau and Elodie Desale for technical assistance.

REFERENCES

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
2. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, Quesniaux V, et al. Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med*. (2006) 203:2715–25. doi: 10.1084/jem.20061401
3. Guiton R, Vasseur V, Charron S, Arias MT, Van Langendonck N, Buzoni-Gatel D, et al. Interleukin 17 receptor signaling is deleterious during *Toxoplasma gondii* infection in susceptible BL6 mice. *J Infect Dis*. (2010) 202:427–35. doi: 10.1086/653738
4. Moussion C, Girard JP. Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. *Nature*. (2011) 479:542–6. doi: 10.1038/nature10540
5. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol*. (2016) 16:676–89. doi: 10.1038/nri.2016.95

6. Carrière V, Arshad MI, Le Seyec J, Lefevre B, Farooq M, Jan A, et al. Endogenous IL-33 deficiency exacerbates liver injury and increases hepatic influx of neutrophils in acute murine viral hepatitis. *Mediat Inflamm*. (2017) 2017:1359064. doi: 10.1155/2017/1359064
7. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. *Nat Immunol*. (2018) 19:375–85. doi: 10.1038/s41590-018-0067-5
8. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci USA*. (2010) 107:18581–6. doi: 10.1073/pnas.1003059107
9. Hodzic Z, Schill EM, Bolock AM, Good M. IL-33 and the intestine: The good, the bad, and the inflammatory. *Cytokine*. (2017) 100:1–10. doi: 10.1016/j.cyto.2017.06.017
10. Pastorelli L, De Salvo C, Vecchi M, Pizarro TT. The role of IL-33 in gut mucosal inflammation. *Mediat Inflamm*. (2013) 2013:608187. doi: 10.1155/2013/608187
11. Sedhom MA, Pichery M, Murdoch JR, Foligné B, Ortega N, Normand S, et al. Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice. *Gut*. (2013) 62:1714–23. doi: 10.1136/gutjnl-2011-301785
12. Villeret B, Brault L, Couturier-Maillard A, Robinet P, Vasseur V, Secher T, et al. Blockade of IL-1R signaling diminishes paneth cell depletion and *Toxoplasma gondii* induced ileitis in mice. *Am J Clin Exp Immunol*. (2013) 2:107–16.
13. Couturier-Maillard A, Froux N, Piotet-Morin J, Michaudel C, Brault L, Le Bérichel J, et al. Interleukin-22-deficiency and microbiota contribute to the exacerbation of *Toxoplasma gondii*-induced intestinal inflammation. *Mucosal Immunol*. (2018) 11:1181–90. doi: 10.1038/s41385-018-0005-8
14. Monticelli LA, Osborne LC, Noti M, Tran SV, Zaiss DM, Artis D. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci USA*. (2015) 112:10762–7. doi: 10.1073/pnas.1509070112
15. Foureau DM, Mielcarz DW, Menard LC, Schulthess J, Werts C, Vasseur V, et al. TLR9-dependent induction of intestinal alpha-defensins by *Toxoplasma gondii*. *J Immunol*. (2010) 184:7022–9. doi: 10.4049/jimmunol.0901642
16. Jones LA, Roberts F, Nickdel MB, Brombacher F, McKenzie AN, Henriquez FL, et al. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with *Toxoplasma gondii*. *Eur J Immunol*. (2010) 40:426–36. doi: 10.1002/eji.200939705
17. Wills-Karp M, Rani R, Dienger K, Lewkowich I, Fox JG, Perkins C, et al. Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med*. (2012) 209:607–22. doi: 10.1084/jem.20110079
18. McBerry C, Egan CE, Rani R, Yang Y, Wu D, Boespflug N, et al. Trefoil factor 2 negatively regulates type 1 immunity against *Toxoplasma gondii*. *J Immunol*. (2012) 189:3078–84. doi: 10.4049/jimmunol.1103374
19. Davidson NJ, Leach MW, Fort MM, Thompson-Snipes L, Kühn R, Müller W, et al. T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J Exp Med*. (1996) 184:241–51.
20. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*. (1993) 75:263–74.
21. Mennechet FJ, Kasper LH, Rachinel N, Minns LA, Luangsay S, Vandewalle A, et al. Intestinal intraepithelial lymphocytes prevent pathogen-driven inflammation and regulate the Smad/T-bet pathway of lamina propria CD4+ T cells. *Eur J Immunol*. (2004) 34:1059–67. doi: 10.1002/eji.200324416
22. Ronet C, Darche S, Leite de Moraes M, Miyake S, Yamamura T, Louis JA, et al. NKT cells are critical for the initiation of an inflammatory bowel response against *Toxoplasma gondii*. *J Immunol*. (2005) 175:899–908. doi: 10.4049/jimmunol.175.2.899
23. Garth JM, Reeder KM, Godwin MS, Mackel JJ, Dunaway CW, Blackburn JP, et al. IL-33 signaling regulates innate IL-17A and IL-22 production via suppression of prostaglandin E2 during lung fungal infection. *J Immunol*. (2017) 199:2140–8. doi: 10.4049/jimmunol.1602186
24. Besnard AG, Togbe D, Couillin I, Tan Z, Zheng SG, Erard F, et al. Inflammasome-IL-1-Th17 response in allergic lung inflammation. *J Mol Cell Biol*. (2012) 4:3–10. doi: 10.1093/jmcb/mjr042
25. Madouri F, Guillou N, Fauconnier L, Marchiol T, Rouxel N, Chenuet P, et al. Caspase-1 activation by NLRP3 inflammasome dampens IL-33-dependent house dust mite-induced allergic lung inflammation. *J Mol Cell Biol*. (2015) 7:351–65. doi: 10.1093/jmcb/mjv012
26. Tan Z, Jiang R, Wang X, Wang Y, Lu L, Liu Q, et al. RORgammat+IL-17+ neutrophils play a critical role in hepatic ischemia-reperfusion injury. *J Mol Cell Biol*. (2013) 5:143–6. doi: 10.1093/jmcb/mjs065
27. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med*. (2000) 191:1069–76. doi: 10.1084/jem.191.6.1069

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Ryffel, Huang, Robinet, Panek, Couillin, Erard, Piotet, Le Bert, Mackowiak, Torres Arias, Dimier-Poisson and Zheng. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 19 June 2019
doi: 10.3389/fimmu.2019.01361

Divergent Effects of Acute and Prolonged Interleukin 33 Exposure on Mast Cell IgE-Mediated Functions

*Elin Rönnberg[1], Avan Ghaib[1,2], Carlos Ceriol[1], Mattias Enoksson[1], Michel Arock[3,4], Jesper Säfholm[5] on behalf of ChAMP Collaborators, Maria Ekoff[1] and Gunnar Nilsson[1,6]**

[1] Immunology and Allergy Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Solna, Sweden, [2] Department of Microbiology, College of Medicine, University of Sulaimani, Sulaimani, Iraq, [3] Molecular and Cellular Oncology, LBPA CNRS UMR 8113, Ecole Normale Supérieure de Cachan, Cachan, France, [4] Laboratoire Central d'Hématologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France, [5] The Unit for Asthma and Allergy Research, The Institute of Environmental Medicine, Karolinska Institutet, Solna, Sweden, [6] Department of Medical Sciences, Uppsala University, Uppsala, Sweden

OPEN ACCESS

Edited by:
Jose Carlos Alves-Filho,
University of São Paulo, Brazil

Reviewed by:
Elzbieta Kolaczkowska,
Jagiellonian University, Poland
Paul Proost,
KU Leuven, Belgium

***Correspondence:**
Gunnar Nilsson
gunnar.p.nilsson@ki.se

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: 16 January 2019
Accepted: 29 May 2019
Published: 19 June 2019

Citation:
Rönnberg E, Ghaib A, Ceriol C, Enoksson M, Arock M, Säfholm J, Ekoff M and Nilsson G (2019) Divergent Effects of Acute and Prolonged Interleukin 33 Exposure on Mast Cell IgE-Mediated Functions. Front. Immunol. 10:1361. doi: 10.3389/fimmu.2019.01361

Background: Epithelial cytokines, including IL-33 and Thymic stromal lymphopoietin (TSLP), have attracted interest because of their roles in chronic allergic inflammation-related conditions such as asthma. Mast cells are one of the major targets of IL-33, to which they respond by secreting cytokines. Most studies performed thus far have investigated the acute effects of IL-33 on mast cells. In the current study, we investigated how acute vs. prolonged exposure of mast cells to IL-33 and TSLP affects mediator synthesis and IgE-mediated activation.

Methods: Human lung mast cells (HLMCs), cord blood-derived mast cells (CBMCs), and the ROSA mast cell line were used for this study. Receptor expression and the levels of mediators were measured after treatment with IL-33 and/or TSLP.

Results: IL-33 induced the release of cytokines. Prolonged exposure to IL-33 increased while TSLP reduced intracellular levels of tryptase. Acute IL-33 treatment strongly potentiated IgE-mediated activation. In contrast, 4 days of exposure to IL-33 decreased IgE-mediated activation, an effect that was accompanied by a reduction in FcεRI expression.

Conclusion: We show that IL-33 plays dual roles in mast cells, in which its acute effects include cytokine release and the potentiation of IgE-mediated degranulation, whereas prolonged exposure to IL-33 reduces IgE-mediated activation. We conclude that mast cells act quickly in response to the alarmin IL-33 to initiate an acute inflammatory response, whereas extended exposure to IL-33 during prolonged inflammation reduces IgE-mediated responses. This negative feedback effect suggests the presence of a novel regulatory pathway that modulates IgE-mediated human mast cell responses.

Keywords: FcεRI, IgE, IL-33, mast cells, TSLP

INTRODUCTION

Compelling evidence suggests that epithelial cell-derived cytokines, such as thymic stromal lymphopoietin (TSLP), and interleukin (IL) 33 (IL-33), are strongly involved in the initiation and/or perpetuation of allergy and chronic inflammatory lung diseases such as asthma (1–3). Data have accumulated over the years from extensive investigations performed in experimental models, both *in vivo* and *in vitro*, as well as from genome wide association studies (4) and clinical trials (5). Both TSLP and IL-33 are released from epithelial cells in response to pathogens, environmental pollutants and allergens, or, in the case of IL-33, as a result of cell damage. Cells that respond to TSLP and IL-33 include T-lymphocytes, type 2 innate lymphoid cells, eosinophils, neutrophils, basophils and mast cells, many of which are often associated with type 2 immune responses, such as allergies (6, 7).

Mast cells are normally located just beneath epithelial cells, and in asthma, they are also found within the intraepithelial cell layer, and they are therefore capable of rapidly responding to TSLP and IL-33 released from epithelial cells (8). In contrast to allergen-induced cross-linkage of the IgE receptor, neither TSLP nor IL-33 causes acute mast cell exocytosis, i.e., the release of histamine, proteases, and other mediators stored in the granules. Instead, Th2 cytokines are prominently induced in response to TSLP and IL-33 (9–11). Interestingly, IL-33 is the sole alarmin that is released from damaged cells that can immediately activate mast cells to induce an inflammatory response and recruit granulocytes (12, 13). Whether mast cells also synthesize lipid mediators, such as prostaglandins and leukotrienes, in response to TSLP and IL-33 is less clear and might depend on species differences and/or the type of mast cell (10–12, 14–17). Although it does not induce mast cell degranulation on its own, IL-33 increases the synthesis and therefore the amount of pre-stored granule mediators (18, 19) and augments IgE-mediated mast cell activation (15, 20, 21), and it can therefore potentially aggravate an allergic reaction.

Most of the pioneering experimental studies that have analyzed the effects of TSLP and IL-33 on mast cell degranulation and cytokine production have explored the acute treatment of mast cells, i.e., a timescale of minutes to a few hours (22). Those results can probably be accurately transferred to an acute inflammatory situation in which mast cells should respond to "danger," such as trauma. However, during prolonged chronic inflammation, such as asthma, the expression of IL-33 is increased in both epithelial cells and airway smooth muscle cells (23, 24), two compartments of the asthmatic lung that are associated with increased mast cell numbers (25–27). We therefore asked how human mast cell functions are affected by acute and prolonged exposure to IL-33 and/or TSLP. The expression profiles of receptors for IL-33 and TSLP were analyzed in human lung mast cells (HLMCs), primary developed mast cells, and mast cell lines. The effects of acute or prolonged exposure to IL-33 and/or TSLP on mediators, receptors, and IgE-mediated activation were analyzed. Our results reveal that IL-33 increases the FcεRI-mediated response when cells are concomitantly exposed to IL-33 and an antigen, whereas prolonged exposure to IL-33 inhibits FcεRI expression and thereby diminishes IgE-mediated mast cell degranulation. IL-33 may therefore play a significant role in the regulation of mast cell reactivity in IgE-associated chronic inflammation.

METHODS

Cell Culture and *in vitro* Stimulations

The human mast cell line ROSAWT KIT (28) was cultured in IMDM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 μg/mL streptomycin, 100 IU/mL penicillin, and 80 ng/mL murine stem cell factor (SCF). Cord blood -derived mast cells (CBMCs) were cultured as previously described (29). Single cell suspensions obtained from human lung tissue, for the analysis of HLMCs, were obtained as previously described (30) and maintained in RPMI 1640 medium (Sigma Aldrich) supplemented with 10% fetal calf serum, 100 ng/ml hSCF, 0.01 M HEPES, 0.5x non-essential amino acids, 2 mM L-glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 48 μM β-mercaptoethanol (Sigma Aldrich). The access to human lung tissue and number of cells obtained was limited therefore these cells were only used for selected experiments, primary CBMC and in some cases the cell-line ROSA cells was used as substitutes. Cells were stimulated with 10 ng/ml TSLP and/or 10 ng/ml IL-33 (Peprotech, Rocky Hill, NJ, USA). The cytokine concentration was chosen based on published data (10, 11, 17). The acute response was analyzed after either 1 h (degranulation and lipid mediator response that occur within 15 min after mast cell activation) or 24 h (cytokine release that occurs later after transcription, translation and secretion) of stimulation and the prolonged response after 4 days with daily addition of the cytokines without media change. To measure the levels of FcεRI receptor (in ROSA cells and CBMCs) and the amount of degranulation induced by FcεRI crosslinking (CBMCs), 10 ng/ml IL-4 (Peprotech) was added 4 days prior (unless otherwise stated) and 1 μg/ml human IgE (Calbiochem, Minneapolis, MN, USA) was added 1 day prior to crosslinking [plasma concentration of IgE in healthy individual is <1 μg/ml, in atopic individuals this is elevated and a plasma concentration above 0.5 μg/ml is predictive of allergy (31)]. After removal of unbound IgE by washing, cells were cross-linked with various concentrations of anti-IgE antibody (Sigma), and calcium ionophore A23187 (2 μM, Sigma) was used as a positive control for activation. In some experiments (indicated in the figure legends) performed to measure lipid mediators, the cells were pretreated with 10 ng/ml IL-4 and 5 ng/ml IL-3 for 4 days.

Measurement of Mediator Release

Released histamine was measured using a histamine release test kit according to the manufacturer's instructions (RefLab, Copenhagen, Denmark). Briefly, this test is based on the adsorption of histamine to glass fiber-coated microtiter plates. The glass fibers bind histamine with high affinity and selectivity. The plates were sent to RefLab, and histamine was detected fluorometrically (OPA-method) by HISTAREADER^{TM} 501-1. PGD_2 was measured using a Prostaglandin D_2-MOX ELISA kit

(Caymen Chemical, Ann Arbor, MI. USA), and the levels of IL-1β, IL-5, MCP-1, MIP-1α, GM-CSF, and TNF were analyzed with Luminex (BioRad, Hercules, CA, USA).

Flow Cytometry

The following antibodies were used: ST2-FITC (clone B4E6, MD Bioproducts, Zürich, Switzerland), IL7R-PE (clone A019D5, Biolegend, San Diego, CA, USA), TSLP-R-PE (clone 1B4, Biolegend), FcεRIα-PE (clone AER-37 (CRA-1), Biolegend), CD63-Pe-Cy7 (Clone H5C6, BD Biosciences, San Jose, CA, USA), tryptase (clone G3, Millipore, Burlington, MA, USA) conjugated in-house with an Alexa Fluor 647 Monoclonal antibody labeling kit (Invitrogen), chymase (clone B7, Millipore) conjugated in-house with a PE Conjugation Kit (Abcam, Cambridge, UK) or CPA3 (clone CA5, a kind gift from Andrew Walls, Southampton, UK) conjugated in-house with an Alexa Fluor™ 488 Antibody Labeling Kit (Thermo Fisher Scientific, Waltham, MA, USA). Human lung cells were stained with BD Horizon™ Fixable Viability Stain 450 (BD Biosciences) and CD45-V500 (Clone HI30, BD Biosciences), CD14- APC-Cy7 (Clone M5E2, Biolegend), and CD117-APC (clone 104D2, BD Biosciences) antibodies; and mast cells were gated as live, $CD45^{+}$, $CD14^{low}$ $CD117^{high}$. For intracellular staining, cells were fixed with 4% PFA and permeabilized using PBS-S buffer (0.1% saponin in PBS with 0.01 M HEPES). Unspecific binding was blocked using blocking buffer (PBS-S with 5% dry milk and 2% FCS). The cells were analyzed using a BD FACSCanto system (BD, Franklin Lakes, NJ, USA), and FlowJo software (FlowJo LLC, Ashland, OR, USA) was used for flow cytometry data analysis.

Quantitative PCR

RNA was extracted using an RNeasy Plus Mini Kit (Qiagen, Hilden, Germany), cDNA was prepared using an iScript cDNA synthesis kit (Bio-Rad), and qPCR was performed using iTaq Universal SYBR Green Supermix (Biorad) on a CFX96 Real-time system (Biorad). The following primers were used: GAPDH (5′- CCACATCGCTCAGACACCAT-3′ and 5′-GGCAACAATATCCACTTTACCAGAG-3′), FcεRIα (5′-CGC GTGAGAAGTACTGGCTA-3′ and 5′-TGTGACCTGCTGCTG AGTTG-3′), FcεRIβ (5′-TGCAGTAAGAGGAAATCCACCA-3′ and 5′-TGTGTTACCCCCAGGAACTC-3′), and FcεRIγ (5′-CCAGCAGTGGTCTTGCTCTT-3′ and 5′-AGGCCCGTGTAA ACACCATC-3′). The results were calculated using the ΔΔCT method, and CT values were normalized to the housekeeping gene GAPDH and related to the unstimulated control.

Statistical Analysis

Data are shown as the mean ± the standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism software version 7.0b. D'Agostino and Pearson normality test or when n was smaller than eight Shapiro-Wilk normality test was performed. For normally distributed data Student's *t*-test was performed when comparing two groups, and when more than two groups were compared, one- or two-way ANOVA with Bonferroni's *post hoc* test was performed. For data that did not pass the normality test, Mann-Whitney or Wilcoxon test (paired data) was performed when comparing two groups, and when comparing more than two groups Kruskal-Wallis with Dunn's multiple comparisons test was performed (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

RESULTS

Expression of Receptors for IL-33 and TSLP on Mast Cells

The surface expression levels of different receptors for IL-33 (ST2) and TSLP (TSLP-R and IL7R) were analyzed in mast cell line ROSA and primary CBMCs and HLMCs by flow cytometry. The surface expression level of TSLP-R was higher in ROSA cells than in the primary CBMCs and HLMCs (**Figure 1A**). IL7R staining was very low in all cells analyzed (**Figure 1B**) but all cells had detectable levels of the IL-33 receptor ST2 (**Figure 1C**). When the mast cells were treated for 4 days with IL-33, the surface expression level of TSLP-R increased, and this effect was counteracted by TSLP, possibly via the internalization of the receptor (**Figure 1D**). The addition of TSLP also reduced surface staining for IL7R (**Figure 1E**). To confirm that TSLP induced the internalization of the receptor we also stained fixed and permeabilized cells, staining both surface, and intracellular TSLP-R and in this case the TSLP-R was equally induced in the IL-33 and the IL-33 plus TSLP group (**Supplementary Figure 1**). None of the cytokines had any significant effect on ST2 receptor expression (**Figure 1F**).

Degranulation After Prolonged Exposure to IL-33 and TSLP

It has been reported that neither IL-33 nor TSLP causes mast cell degranulation (9–11, 20, 32), and our results confirm this finding because no histamine was released after 1 h of stimulation (**Figures 2A,C**); however, after 4 days of stimulation, IL-33 caused a small but significant increase in the release of histamine in CBMCs (**Figure 2D**), and the combined addition of IL-33 and TSLP induced histamine release in ROSA cells (**Figure 2B**).

Prostaglandin D_2 and Cytokine Release Induced by IL-33

The results of reports regarding whether IL-33 induces the release of lipid mediators in mast cells have been inconsistent (10, 12, 14–17). We observed considerable variation in the basal levels of PGD_2 released from different CBMC cultures: in some, there appeared to be an increase following IL-33 stimulation, whereas there was no change in others. Collectively, our results indicated there was no significant increase overall (**Figure 3A**). Pretreating the cells with IL-4 and IL-3 for 4 days increased the amount of PGD_2 released but did not increase the number of CBMC cultures that responded to IL-33 (**Figure 3B**). Increasing the IL-33 concentration from 10 to 100 ng/ml did not increase the number of CBMC cultures that responded either (data not shown). TSLP did not induce any PGD_2 release (data not shown). All cultures treated with calcium ionophore A23187 (used as a positive control) exhibited a substantial increase in the amount of PGD_2 released following this treatment (**Figure 3C**). There

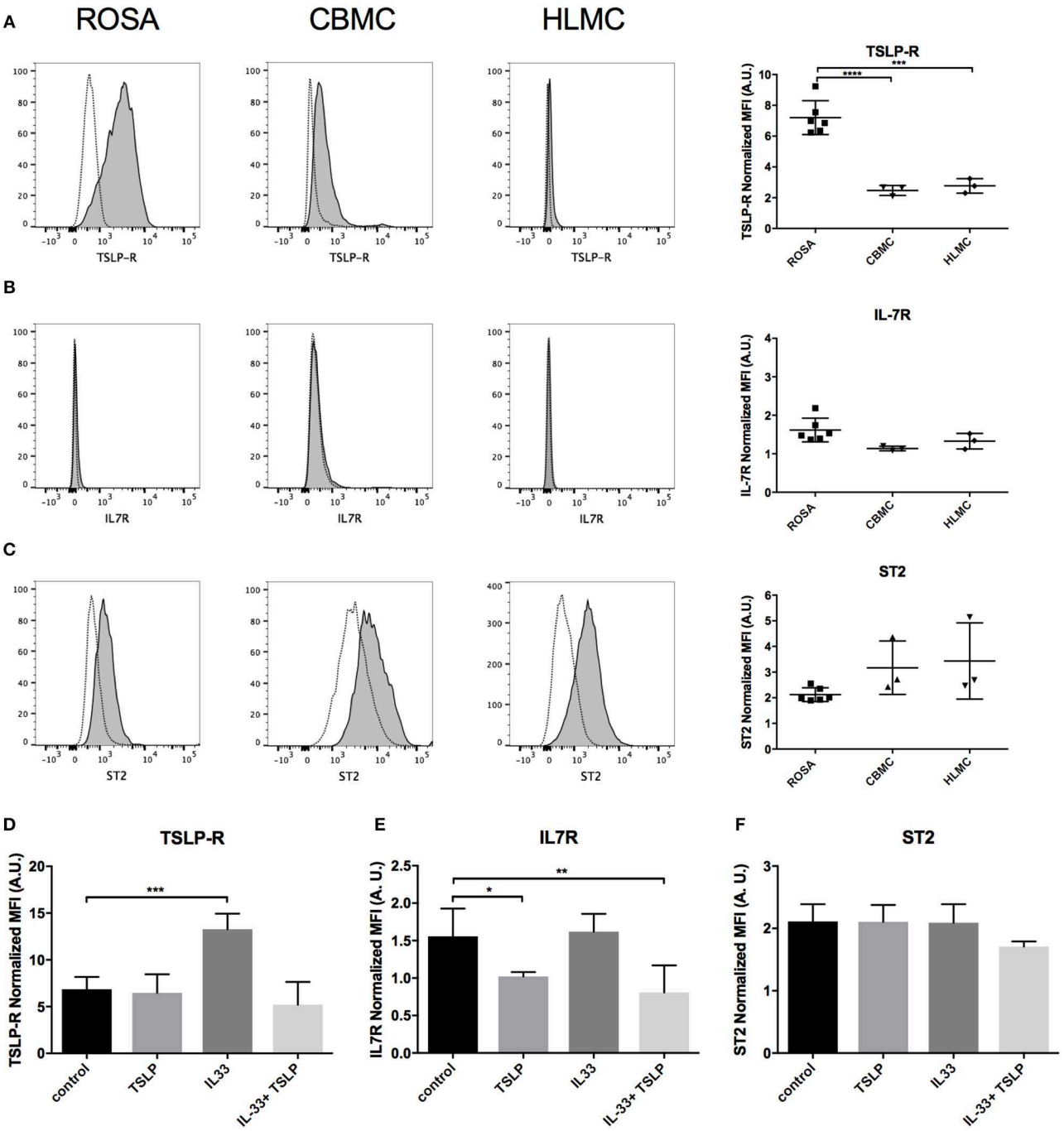


FIGURE 1 | Expression of receptors for IL-33 (ST2) and TSLP (TSLP-R/IL7R) on various human mast cells. Surface expression of TSLP-R **(A)**, IL7R **(B),** and ST2 **(C)** on ROSA **(A–F)**, CBMC **(A–C),** and HLMCs **(A–C)** was measured by flow cytometry. Representative histograms in which dotted lines represent the respective isotype controls are shown for different cell lines to the left, and quantification of the data is shown to the right **(A–C)**. Mast cells were treated with 10 ng/ml IL-33, TSLP or a combination or both repeatedly for 4 days; thereafter, surface expression of TSLP-R **(D)**, IL7R **(E),** and ST2 **(F)** was measured by flow cytometry. The median fluorescent intensity (MFI) of the receptors was normalized to the respective isotype control. Data shown were pooled from three independent experiments, $n = 3–6$.

was no detectable release of cysteine leukotrienes in response to IL-33 or TSLP (data not shown). Mast cells can store some cytokines in their granules and release them directly upon degranulation, but activation can also induce *de-novo* synthesis of cytokines (33). Since we did not detect any degranulation by IL-33 (**Figures 2A,C**) we measured cytokine release after 24 h to detect *de-novo* synthesized cytokines. IL-33 caused a significant increase in the cytokines IL-5, GM-CSF and TNF (**Figures 3D–F**)

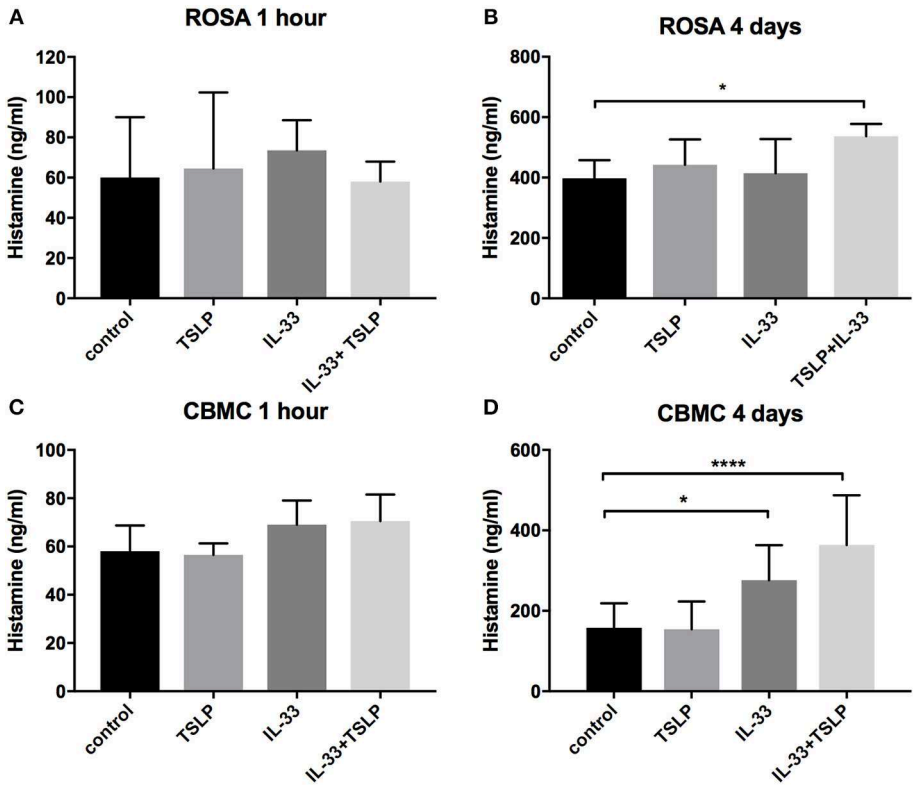


FIGURE 2 | Histamine release induced by prolonged exposure to IL-33. ROSA cells **(A,B)** or CBMCs **(C,D)** were treated with 10 ng/ml IL-33, TSLP or a combination of both for 1 h **(A,C)** or treated repeatedly (every day) for 4 days **(B,D)**. Thereafter, supernatants were collected, and histamine was measured. Data shown were pooled from 3 to 5 independent experiments, $n = 6–10$.

but did not affect the amount of IL-1β, MCP-1 (CCL2), and MIP-1α (CCL3) released (**Figures 3G–I**).

Effects on Mediator Storage by IL-33 and TSLP

Next, we investigated whether the storage of mast cell mediators would be affected by 4 days of exposure to IL-33 and TSLP. Intracellular tryptase was increased by IL-33 but decreased by TSLP in ROSA cells (**Figure 4A**). IL-33 also increased tryptase storage in CBMCs, while TSLP alone had no effect in these mast cells (**Figure 4B**). Neither IL-33 nor TSLP had any effect on the intracellular storage of the mast cell proteases chymase and CPA3 in CBMCs (**Figures 4C,D**). Chymase was not detectable and CPA3 was very low in ROSA cells, and the addition of IL-33 and/or TLSP did not affect the levels (data not shown). Nor did IL-33 and/or TLSP affect the storage of intracellular histamine (**Figures 4E,F**).

Effects on IgE-Mediated Degranulation by IL-33

It has previously been demonstrated that IL-33 potentiates IgE-mediated degranulation (15, 19–21). Pretreatment with IL-33 for 1 h prior to FcεRI crosslinking increased both surface CD63 expression and histamine release (**Figures 5A,C**). In contrast, when CBMCs were repeatedly treated with IL-33 for 4 days, IgE-mediated degranulation was significantly decreased, with reduced induction of CD63 surface expression and histamine release compared to the untreated group (**Figures 5B,D**). Pretreatment with TSLP for 4 days had no effect on IgE-mediated degranulation (data not shown).

FcεRI Surface Receptor Expression Is Decreased by 4 Days of IL-33 Treatment

To further investigate a possible mechanism that could underlie the observed decrease in degranulation, we next investigated the effect of IL-33 and TSLP on surface expression of the FcεRI receptor. We found that 4 days of treatment with IL-33 caused a dramatic decrease in the surface expression of the FcεRI receptor in ROSA cells, CBMCs, and HLMCs (**Figures 6A–C**). In ROSA cells, TSLP also caused a significant drop in FcεRI surface staining (**Figure 6A**). Since human mast cells grown in culture express very low levels of FcεRI, we added IL-4 to upregulate the receptor in ROSA cells and CBMCs (**Figures 6A,B**). To investigate whether IL-33 reduces receptor expression simply by blocking IL-4-mediated receptor upregulation, IL-4 was added 4 days prior to a media change, and IL-33 and TSLP addition. Also, in this experiment, IL-33 downregulated the FcεRI receptor, indicating that a blockade of IL-4-mediated upregulation is not the mechanism that reduces FcεRI receptor

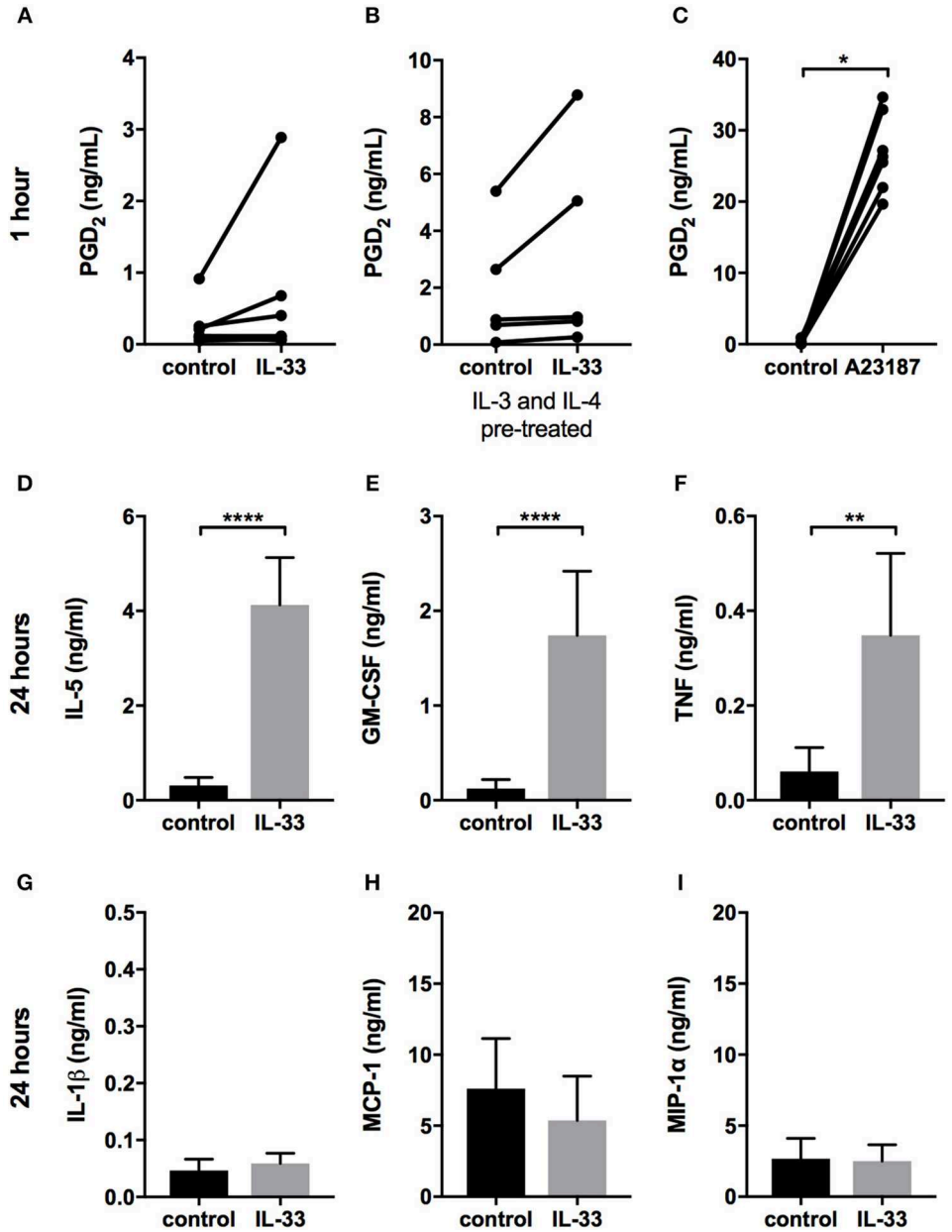


FIGURE 3 | Prostaglandin D_2 and cytokine release in response to IL-33. CBMCs were treated for 1 h **(A–C)** or 24 h **(D–I)** with 10 ng/ml IL-33 or 2 μM A23187, and supernatants were collected. PGD_2 **(A–C)** was measured using ELISA, and cytokines were measured using Luminex assays **(D–I)**. The CBMCs shown in **(B)** were pretreated with 10 ng/ml IL-4 and 5 ng/ml IL-3 for 4 days. Data shown were pooled from 5 to 9 independent experiments, $n = 5–9$.

surface expression. However, the reduction of FcεRI by TSLP was absent in this case, indicating that TSLP is blocking the IL-4 mediated upregulation (**Figures 6D,E**). In addition, we exposed ROSA cells to IL-33 and TSLP without the addition of IL-4 and although the receptor expression is low in this case, IL-33 caused a reduction (**Supplementary Figure 2**). HLMC that are extracted from human lung tissue have a natural high FcεRI expression therefore IL-4 was not added to these cells and IL-33 caused a decrease also in these cells (**Figure 6C**). Altogether, these data indicate that the downregulation of FcεRI by IL-33 is independent of IL-4.

IL-33 Decreases the Expression of FcεRI

The FcεRI receptor consists of four chains, including one α-, one β- and two γ-chains, which are regulated at both the protein and mRNA levels (34). We therefore next investigated whether IL-33 affects the mRNA expression of the different subunits of this receptor. We found that 4 days of treatment with IL-33

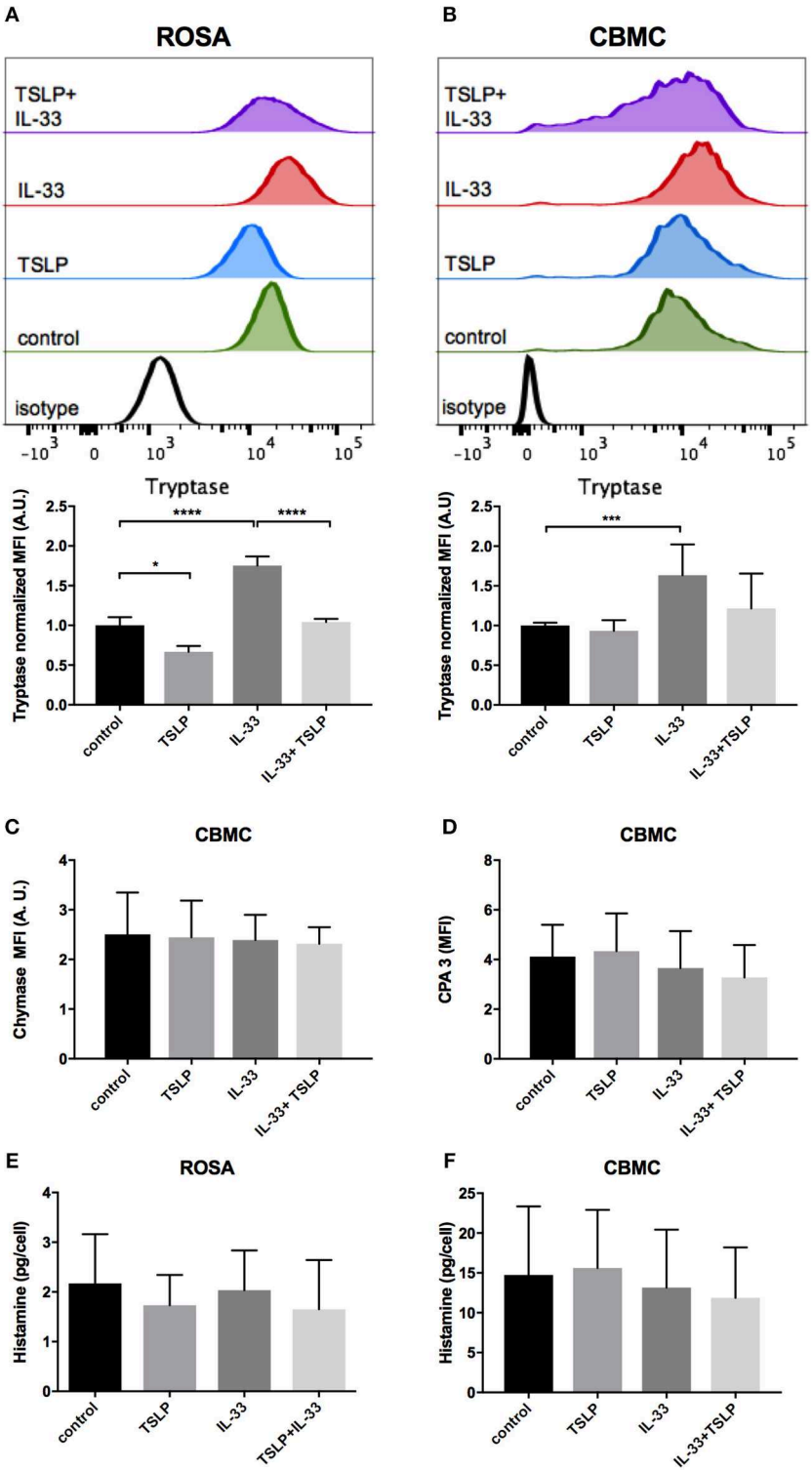


FIGURE 4 | Changes in mediator storage after 4 days of treatment with IL-33 and TSLP. ROSA cells **(A,E)** or CBMCs **(B–D,F)** were repeatedly treated every day for 4 days with 10 ng/ml IL-33, TSLP or a combination of both; thereafter, the levels of the proteases tryptase **(A,B)**, chymase **(C)**, and CPA3 **(D)** were measured by intracellular flow cytometry staining, and total histamine content **(E,F)** was measured by a histamine release test kit. The MFI of protease expression was normalized to the respective isotype control. **(A)** a representative of three independent experiments, $n = 3$. Data shown were pooled from 5 **(B)**, 2 **(C,D)**, 3 **(E),** and 6 **(F)** independent experiments, $n = 4–12$.

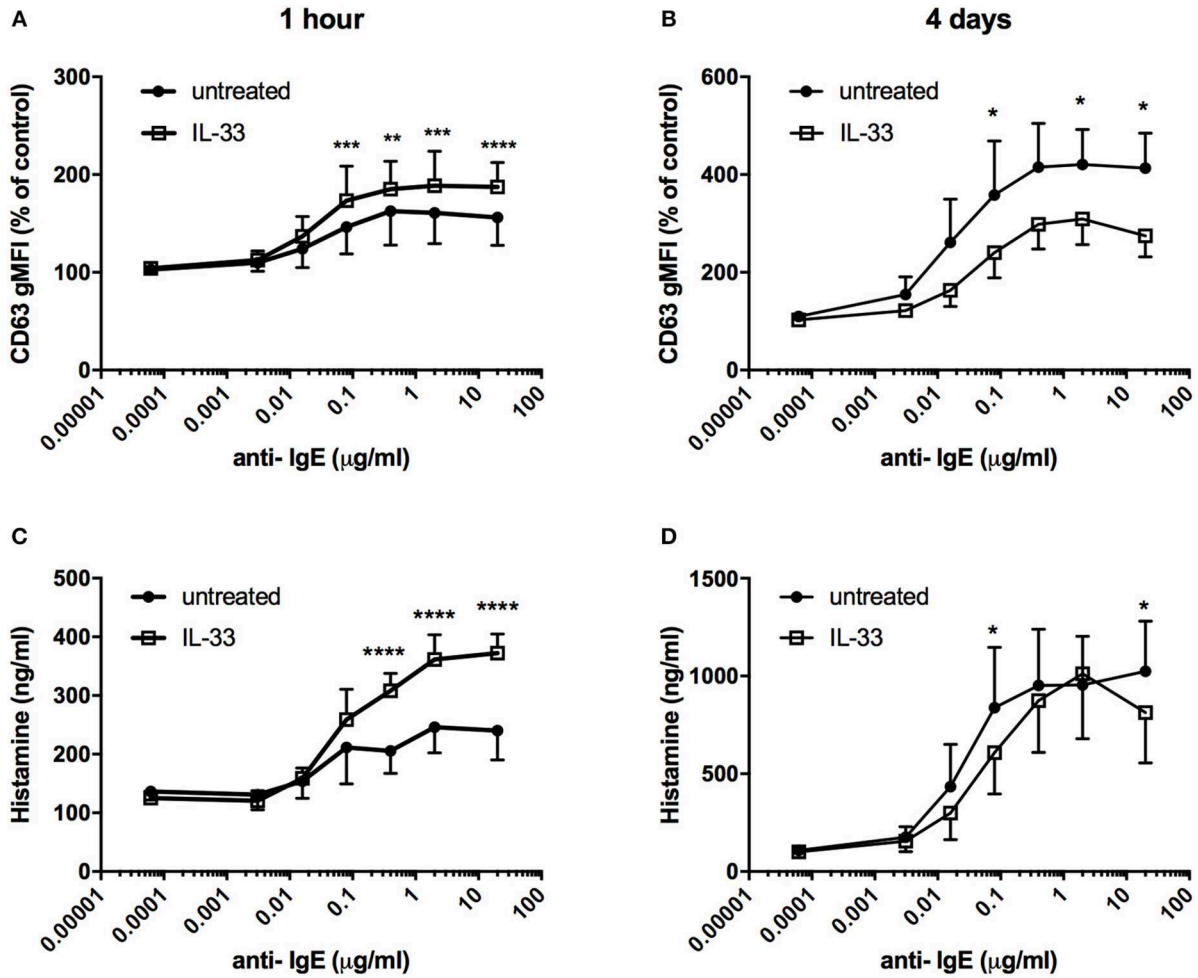


FIGURE 5 | Effect of IL-33 on mast cell degranulation by FcεRI crosslinking. CBMCs were treated with 10 ng/ml IL-33 for 1 h **(A,C)** or repeatedly every day for 4 days **(B,D)**; thereafter, they were stimulated various concentrations of anti-IgE. Degranulation was measured by the detection of surface CD63 with flow cytometry **(A,B)** or as histamine release **(C,D)**. Data shown were pooled from 3 independent experiments, n = 3.

significantly reduced the levels of all subunits, indicating that the relevant regulatory mechanism occurs at the mRNA level (**Figures 7A–C**).

DISCUSSION

Compelling evidence indicates that IL-33 acts as an alarmin to activate mast cells in an acute manner, resulting in the release of pro-inflammatory mediators (22). In the present study, we demonstrate an opposite effect of IL-33 by which prolonged exposure to IL-33, down-regulates the high-affinity IgE receptor and reduces the allergic response (**Figures 5**, **6**). Thus, IL-33 appears to have divergent functions on mast cells, including an acute effect by which it induces the release of cytokines (**Figure 3**) and consequentially acute inflammation and a dampening effect observed in cells exposed to IL-33 for a longer period, such as during chronic inflammation (2).

We also investigated the surface expression of receptors for IL-33 and TSLP, including ST2, TSLP-R and IL7R, in the mast cell line ROSA as well as primary CBMCs and HLMCs. Similar to previously published reports, we found that all of these cells expressed the ST2 receptor (**Figure 1C**) (10, 11, 14, 35); however, contrary to Kaur et al. (35), we did not observe any increase in the surface expression of ST2 after IL-33 treatment (**Figure 1F**), possibly because we exposed the cells to IL-33 for different periods of time (24 h vs. 4 days). TSLP-R surface staining was stronger in the mast cell lines than in primary mast cells (**Figure 1A**), and the ROSA mast cells were the only cells that responded to TSLP, with no response observed in the primary cells (**Figures 4A,B, 6A–E**). However, exposure to IL-33 upregulated TSLP-R surface expression (**Figure 1D**), and in some cases, we also observed that TSLP affected primary cells when added in combination with IL-33 (**Figure 2D**), possibly because of the upregulation of its receptor. IL7R surface staining was very low in all cells, but when cells were treated with TSLP, it was

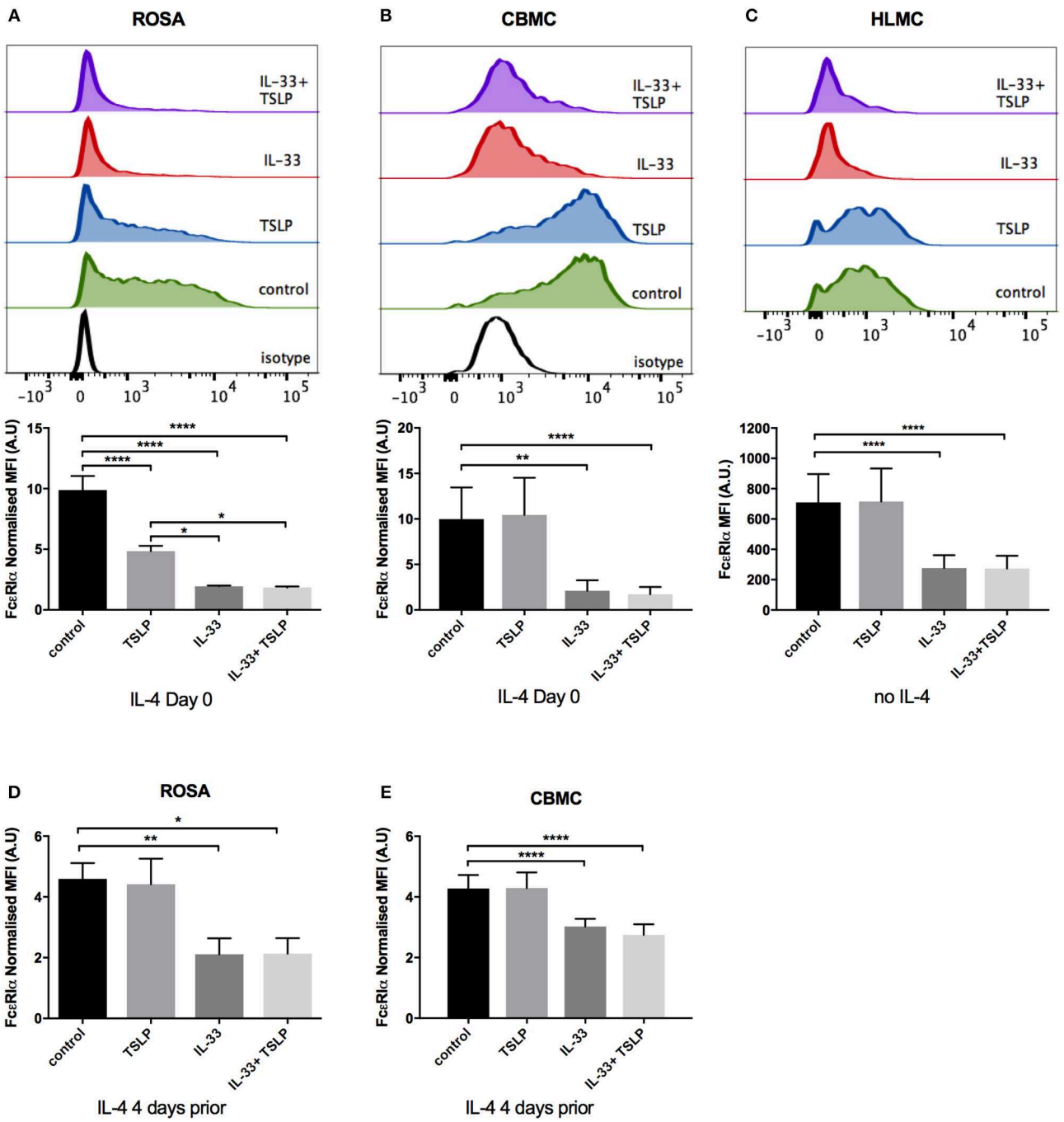


FIGURE 6 | Decrease in FcεRI surface expression by IL-33. ROSA cells **(A,D)**, CBMCs **(B,E)**, or HLMCs **(C)** were repeatedly treated every day for 4 days with 10 ng/ml IL-33, TSLP or a combination of both. In addition, IL-4 was added on day 0 **(A,B)** or 4 days prior to the addition of IL-33 and TSLP **(D,E)** to upregulate the FcεRI receptor. IL-4 was not added to HLMCs **(C)**. Surface FcεRIα receptor expression was measured by flow cytometry; representative histograms are shown in **(A–C)** and quantification of the expression is shown below. The MFI of FcεRI was normalized to that of the isotype control. Data shown were pooled from 3 to 5 independent experiments, $n = 6–10$.

even lower, suggesting that although it is expressed at very low levels, it is still functional and internalized upon ligand binding (**Figure 1E**).

Several studies have previously demonstrated that IL-33 induces the release of cytokines from mast cells (10–12, 14, 15, 20, 21, 32, 36). Studies have also shown that IL-33 induces the

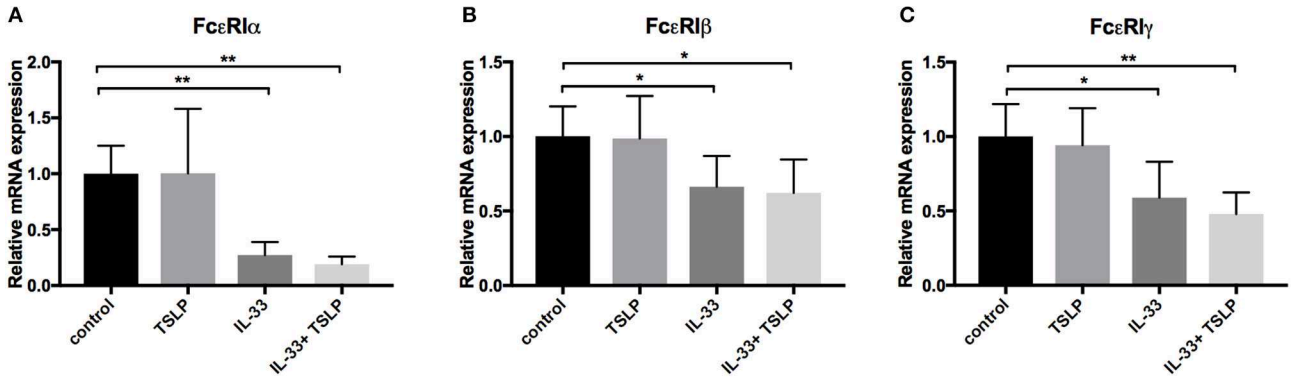


FIGURE 7 | Decrease in mRNA expression of the FcεRI receptor by IL-33. CBMCs were repeatedly treated every day for 4 days with 10 ng/ml IL-33, TSLP or a combination of both. In addition, IL-4 was added on day 0. The mRNA expression level of the different subunits of the FcεRI receptor were quantified using qPCR **(A–C)**. Expression was normalized to that of the housekeeping gene GAPDH and thereafter to the unstimulated control. Data shown were pooled from six independent experiments, $n = 6$.

release of lipid mediators (12, 14, 16, 17), while other studies have reported no lipid mediator release in response to IL-33 (10, 11, 15). We also observed that IL-33 induced the release of cytokines (**Figures 3D–I**); however, there was high donor to donor variation in PGD_2 release, resulting in no significant change overall (**Figures 3A,B**). The reason for this variation between donors remains unresolved, and further studies are needed to determine why some patients do not release PGD_2 in response to IL-33.

IL-33 has been proposed to be important for the maturation of mast cells because it increases storage of the mast cell proteases tryptase and CPA3 as well as the amine serotonin (10, 16, 18, 19). In our experiments, 4 days of exposure to IL-33 also increased, whereas TSLP decreased, the intracellular level of tryptase. These findings are in contrast to the results presented by Lai et al. who found that storage of tryptase was increased by TSLP (16). This difference could be because we used mature CBMCs (more than 8 weeks in culture), while they added TSLP when the cultures of cord blood cells were begun in order to develop them into mast cells. They also cultured the cells in the presence of TSLP for 3 weeks, while we did so for only 4 days. Interestingly, this was the only experiment in our study in which IL-33 and TSLP exerted opposing effects. We did not observe any change in chymase and CPA3 expression after 4 days of stimulation, in agreement with the results presented in Lai et al. (16). However, they reported that CPA3 expression was increased after 3 weeks of treatment. Human mast cells do not contain as much serotonin as is contained in mouse mast cells, but they store more histamine (37). We therefore investigated whether the storage of histamine was affected by IL-33 and TSLP, but we observed no change after 4 days of treatment (**Figures 4E,F**).

Previous studies have shown that neither IL-33 nor TSLP alone induces acute mast cell degranulation (21, 38, 39), and this finding was also confirmed in this study (**Figures 2A,C**). However, 4 days of exposure to IL-33 induced partial exocytosis and histamine release (**Figures 2B,D**). Similar to previous studies (21, 39), we found that 1 h of pretreatment with IL-33 strongly potentiated the mast cell degranulation induced by crosslinkage of the IgE- receptor (**Figures 5A,C**). In contrast, 4 days of exposure to IL-33 had the opposite effect, with the treated cells exhibiting less degranulation than was observed in the untreated group (**Figures 5B,D**). Jung et al. also showed that prolonged exposure to IL-33 induced a hyporesponsive phenotype in mast cells. They investigated the cause of this hyporesponsiveness in mouse mast cells and observed that while there was no difference in FcεRI receptor expression, but Hck expression was decreased (40). We did not observe any change in Hck expression (data not shown) in human mast cells, but FcεRI surface receptor expression dropped dramatically in both human ROSA cells and primary CBMCs as well as in primary mast cells isolated from human lungs (**Figures 6A–C**). FcεRI expression can be regulated at both the mRNA and protein level (34); IL-33 mediated the downregulation of FcεRI by decreasing the mRNA expression levels of the different subunits (**Figures 7A–C**). Since IL-33 activates mast cells to release various cytokines it is not clear if the observed long-term effect of IL-33 is a direct effect or if it is a secondary consequence of the mediators that are released by IL-33 activation. This warrants further investigation to clarify.

Mast cells are very potent pro-inflammatory cells, and the systemic activation of mast cells can lead to anaphylaxis and potentially death. Mast cell activation must therefore be tightly regulated (41). IL-33 is a potent pro-inflammatory cytokine, and it is clear that acute exposure to IL-33 activates mast cells and increases their responsiveness to antigens. This is a potentially dangerous situation, and we therefore suggest that after prolonged exposure to IL-33, mast cells down-regulate the FcεRI receptor in a negative feedback loop to prevent damage caused by excessive mast cell activation.

ETHICS STATEMENT

The local ethics committee approved the experiments involving human subjects, i.e., the collection of lung tissue from patients

undergoing lobectomies, and all patients provided informed consent. In accordance with Swedish legislation, ethics approval was not needed for the anonymous collection of cord blood because the samples cannot be traced to a specific person.

AUTHOR CONTRIBUTIONS

ER involved in conception and designed of study, performed experiments, analyzed data, and wrote manuscript. GN involved in conception and design of study and wrote manuscript. AG, CC, MatE, and MarE planned and performed experiments. JS provided clinical samples. MA provided reagents. All authors contributed to manuscript writing.

ACKNOWLEDGMENTS

This manuscript has been released as a Pre-Print at bioRxiv (42). We thank SOBI, Stockholm, Sweden, for generously gifting SCF and Andrew Walls for generously gifting the CPA3 antibody. This study was supported by grants from the Swedish Research Council; the Heart-Lung Foundation; the Ollie and Elof Ericssons foundation; the Ellen, Walter and Lennart Hesselman's foundation; Tore Nilssons Foundation; the Lars Hiertas memory fund; the Konsul Th C Berghs Foundation; the Tornspiran Foundation; the O. E. and Edla Johanssons Foundation; the Swedish Society for Medical Research; The ChAMP (Centre for Allergy Research Highlights Asthma Markers of Phenotype) consortium funded by the Swedish Foundation for Strategic Research; the AstraZeneca and Science for Life Laboratory Joint Research Collaboration; and the Karolinska Institutet.

ChAMP collaborators: Ann-Charlotte Orre, Mamdoh Al-Ameri, Mikael Adner, and Sven-Erik Dahlén.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01361/full#supplementary-material

REFERENCES

1. Ohno T, Morita H, Arae K, Matsumoto K, Nakae S. Interleukin-33 in allergy. *Allergy.* (2012) 67:1203–14. doi: 10.1111/all.12004
2. Makrinioti H, Toussaint M, Jackson DJ, Walton RP, Johnston SL. Role of interleukin 33 in respiratory allergy and asthma. *Lancet Respir Med.* (2014) 2:226–37. doi: 10.1016/S2213-2600(13)70261-3
3. Mitchell PD, O'byrne PM. Epithelial-derived cytokines in asthma. *Chest.* (2017) 151:1338–44. doi: 10.1016/j.chest.2016.10.042
4. Vicente CT, Revez JA, Ferreira MAR. Lessons from ten years of genome-wide association studies of asthma. *Clin Transl Immunol.* (2017) 6:e165. doi: 10.1038/cti.2017.54
5. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in adults with uncontrolled asthma. *N Engl J Med.* (2017) 377:936–46. doi: 10.1056/NEJMoa1704064
6. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89. doi: 10.1038/nri.2016.95
7. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol.* (2017) 8:475. doi: 10.3389/fimmu.2017.00475
8. Erjefalt JS. Mast cells in human airways: the culprit? *Eur Respir Rev.* (2014) 23:299–307. doi: 10.1183/09059180.00005014
9. Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S, et al. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med.* (2007a) 204:253–8. doi: 10.1084/jem.20062211
10. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol.* (2007b) 179:2051–4. doi: 10.4049/jimmunol.179.4.2051
11. Iikura M, Suto H, Kajiwara N, Oboki K, Ohno T, Okayama Y, et al. IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest.* (2007) 87:971–8. doi: 10.1038/labinvest.3700663
12. Enoksson M, Lyberg K, Moller-Westerberg C, Fallon PG, Nilsson G, Lunderius-Andersson C. Mast cells as sensors of cell injury through IL-33 recognition. *J Immunol.* (2011b) 186:2523–8. doi: 10.4049/jimmunol.1003383
13. Enoksson M, Moller-Westerberg C, Wicher G, Fallon PG, Forsberg-Nilsson K, Lunderius-Andersson C, et al. Intraperitoneal influx of neutrophils in response to IL-33 is mast cell dependent. *Blood.* (2013) 121:530–6. doi: 10.1182/blood-2012-05-434209
14. Moulin D, Donze O, Talabot-Ayer D, Mezin F, Palmer G, Gabay C. Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine.* (2007) 40:216–25. doi: 10.1016/j.cyto.2007.09.013
15. Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res.* (2010) 59:207–18. doi: 10.1007/s00011-009-0088-5
16. Lai Y, Altemeier WA, Vandree J, Piliponsky AM, Johnson B, Appel CL, et al. Increased density of intraepithelial mast cells in patients with exercise-induced bronchoconstriction regulated through epithelially derived thymic stromal lymphopoietin and IL-33. *J Allergy Clin Immunol.* (2014) 133:1448–55. doi: 10.1016/j.jaci.2013.08.052
17. Buchheit KM, Cahill KN, Katz HR, Murphy KC, Feng C, Lee-Sarwar K, et al. Thymic stromal lymphopoietin controls prostaglandin D2 generation in patients with aspirin-exacerbated respiratory disease. *J Allergy Clin Immunol.* (2016) 137:1566–76 e1565. doi: 10.1016/j.jaci.2015.12.777
18. Kaieda S, Shin K, Nigrovic PA, Seki K, Lee RT, Stevens RL, et al. Synovial fibroblasts promote the expression and granule accumulation of tryptase via interleukin-33 and its receptor ST-2 (IL1RL1). *J Biol Chem.* (2010) 285:21478–86. doi: 10.1074/jbc.M110.114991
19. Sjoberg LC, Gregory JA, Dahlen SE, Nilsson GP, Adner M. Interleukin-33 exacerbates allergic bronchoconstriction in the mice via activation of mast cells. *Allergy.* (2015) 70:514–21. doi: 10.1111/all.12590
20. Andrade MV, Iwaki S, Ropert C, Gazzinelli RT, Cunha-Melo JR, Beaven MA. Amplification of cytokine production through synergistic activation of NFAT and AP-1 following stimulation of mast cells with antigen and IL-33. *Eur J Immunol.* (2011) 41:760–72. doi: 10.1002/eji.201040718
21. Joulia R, L'faqihi FE, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. *J Allergy Clin Immunol.* (2017) 140:497–509 e410. doi: 10.1016/j.jaci.2016.09.049
22. Saluja R, Zoltowska A, Ketelaar ME, Nilsson G. IL-33 and thymic stromal lymphopoietin in mast cell functions. *Eur J Pharmacol.* (2016) 778:68–76. doi: 10.1016/j.ejphar.2015.04.047
23. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol.* (2009) 183:5094–103. doi: 10.4049/jimmunol.0802387
24. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol.* (2010) 125:752–4. doi: 10.1016/j.jaci.2009.12.935

25. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Eng J Med.* (2002) 346:1699–705. doi: 10.1056/NEJMoa012705
26. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, et al. Mast cell phenotype, location, and activation in severe asthma: data from the severe asthma research program. *Am J Respir Crit Care Med.* (2011) 183:299–309. doi: 10.1164/rccm.201002-0295OC
27. James A, Gyllfors P, Henriksson E, Dahlén S, Adner M, Nilsson G, et al. Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi. *Allergy.* (2012) 67:958–61. doi: 10.1111/j.1398-9995.2012.02836.x
28. Saleh R, Wedeh G, Herrmann H, Bibi S, Cerny-Reiterer S, Sadovnik I, et al. A new human mast cell line expressing a functional IgE receptor converts to tumorigenic growth by KIT D816V transfection. *Blood.* (2014) 124:111–20. doi: 10.1182/blood-2013-10-534685
29. Enoksson M, Ejendal KF, Mcalpine S, Nilsson G, Lunderius-Andersson C. Human cord blood-derived mast cells are activated by the Nod1 agonist M-TriDAP to release pro-inflammatory cytokines and chemokines. *J Innate Immun.* (2011a) 3:142–9. doi: 10.1159/000321933
30. Ravindran A, Ronnberg E, Dahlin JS, Mazzurana L, Safholm J, Orre AC, et al. An optimized protocol for the isolation and functional analysis of human lung mast cells. *Front Immunol.* (2018) 9:2193. doi: 10.3389/fimmu.2018.02193
31. Amarasekera M. Immunoglobulin E in health and disease. *Asia Pac Allergy.* (2011) 1:12–5. doi: 10.5415/apallergy.2011.1.1.12
32. Ho LH, Ohno T, Oboki K, Kajiwara N, Suto H, Iikura M, et al. IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcepsilonRI signals. *J Leukoc Biol.* (2007) 82:1481–90. doi: 10.1189/jlb.0407200
33. Zhang Y, Ramos BF, Jakschik BA. Neutrophil recruitment by tumor necrosis factor from mast cells in immune complex peritonitis. *Science.* (1992) 258:1957–9. doi: 10.1126/science.1470922
34. Kraft S, Kinet JP. New developments in FcepsilonRI regulation, function and inhibition. *Nat Rev Immunol.* (2007) 7:365–78. doi: 10.1038/nri2072
35. Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R, et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. *Allergy.* (2015) 70:556–67. doi: 10.1111/all.12593
36. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. *Eur J Immunol.* (2015) 45:3034–44. doi: 10.1002/eji.201545501
37. Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A, Metcalfe DD. Human mast cells are capable of serotonin synthesis and release. *J Allergy Clin Immunol.* (2007) 119:498–9. doi: 10.1016/j.jaci.2006.09.003
38. Fux M, Pecaric-Petkovic T, Odermatt A, Hausmann OV, Lorentz A, Bischoff SC, et al. IL-33 is a mediator rather than a trigger of the acute allergic response in humans. *Allergy.* (2014) 69:216–22. doi: 10.1111/all.12309
39. Cop N, Ebo DG, Bridts CH, Elst J, Hagendorens MM, Mertens C, et al. Influence of IL-6, IL-33, and TNF-alpha on human mast cell activation: lessons from single cell analysis by flow cytometry. *Cytometry B Clin Cytom.* (2017) 94:405–11. doi: 10.1002/cyto.b.21547
40. Jung MY, Smrz D, Desai A, Bandara G, Ito T, Iwaki S, et al. IL-33 induces a hyporesponsive phenotype in human and mouse mast cells. *J Immunol.* (2013) 190:531–8. doi: 10.4049/jimmunol.1201576
41. Halova I, Ronnberg E, Draberova L, Vliagoftis H, Nilsson GP, Draber P. Changing the threshold-Signals and mechanisms of mast cell priming. *Immunol Rev.* (2018) 282:73–86. doi: 10.1111/imr.12625
42. Rönnberg E, Ghaib A, Ceriol C, Enoksson E, Arock M, Säfholm J, et al. Divergent effects of acute and prolonged interleukin 33 exposure on mast cell IgE-mediated functions. *bioRxiv.* (2018). doi: 10.1101/463950

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Rönnberg, Ghaib, Ceriol, Enoksson, Arock, Säfholm, Ekoff and Nilsson. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 21 June 2019
doi: 10.3389/fimmu.2019.01394

Interleukin 33/ST2 Axis Components Are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer

*Glauben Landskron[1], Marjorie De la Fuente López[1,2], Karen Dubois-Camacho[1], David Díaz-Jiménez[1], Octavio Orellana-Serradell[1], Diego Romero[3], Santiago A. Sepúlveda[3], Christian Salazar[1], Daniela Parada-Venegas[1], Rodrigo Quera[4], Daniela Simian[2], María-Julieta González[5], Francisco López-Köstner[6], Udo Kronberg[6], Mario Abedrapo[6,7], Iván Gallegos[8], Héctor R. Contreras[9], Cristina Peña[10], Guillermo Díaz-Araya[11], Juan Carlos Roa[3] and Marcela A. Hermoso[1]**

[1] Immunology Program, Innate Immunity Laboratory, Faculty of Medicine, Biomedical Sciences Institute, Universidad de Chile, Santiago, Chile, [2] Research Sub-direction, Academic Direction, Clinica Las Condes, Santiago, Chile, [3] Pathology Department, Faculty of Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile, [4] Inflammatory Bowel Disease Program, Gastroenterology Department, Clinica Las Condes, Santiago, Chile, [5] Cell and Molecular Biology Program, Faculty of Medicine, Institute of Biomedical Sciences, Universidad de Chile, Santiago, Chile, [6] Coloproctology Department, Clinica Las Condes, Santiago, Chile, [7] Coloproctology Surgery Department, Hospital Clinico Universidad de Chile, Santiago, Chile, [8] Pathology Department, Hospital Clinico Universidad de Chile, Santiago, Chile, [9] Department of Basic and Clinic Oncology, Faculty of Medicine, Universidad de Chile, Santiago, Chile, [10] Medical Oncology Department, Ramon y Cajal University Hospital, IRYCIS, CIBERONC, Madrid, Spain, [11] Molecular Pharmacology Laboratory, Faculty of Chemical Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile

OPEN ACCESS

Edited by:
Hui-Rong Jiang, University of Strathclyde, United Kingdom

Reviewed by:
Remo Castro Russo, Federal University of Minas Gerais, Brazil
Paul Proost, Rega Institute for Medical Research, KU Leuven, Belgium

***Correspondence:**
Marcela A. Hermoso
mhermoso@med.uchile.cl

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *22 September 2018*
Accepted: *03 June 2019*
Published: *21 June 2019*

Citation:
Landskron G, De la Fuente López M, Dubois-Camacho K, Díaz-Jiménez D, Orellana-Serradell O, Romero D, Sepúlveda SA, Salazar C, Parada-Venegas D, Quera R, Simian D, González M-J, López-Köstner F, Kronberg U, Abedrapo M, Gallegos I, Contreras HR, Peña C, Díaz-Araya G, Roa JC and Hermoso MA (2019) Interleukin 33/ST2 Axis Components Are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer. Front. Immunol. 10:1394. doi: 10.3389/fimmu.2019.01394

In colorectal cancer (CRC), cancer-associated fibroblasts (CAFs) are the most abundant component from the tumor microenvironment (TM). CAFs facilitate tumor progression by inducing angiogenesis, immune suppression and invasion, thus altering the organization/composition of the extracellular matrix (i.e., desmoplasia) and/or activating epithelial-mesenchymal transition (EMT). Soluble factors from the TM can also contribute to cell invasion through secretion of cytokines and recently, IL-33/ST2 pathway has gained huge interest as a protumor alarmin, promoting progression to metastasis by inducing changes in TM. Hence, we analyzed IL-33 and ST2 content in tumor and healthy tissue lysates and plasma from CRC patients. Tissue localization and distribution of these molecules was evaluated by immunohistochemistry (using localization reference markers α-smooth muscle actin or α-SMA and E-cadherin), and clinical/histopathological information was obtained from CRC patients. *In vitro* experiments were conducted in primary cultures of CAFs and normal fibroblasts (NFs) isolated from tumor and healthy tissue taken from CRC patients. Additionally, migration and proliferation analysis were performed in HT29 and HCT116 cell lines. It was found that IL-33 content increases in left-sided CRC patients with lymphatic metastasis, with localization in tumor epithelia associated with abundant desmoplasia. Although ST2 content showed similarities between tumor and healthy tissue, a decreased immunoreactivity was observed in left-sided tumor stroma, associated to metastasis related factors (advanced stages, abundant desmoplasia, and presence of tumor budding). A principal component analysis (including stromal and epithelial IL-33/ST2 and α-SMA immunoreactivity with

extent of desmoplasia) allowed us to distinguish clusters of low, intermediate and abundant desmoplasia, with potential to develop a diagnostic signature with benefits for further therapeutic targets. IL-33 transcript levels from CAFs directly correlated with CRC cell line migration induced by CAFs conditioned media, with rhIL-33 inducing a mesenchymal phenotype in HT29 cells. These results indicate a role of IL-33/ST2 in tumor microenvironment, specifically in the interaction between CAFs and epithelial tumor cells, thus contributing to invasion and metastasis in left-sided CRC, most likely by activating desmoplasia.

Keywords: colorectal cancer, cancer associated fibroblasts, interleukin 33, desmoplasia, epithelial-mesenchymal transition

INTRODUCTION

Colorectal cancer (CRC) is one of the most frequent types of cancer, with the third highest incidence in men and the second in women worldwide; with more than half of all cases occurring in developed countries (1). In Chile, crude death rate has duplicated in the past years, being the fourth most deadly cancer in men and third on women (1, 2). CRC is also a multifactorial pathology that occurs with the formation of a focus of aberrant crypt, progresses with the appearance of polyps, adenoma and finally carcinoma, which comprises the final stage of malignant epithelial transformation (3, 4).

The tumor microenvironment is a very complex structure represented by several cells, including tumor cells, resident fibroblasts, endothelial cells, and recruited macrophages/lymphocytes, that establish communications with each other and with tumor cells by means of soluble factors and cell-cell contact (5, 6). Depending on the context and the predominant cytokine profile, immune response can favor, or delay tumor progression (7, 8), and in some tumors a fibrotic response is also detected. Among all cell types, cancer associated fibroblasts (CAFs) are the most abundant stromal cells and can mediate a fibrotic response to a chronic inflammatory milieu (9).

During the tumor formation, not only the epithelial cells undergo changes, but also the stroma, with morphological alterations such as desmoplasia, angiogenesis and inflammatory or immune cell infiltration (10, 11). Desmoplasia corresponds to a stromal reaction to the tumor, where CAFs supply matrix remodeling molecules [e.g., tenascin, metalloproteinases (MMPs)] affecting extracellular matrix component deposition in the invasion front (12), and organizing protein fibers toward an ordered pattern favoring tumor cell migration (13).

In CRC, desmoplasia, together with advanced invasion stages and lymph node (LN) metastasis, constitutes an independent factor of poor prognosis (low free survival of recurrence at 5 years) (14, 15). In addition, desmoplastic reaction can be associated with tumor dedifferentiation, thus contributing to tumor invasion (16). A high α-smooth muscle actin (α-SMA) content reflects desmoplasia and is associated with poor prognosis (17), and also with high M2-type macrophage content (18), confirming the interaction previously described between CAFs and M2 macrophages in processes of fibrosis and tumor progression (19, 20).

IL-33 is a cytokine belonging to the family of IL-1, which is expressed not only in non-hematopoietic cells (fibroblasts, adipocytes, endothelial, smooth muscle, and epithelial cells), but also in macrophages and dendritic cells (21–23). The IL-33 receptor is called ST2 (encoded by *IL1RL1* gene), of which there are two variants: one membrane-anchored called ST2L or IL-33R, which exerts the cellular effects of IL-33 and a soluble variant, the sST2, lacks the transmembrane portion acting as decoy receptor of IL-33 (24). When IL-33 binds to the receptor complex formed by ST2L and the IL-1 receptor accessory protein (IL1RAcP), activation of a signaling pathway mediated by MAP kinases (mitogen-activated protein) and NF-κB takes place (21). In cells of the immune system (mast cells, nuocytes, or innate lymphoid cell type 2 and eosinophils), expressing ST2, IL-33 induces the synthesis and secretion of Th2-related cytokines (IL-4, IL-13, or IL-5) (21, 25, 26).

Previously evaluated for its role as a pro-inflammatory cytokine in the pathophysiology of inflammatory bowel diseases, mainly ulcerative colitis (27), IL-33 has been implicated in tumorigenic processes in murine and *in vitro* models (28–30). Furthermore, elevated serum IL-33 levels have been detected in patients with lung, gastric, and hepatocellular cancer (30), although conversely, IL-33 activates CD8+ T lymphocytes and NK effector cells in the antitumor response in murine models of immunotherapy and lung and melanoma cancer, exerting a protective role (31, 32).

Moreover, IL-33 secreted by CAFs from patients with head and neck cancer has been associated with invasion by activating Epithelial to Mesenchymal Transition (EMT) (33), which emphasize the importance of studying IL-33 in metastasis.

In CRC, the IL-33/ST2 axis activates the tumor stroma fibroblasts promoting polyp formation in Adenomatous Poliposis Coli $(APC)^{Min/+}$ mice model (34). In addition, IL-33, and total ST2 mRNA content were found elevated in tumor tissue of patients (adenomas > carcinomas) vs. normal tissue (35, 36), and were related to increased invasion and metastasis in CRC tumor cells and xenograft murine models of a IL-33-overexpressing tumor cell line (36). Additionally, high IL-33 immunoreactivity in metastatic CRC tumor cells has been associated with shorter survival (37), confirming sST2 as a protective tumorigenesis factor by counteracting protumoral IL-33 effects such as angiogenesis induction and modification of tumor microenvironment (38).

However, the content of sST2 is diminished in tumor tissue of CRC patients and inversely correlates with more advanced tumors, as well as IL-33 content correlation to tumor progression (38, 39), suggesting that IL-33/ST2 axis participates in the progression to CRC metastasis.

Regarding the role of IL-33/ST2 in CRC, studies showed activation of stroma in intestinal human myofibroblast cell lines and murine models of CRC stimulated both by IL-33 (34), however, the impact of IL-33/ST2 axis in desmoplasia of CRC patients is unknown. From this perspective, using a cohort of Chilean patients, we analyzed the association of IL-33/ST2 content and distribution with CRC progression and clinical/histopathological features (TNM staging, desmoplasia, tumor localization, among others) in tumor and healthy tissue.

PATIENTS AND METHODS

Patients

Samples from 62 patients (mean age 65.1 ± 14 years old, 38% women) with CRC were included from three health centers (Tissue Biobank of Universidad de Chile Clinical Hospital, Coloproctology Departments from Universidad de Chile Clinical Hospital and Clinica Las Condes) between 2015 and 2017. Patients undergoing surgery for tumor resection had to be older than 18 years old and not have received chemotherapy or neoadjuvant therapy prior to total or partial colectomy. Tumor staging was classified according to the TNM classification (The Union for International Cancer Control; UICC) (40).

Immediately after surgery, samples of fresh tumor including core and invasive front and healthy intestinal mucosa (at least 10 cm away from tumor) were macroscopically selected by a pathologist from each center in consensus to ensure homogeneity between samples. A small fragment from each selected tissue was stored at −80°C until posterior delivery to the Innate Immunity Lab for protein extraction. Biopsy size-samples of tumor and healthy tissue were fixated in 2% paraformaldehyde and embedded in paraffin for a tissue microarray (TMA) construction and immunohistochemistry analysis. Histology sections from primary tumor and healthy intestinal mucosa, metastatic and healthy LNs were also used for immunofluorescence analysis. Plasma samples from 34 CRC patients and 15 age-matched healthy controls were also obtained after signed informed consent. Clinical data from CRC patients and controls were also obtained for association analysis. Histological evaluation was conducted by a pathologist blinded to the patients' information.

The clinical and demographic information of CRC patients and controls is summarized in **Table 1** and classified by the type of sample. Tumor localization proximal to the splenic flexure was defined was right-sided cancers and those at or distal to the splenic flexure as left-sided cancer (41). We were not able to obtain the same variety of samples from CRC patients; hence, different sample sizes are shown.

TABLE 1 | Demographic characteristics from colorectal cancer patients included in study.

	Total CRC patients	Frozen tissue	Plasma from CRC patients	Tissue microarray (TMA)	Plasma from healthy controls
Sample size	**62**	41	34	22	**15**
Gender (F/M)	**24/38**	19/22	14/20	8/14	**7/8**
Age (range)	**65.1 (32–91)**	66.6 (32–91)	63.8 (42–89)	65.1 (33–85)	**50.7 (29–79)**
Localization					
Left-colon	**41**	26	24	14	
Right-colon	**21**	14	9	8	
Tnm					
1	**8**	5	4	5	
2	**22**	15	15	7	
3	**30**	20	15	8	
4	**1**	0	0	1	

Bold values indicate the total number or age (range) of patients or controls.

Ethics Statement

This study was carried out in accordance with the recommendations of the Review Boards and Local Ethical Committees from Universidad de Chile Clinical Hospital, Tissue Biobank of Universidad de Chile and Clinica Las Condes, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki and were identified only by codification established by their respective Center to keep anonymity. The protocol was approved by the Local Ethical Committees from Universidad de Chile Clinical Hospital, Tissue Biobank of Universidad de Chile and Clinica Las Condes.

Cell Lines and Reagents

HT29 and HCT116 adenocarcinoma cell lines were kindly donated from Dr. Julio Tapia (Universidad de Chile, Santiago, Chile) and Dr. Jorge Toledo (Universidad de Concepcion, Concepcion, Chile), respectively. These cells were cultured in DMEM high-glucose cell culture media (Corning Life Sciences, Tewksbury, MA, USA) with 10% Fetal Bovine Serum (FBS, Gibco, Thermo Scientific, Waltham, MA, USA) + 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco, Thermo Scientific, Waltham, MA, USA). Each cell passage was made with 0.025% trypsin/EDTA (Gibco, Thermo Scientific, Waltham, MA, USA) and sterile PBS (Sigma–Aldrich, St. Louis, MO, USA).

Quantification of IL-33 and ST2 Protein From Tissue and Plasma

Frozen tumor and healthy adjacent tissues from patients with CRC were lysed with radioimmunoprecipitation (RIPA) buffer (Sigma–Aldrich, St. Louis, MO, USA) containing protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany) and lysates total protein was quantified with Pierce BCA Protein Assay Kit (Pierce Biotechnologies, Rockford, IL, USA). Plasma of CRC patients and healthy controls was collected from whole blood in BD Vacutainer® EDTA tubes. The determination of IL-33 and ST2 in tissue extracts and plasma were performed in duplicate by enzyme-linked immunosorbent assay (ELISA) DuoSet kits (R&D Systems, Minneapolis, MN, USA). The

IL-33 and ST2 protein absorbance were read at 450 nm, by spectrophotometric analysis (Synergy 2, Biotek Instruments, Inc., Winooski, VT, USA). Protein levels from tissue extracts are expressed in pg/mg and normalized to the total protein content (mg), plasma results are expressed in pg/mL.

Tissue Microarray (TMA) Assembly and Histological Characterization

A TMA was generated from formalin-fixed paraffin-embedded tissue from 31 patients with CRC. A representative zone was selected by a pathologist through careful observation of the Hematoxylin/Eosin stain. With a 2 mm diameter punch (Beecher Instruments, Silver Spring, MD, USA), cores from paraffin-embedded tissue were transferred to a new paraffin block with a 6 × 4 matrix distribution, one TMA for tumor and one TMA for healthy adjacent tissue. Two cores from renal tissue were included for orientation purposes. Then, 2 μm sections were transferred to positively activated glass slides and analyzed by immunohistochemistry.

A histological section of paraffin-embedded tumor and healthy adjacent colonic tissue of 21 patients with CRC (of 2-μm thickness) was stained with Hematoxylin/Eosin and evaluated by a pathologist, blinded to the patients's data. A score of 1 to 3 was assigned according to the tumor grade [well-differentiated (1), moderately differentiated (2) and poorly differentiated (3)], the amount of desmoplastic reaction [activation of myofibroblasts in the tumor, low (1), moderate (2), abundant (3)] and the degree of inflammatory infiltrate [low (1), moderate (2), abundant (3)]. These characteristics were considered to evaluate the association with the immunohistochemical markers and the TNM stage of patients. The presence of tumor budding (TB, or focal budding) was evaluated with the immunostaining of E-cadherin, observing foci of 5 or more tumor cells in the invasive front of the tumor, which were counted and considered positive when more than 5 foci per 20X field were observed.

Immunohistochemistry From Histological Sections

First, sections were deparaffinized and rehydrated with deionized water. Then, they were heated in an EDTA-based buffer at pH 9.0 (Buffer EnVision Flex Antigen Retrieval, Dako, Carpinteria, CA, USA), using an electric pressure cooker for 3 min at 12–15 pounds/square inch at ∼120°C, and cooled for 10 min before immunostaining. Then, all sections were incubated with 3% H_2O_2 (blockade of endogenous peroxidases) for 10 min and subsequently incubated with goat anti-hIL-33 (R&D Systems, Minneapolis, MN, USA), goat anti-hST2 (R&D Systems, Minneapolis, MN, USA), mouse anti-α-SMA (Sigma–Aldrich, St. Louis, MO, USA) and mouse anti-E-cadherin (BD Biosciences, Franklin Lakes, NJ, USA) primary antibody for 30 min each. Tissue sections were incubated with a goat–rabbit IgG linker (for samples incubated with goat polyclonal antibodies) and then incubated with the secondary antibody–universal polymer (EnVision Flex-HRP, Dako, Carpinteria, CA, USA). Sections were revealed with substrate + DAB and counterstained with Harris Hematoxylin. Coverslips were mounted with Tissue-Tek SCA (Sakura Finetek USA. Inc, Torrance, CA, USA). Positive and negative controls were run with each batch of patient/study slides tested.

Immunostaining Analysis

Images were captured with Aperio ScanScope (Leica Biosystems, Wetzlar, Germany). The analysis of the images was evaluated with the Aperio ImageScope Software and the algorithm to evaluate the positive pixels was Positive Pixel Count 9. The proportion of positive pixels respective to the total pixels per area (positive and negative), were considered for association with the clinical variables and histological features [as Positivity per area, Pos/area (μm^2)]. The Pos/area was then validated by two experienced pathologists.

Indirect Immunofluorescence

Paraffin histological sections derived from primary CRC tumor, metastatic and healthy LNs were evaluated for the co-expression of IL-33/α-SMA and ST2/E-cadherin by immunofluorescence. Briefly, the sections were subjected to deparaffinization (NeoClear, Merck KGaA, Darnstadt, Germany), then rehydrated with a battery of alcohols from absolute ethanol to 70° ethanol. The antigenic recovery was performed with EDTA buffer (pH 8) for IL-33/α-SMA and with sodium citrate buffer (pH 6) for ST2/Ecad. Then, the sections were incubated with 100 mM glycine and 2% bovine serum albumin (BSA) (Sigma–Aldrich, St. Louis, MO, USA) plus 1% normal donkey serum in 1X PBS (Sigma–Aldrich, St. Louis, MO, USA) (for autofluorescence and non-specific proteins blocking, respectively). The sections were incubated at room temperature for 1 h with the following primary antibodies: anti-IL-33 (1/50) (Polyclonal Goat anti human, AF3625, R&D Systems, Minneapolis, MN, USA) in conjunction with anti-α-SMA (1/500) (monoclonal mouse antibody, A2547, Sigma–Aldrich, St. Louis, MO, USA) and anti-ST2 (1/100) (Polyclonal Goat anti human, AF523, R&D Systems, Minneapolis, MN, USA) in conjunction with anti-E-cadherin (1/500) (monoclonal mouse antibody, 610181, BD Biosciences, Franklin Lakes, NJ, USA). After PBS rinse, tissue sections were incubated for 1 h at room temperature with secondary antibodies (Thermo Scientific, Waltham, MA, USA) Donkey Anti-Goat IgG conjugated with Alexa Fluor 594 (1/200) and Donkey Anti-Mouse IgG conjugated with Alexa Fluor 488 (1/200). Hoechst 33342 (1/500) was used as a nuclear counterstain. Finally, slides were covered with a coverslip plus mounting solution (Dako, Agilent Technologies Inc., Santa Clara, CA, USA). Slides were visualized by C2+ confocal microscope at 20x and 60x objectives (Nikon Instruments Inc, Melville, NY, USA).

Microarray Analysis of IL-33 mRNA in CAFs

The IL-33 mRNA expression levels from a group of colorectal CAFs by RNA microarray (GEO accession number GSE51257), were re-analyzed to correlate the IL-33 expression levels and their promigratory potential. This microarray was initially analyzed to establish a functional heterogeneity of CAFs based on their ability to induce cell migration in the CRC adenocarcinoma cell lines LIM1215 and SW480 (42).

RNA Extraction and RT-qPCR

HT29 and HCT116 cell lines were stimulated with 50 ng/mL of rhIL-33 (R&D Systems, Minneapolis, MN, USA) for 6 h, and the expression analyses were performed using real-time qPCR (RT-qPCR). Total RNA from each sample was extracted with RNEasy Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, integrity was analyzed by electrophoresis in 1% agarose gel and concentration was determined by spectrophotometric analysis (Synergy 2, Biotek Instruments, Inc., Winooski, VT, USA). Then, two μg of RNA was used to synthesize cDNA using oligo-dT (Thermo Fisher Scientific, Waltham, MA, USA) and RT-affinity Script enzyme (Agilent Technologies Inc., Santa Clara, CA, USA) in a final volume of 20 μL. All mRNAs expression analyses were performed by real-time qPCR (RT-qPCR) using the Brilliant® II kit SYBR® Green QPCR Master Mix (Agilent Technologies Inc., Santa Clara, CA, USA) and primers for E-Cadherin, N-Cadherin and Vimentin at a final concentration of 250 nM in a final volume of 20 μL with 100 ng of cDNA. Amplification was performed with Mx3000 P QPCR System (Agilent Technologies Inc., Santa Clara, CA, USA). 18s was used as a reference gene to normalize mRNA levels. To analyze qPCR results $2^{-\Delta\Delta CT}$ method was used.

Cell Viability Assay

HT29 and HCT116 cell lines were cultured at 2×10^5 cells/well (in a 12-well plate) in DMEM medium with two different concentrations of FBS (10 and 0.5%). Cells containing each concentration of FBS were stimulated with 50 ng/mL recombinant human IL-33 (R&D Systems, Minneapolis, MN, USA). Cells were counted with trypan blue staining in a Neubauer chamber at 6, 12, 24, 48, and 72 h post-stimulus.

Wound Healing Assay

HT29 and HCT116 cell lines stimulated with 50 ng/mL of rhIL-33 (R&D Systems, Minneapolis, MN, USA) were cultured at 6×10^5 and 7×10^5 cells/well, respectively (24-well plates) with DMEM 10% FBS medium, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco, Thermo Scientific, Waltham, MA, USA) until complete adherence to the plate (6 and 9 h for HT29 and HCT116, respectively). Then, medium was replaced by DMEM 0.5% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco, Thermo Scientific, Waltham, MA, USA) until the next day. Then, the wound was made with a 24-well SPL Scar Scratcher (SPL Life Sciences, Naechon-Myeon, Pocheon, South Korea). After two gentle washes with PBS to remove the detached cells, the medium was replaced with 1 mL of fresh medium with low FBS (0.5%) together with rhIL-33, except for two wells with FBS 10% or 5 ng/mL TGFβ, as a positive control. Images were acquired with Cytation 3 Cell Imaging Reader (Biotek Instruments, Inc., Winooski, VT, USA). Subsequently, the plate was incubated at 37°C/5% CO_2 and visualized at 24 h in the same coordinates. The analysis was performed with the ImageJ software by calculating the proportional free area at 24 h normalized to time zero and comparing each stimulus with the control at the respective times.

Statistical Analysis

First, the D'Agostino & Pearson test was used to evaluate normality of data from ELISA results. In those with normal distribution, the results were expressed as means plus standard deviation using unpaired *t*-test or one-way ANOVA for the comparison of quantitative variables. In the non-parametric data, the results were expressed in median plus interquartile range. For the comparison of paired and unpaired data between two groups, the Wilcoxon and Mann Whitney tests were used, respectively. To compare more than two groups the Kruskal Wallis test was used. The Chi square test was used for contingency analysis. The associations of the histopathological characteristics with the positivity index of the IL-33/ST2 markers in epithelium and stroma were evaluated by linear regression and Spearman coefficient. The correlation between the IL-33 transcript and migration induction was determined with a linear regression test. A p-value <0.05 was considered significant. A cluster analysis among Pos/area from IHC allowed us to observe those markers related to each other and grouped according to the degree of desmoplasia. The distances between the column clusters and the Heatmap rows were hierarchized using Euclidean and Ward distance methods (unsquared). Then, a principal component analysis (PCA) was applied through the Clustvis web platform, (https://biit.cs.ut.ee/clustvis/), grouping together, those tumor markers that explain the variability observed in patients with different levels of desmoplasia.

RESULTS

Clinical and Histopathological Features

Table 1 summarizes clinical information from the total 62 CRC patients included in this study, divided by type of sample. Thirty patients were diagnosed with local metastasis to lymph nodes and one patient diagnosed with distant metastasis (TNM stage III and IV, respectively). Over sixty percent (66.1%) of patients had left-sided CRC.

Tumor IL-33 From Left-Colon CRC Patients Increases in TNM Stage With Lymphatic Metastasis

First, we evaluated IL-33 and ST2 levels in tumor, distant non-tumor tissue and plasma from CRC patients. We found similar levels between tumor IL-33 compared to distant healthy tissue (**Figure 1A**), as well as IL-33 levels according to TNM staging (**Supplementary Figure 1A**). Similar results were found with ST2 analysis (**Figure 1B**). Interestingly, tumor IL-33 levels were increased in those patients with LN metastasis (**Figure 1C**). Conversely, we found comparable ST2 levels between tumor and healthy tissue, regardless of presence of LN metastasis and TNM stage (**Figure 1D** and **Supplementary Figure 1B**, respectively).

Considering that tumor localization has been reported to impact on immune response and CRC patient outcomes (43), we analyzed separately IL-33 and ST2 levels in left-sided or right-sided CRC. In general, IL-33 levels are increased in left-sided CRC compared to right-sided CRC (**Supplementary Figure 1C**). Here again, we found that tumor IL-33 levels from left-sided

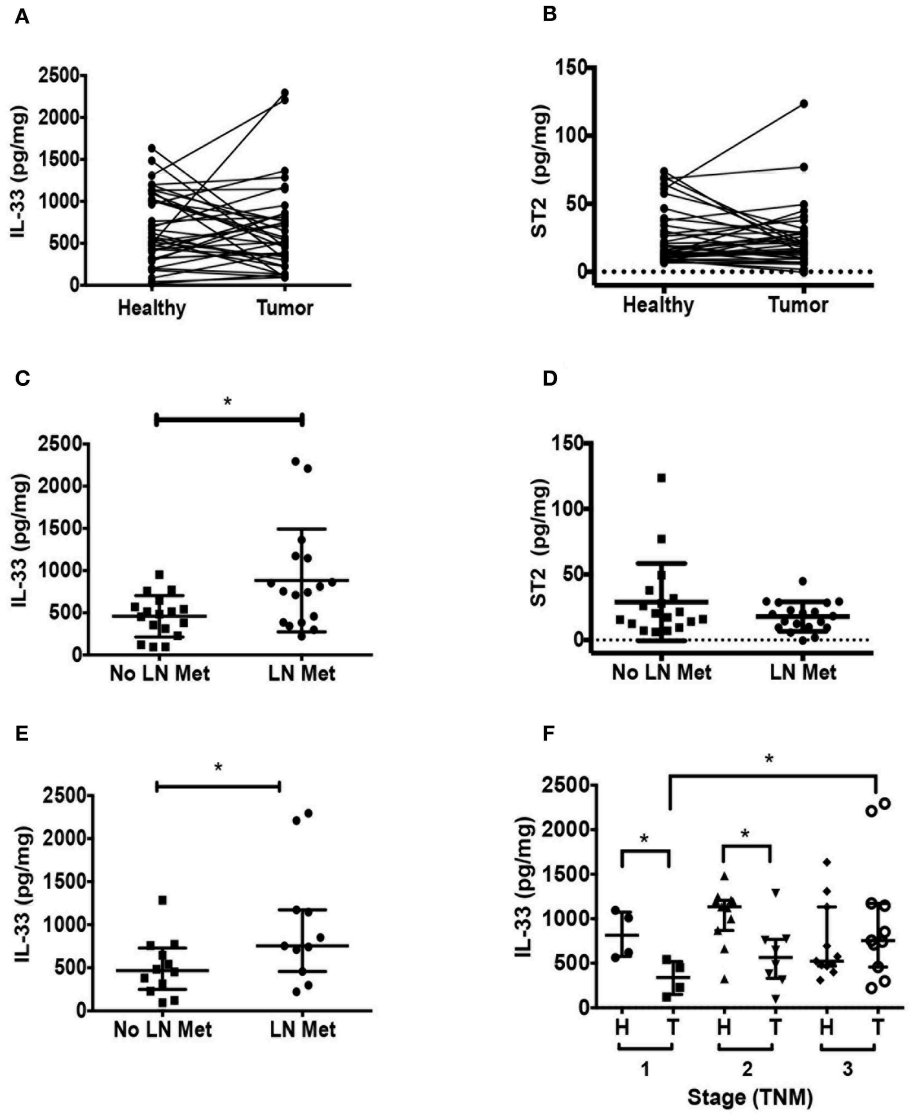


FIGURE 1 | IL-33 is increased in tumors from left-colon colorectal cancer (CRC) patients with lymph node (LN) metastasis. Total IL-33 **(A)** and ST2 **(B)** protein levels were determined by ELISA in tumor and non-tumor tissue lysates from 38 CRC patients by TNM staging, Wilcoxon paired test were performed in both analyses. Total IL-33 **(C)** and ST2 **(D)** protein levels in tumor lysates from patients with and without LN metastasis using *t*-Student test to compare between groups. In **(E)**, tumor IL-33 protein levels from left-sided CRC patients with and without LN metastasis. In **(F)**, tumor and non-tumor IL-33 protein levels from left-sided CRC patients classified by TNM stage, Kruskal Wallis and Dunn's multiple comparisons post-test were performed. *$P < 0.05$.

CRC patients were increased in those with LN metastasis (**Figure 1E**, $p = 0.04$). At early stages, tumor IL-33 levels were lower than its corresponding non-tumor distant tissue ($p = 0.02$ and 0.03 for TNM stage 1 and 2), however, stage 3 tumor tissue show increased IL-33 compared to stage 1 ($p = 0.03$, **Figure 1F**). ST2 levels did not show any difference in the same analysis (**Supplementary Figure 1D**). Right-sided tumor IL-33 and ST2 levels were similar in those patients with regional LN metastasis compared to those without metastasis (**Supplementary Figures 1E,F**, respectively).

Circulating IL-33 levels evaluated from plasma were similar between CRC patients vs. controls (Mean ± SD (pg/mL): 350 ± 386.9 vs. 226.2 ± 35.41, respectively. Mann Whitney test, $p = 0.0547$), with five patients showing higher IL-33 plasma levels than the rest of patients and controls, being mostly (4/5) of pT3 stage and left-sided CRC. The other patient had right-sided CRC but with LN metastasis. Similarly, circulating ST2 levels did not show differences between patients vs. controls (Mean ± SD (pg/mL): 380 ± 303.4 vs. 391.6 ± 194.8, respectively. Mann Whitney test, $p = 0.33$). As for IL-33, ST2 plasma levels of 4 patients were detected as outliers with levels higher than controls and the rest of the patients. However, clinically, these patients were completely different from each other.

IL-33 and ST2 Expression in Epithelial and Stromal Compartments of Primary Tumors From CRC Patients

Since we analyzed total IL-33 and ST2 protein content, we therefore evaluated IL-33 and ST2 distribution in stroma and epithelium in a tissue microarray. An initial association analysis showed that both the degree of differentiation and the amount of desmoplasia are associated to the tumor stage (TNM) (Chi-Square test, $p = 0.0015$ and 0.02, respectively), in contrast to the lymphocytic inflammatory infiltration (**Supplementary Figure 2A**). In addition, CRC patients with abundant desmoplasia are associated with a higher proportion of lymphatic metastasis ($p = 0.012$) (**Supplementary Figure 2B**).

The content of IL-33 in healthy colon was limited to endothelial nuclear staining and some cytoplasmic staining in lamina propria mononuclear cells (LPMNCs), and scarcely observed in epithelium (**Figure 2A**). While the content of α-SMA in healthy colon was limited to subepithelial myofibroblasts, vessels and the muscularis mucosa (**Supplementary Figure 2C**), the co-expression of both markers was only observed in blood vessels (**Figure 2B**). In the tumor, IL-33 distribution is heterogeneous among patients, observing both nuclear and cytoplasmic staining in tumor epithelial cells, with stromal staining in fibroblast-like cells, mononuclear cells and endothelium (**Figure 2A**). Co-expression of IL-33 and α-SMA in tumors with advanced invasion (higher than pT3) showed a high proportion of IL-33$^+$/α-SMA$^+$ cells, suggesting that CAFs can express IL-33 in more invasive stages (**Figure 2B**).

The ST2 content in healthy colon was observed mainly in LPMNCs, being almost absent in epithelium (**Figure 2A**). E-cadherin distribution was only limited to healthy epithelium and homogeneous in all samples (**Supplementary Figure 2C**), while co-expression of both markers in healthy colon was not observed (**Figure 2B**). In tumor, ST2 expression was heterogeneous among patients and cytoplasmic staining of epithelial tumor cells as well as in fibroblasts and mononuclear cells in stroma (**Figure 2A**). The co-expression of ST2 and E-cadherin in the advanced invasion tumors (pT3) was observed mainly in some areas of epithelial tumor cells, although not necessarily co-localized in the same subcellular compartment (**Figure 2B**).

Epithelial Tumor IL-33 and ST2, and Stromal ST2 Correlates With α-SMA and Desmoplasia by Hierarchical Analysis and PCA

First, the association analysis of the IL-33/ST2 distribution in tumor epithelium shows that IL-33 immunoreactivity is directly associated with a greater amount of desmoplasia (**Figure 2C**), observing a similar trend with ST2 immunoreactivity (**Supplementary Figure 3A**). Both markers are moderately correlated (Spearman $r = 0.5$, $p = 0.01$), suggesting that IL-33 and ST2 variants present in the tumor are related to fibroblast activation and could potentiate the desmoplastic reaction of the tumor. ST2 immunoreactivity in the stroma was found to be inversely associated with stage and with the amount of desmoplasia (**Figure 2D**).

Analysis according to the degree of desmoplasia allowed to classify three important clusters (**Figure 3A**), the first concentrates the cases of abundant and moderate desmoplasia, the third concentrates the cases of scarce desmoplasia; with an intermediate one that has different degrees of desmoplasia. This hierarchization shows a close relationship between variations of tumor and stromal IL-33 content together with stromal ST2 in relation to desmoplasia. Alternatively, the content of tumor ST2 shows a smaller distance or variability with the content of α-SMA. The markers together were then evaluated with a PCA, determining components (set of markers) that allow data grouping according to the variability intra and inter groups. This analysis allowed us to classify the patients who showed scarce (1) from abundant desmoplasia (3) (**Figure 3B**).

Decreased Stromal ST2 and Poor Prognosis Factors Correlates With M2 Macrophage Markers in CRC Patients

Since stromal ST2 showed inverse correlation with stage and amount of desmoplasia, we evaluated other clinical factors involved in CRC prognosis, such as LN metastasis, tumor budding (TB) and macrophage markers (general marker CD68, M2-marker CD163, and M1-marker iNOS), analyzed by IHC. ST2 immunoreactivity in the stroma is diminished both in patients with lymphatic metastasis [**Figure 4A**, Mean positivity per area ± SD (Pos/area AU, arbitrary units): 0.232 ± 0.06 vs. 0.377 ± 0.14 without LN metastasis, respectively. Student *t* test, $p = 0.0085$] and in left colon tumors [**Figure 4B**, Mean pos/area ± SD (AU, arbitrary units): 0.275 ± 0.09 vs. 0.463 ± 0.23 in right-sided CRC, respectively, Student *t* test, $p = 0.0359$].

Alternatively, patients with tumor budding, possibly a poor prognosis factor, were associated with abundant desmoplasia (**Supplementary Figure 3B**, Chi-square for trends, $p = 0.02$) and decreased stromal ST2 [**Figure 4C**, Mean pos/area ± SD (AU): 0.26 ± 0.091 vs. 0.415 ± 0.204 without TB, $p = 0.031$]. Interestingly, patients with TB also showed increased α-SMA (**Supplementary Figure 3C**, $p = 0.009$) and decreased E-cadherin (**Supplementary Figure 3D**, $p = 0.001$) positivity per area.

We also evaluated the positivity per area of stromal ST2 associated with macrophage markers, observing a direct association between stromal ST2 with CD68 and CD163, suggesting that a M2-macrophage rich milieu would be important in ST2 stromal expression, at least on early stages (**Figure 4D** and **Supplementary Figure 3E**).

IL-33/ST2 Distribution in Metastatic CRC Ganglia Resembles Primary Tumor Distribution

In the healthy LN, the content of α-SMA is limited to walls of blood vessels, the content of IL-33 to the nucleus of endothelial cells (scarcely in mononuclear cells), ST2 in the cytoplasm of mononuclear cells and E-cadherin is absent (**Supplementary Figure 4**).

The metastatic LN (**Figure 5A**) presents variable α-SMA (desmoplasia) and IL-33 is present in cells with fibroblast-like

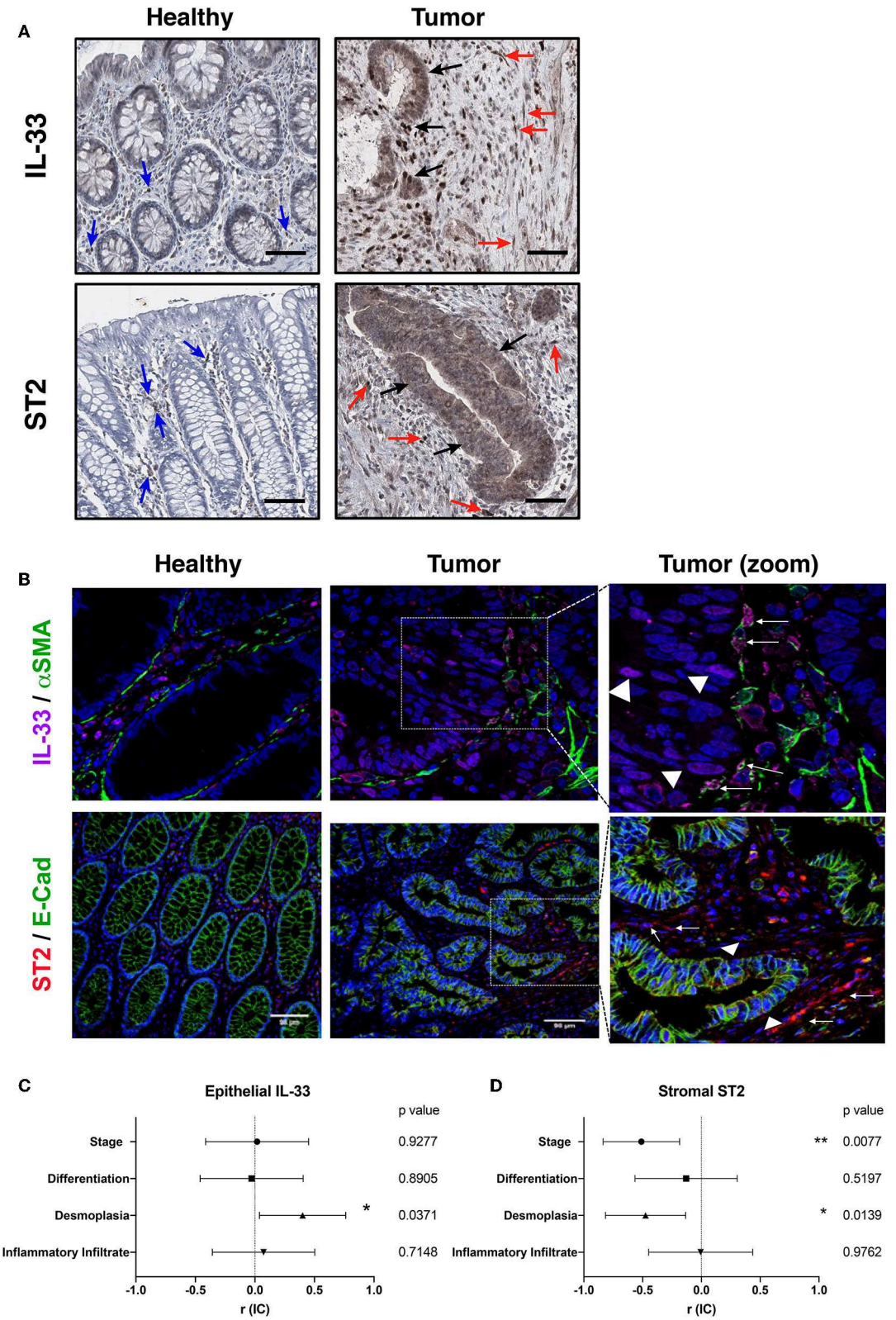


FIGURE 2 | IL-33, ST2, α-SMA, and E-cadherin tissue distribution in human CRC samples. Representative images of IL-33 and ST2 **(A)** distribution in healthy colon and tumor by immunohistochemistry (in brown) (20X objective). Blue arrows indicate positive cells in healthy lamina propria, black arrows indicate IL-33^{+} or ST2^{+} tumor cells and red arrows indicate IL-33^{+} or ST2^{+} stromal cells (as may be the case). In **(B)**, co-expression by indirect immunofluorescence of IL-33 (magenta),

(Continued)

FIGURE 2 | α-SMA (green), and ST2 (red) with E-cadherin (green), Hoechst was used as nuclear counterstain (blue). Right images are a zoom from the depicted square, objective 20X. Arrows indicate fibroblast-like α-SMA$^+$ cells IL-33$^+$ or ST2$^+$ (as may be the case). Triangles indicate epithelial tumor cells IL-33$^+$ or ST2$^+$. In **(C,D)**, Spearman correlation analyses were performed between positivity per area index of epithelial IL-33 or stromal ST2 immunostaining and histological features (TNM staging, differentiation grade, amount of desmoplasia and amount of inflammatory infiltrate), "r" coefficient and interval of confidence are depicted for each variable. *$P < 0.05$, **$P < 0.01$.

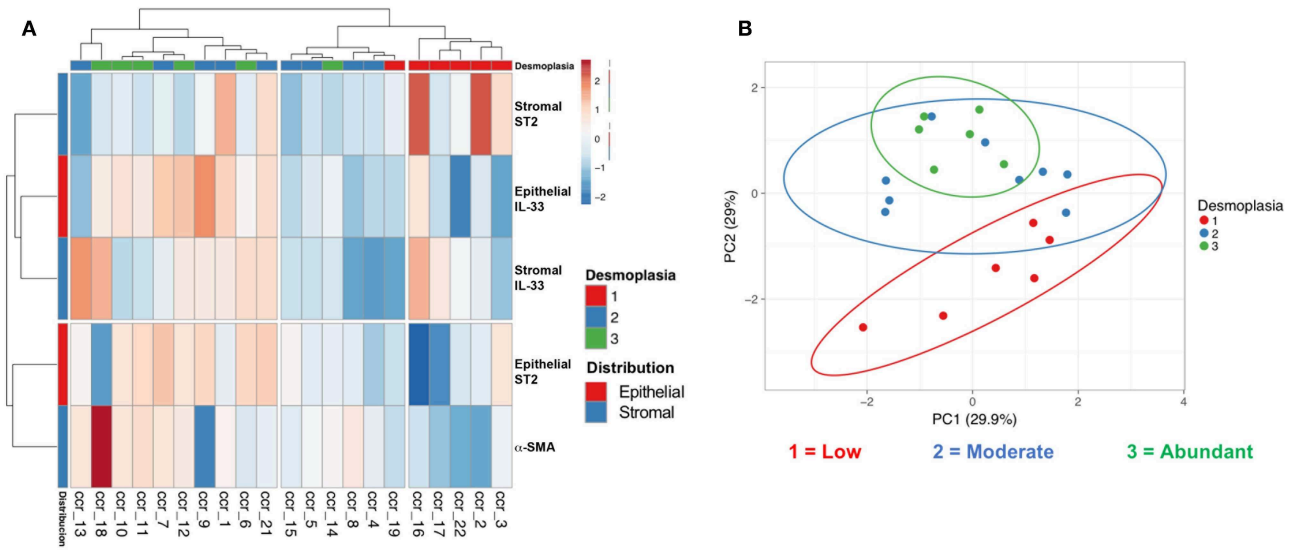


FIGURE 3 | Hierarchical distribution and principal component analysis of epithelial and stromal IL-33, ST2, and α-SMA markers with desmoplasia. Clustering **(A)** and principal component analysis **(B)** including stromal and epithelial IL-33/ST2 and α-SMA immunoreactivity (with extent of desmoplasia) allowed us to distinguish clusters of low, intermediate and abundant desmoplasia.

morphology in areas with high desmoplasia and tumor invading cells (**Figure 5B**). Almost all of the tumor cells expressed membrane E-cadherin and in some areas the tumor cells express cytoplasmic ST2 (**Figure 5C**); however, localization of membrane E-cadherin decreases in the tumor cells that co-express higher intensity of ST2 (**Figure 5D**).

These results suggest that IL-33 and ST2 content in metastatic LN resembles to primary tumor and ST2 expression in the tumor epithelium (with reduced E-cadherin content) could be related to activation of a mesenchymal phenotype and potential tumor progression.

IL-33 Induces a Migrating Mesenchymal Transcript Profile in HT29 Cells

Initially, IL-33 transcript levels from a previous study (extracted from an analysis of mRNA microarray) correlated directly with the ability of conditioned media from colorectal CAFs to induce the migration of tumor lines of colon adenocarcinoma (**Figure 6A**). Then, the effect of IL-33 on the migration of the HT29 and HCT116 cell lines was evaluated through the wound closure assay at 24 h. TGFβ and FBS 10% were used as controls. At 24 h, cell proliferation was increased in high FBS but not under low FBS conditions (**Supplementary Figures 5A–D**). Therefore, HT29 and HCT116 cell lines were stimulated with IL-33 (50 ng/mL) with low FBS or IL-33 with 10% FBS (IL-33 + FBS), TGFβ (5 ng/mL), or 10% FBS. IL-33, in low FBS, favors the migration of HT29 cells at 24 h compared to control (**Figure 6B**), this effect does not occur in HCT116 cells (**Supplementary Figure 6A**). After, HT29 and HCT116 cell lines were exposed to IL-33 (50 ng/mL) and TGFβ (5 ng/mL) as a positive control, the transcripts of E-cadherin, N-cadherin and Vimentin were determined. IL-33 decreases the mRNA of E-cadherin in both cell lines, additionally increasing N-cadherin and vimentin mRNA only in HT29 cells, not in HCT116 cells (**Figures 6C–E** and **Supplementary Figures 5C,D**, respectively). These results suggest that IL-33 activates the onset of the mesenchymal phenotype in HT29 cells.

DISCUSSION

In the present study, we observed an association between tumor and stromal IL-33 and ST2 localization with desmoplasia in CRC patients, coupled with IL-33 increased in left-sided CRC patients with LN metastasis. Also, stromal ST2 was decreased in patients with left-sided cancer and LN metastasis and inversely correlated with desmoplasia, suggesting that IL-33/ST2 axis participates in CRC desmoplasia and tumor progression in this subgroup of patients.

The total content of IL-33 and ST2 were similar in tumor and normal tissue, however, it seems to depend, for IL-33, of the TNM stage. This is the first report describing quantitative analysis of tissue IL-33/ST2, since previous literature mainly

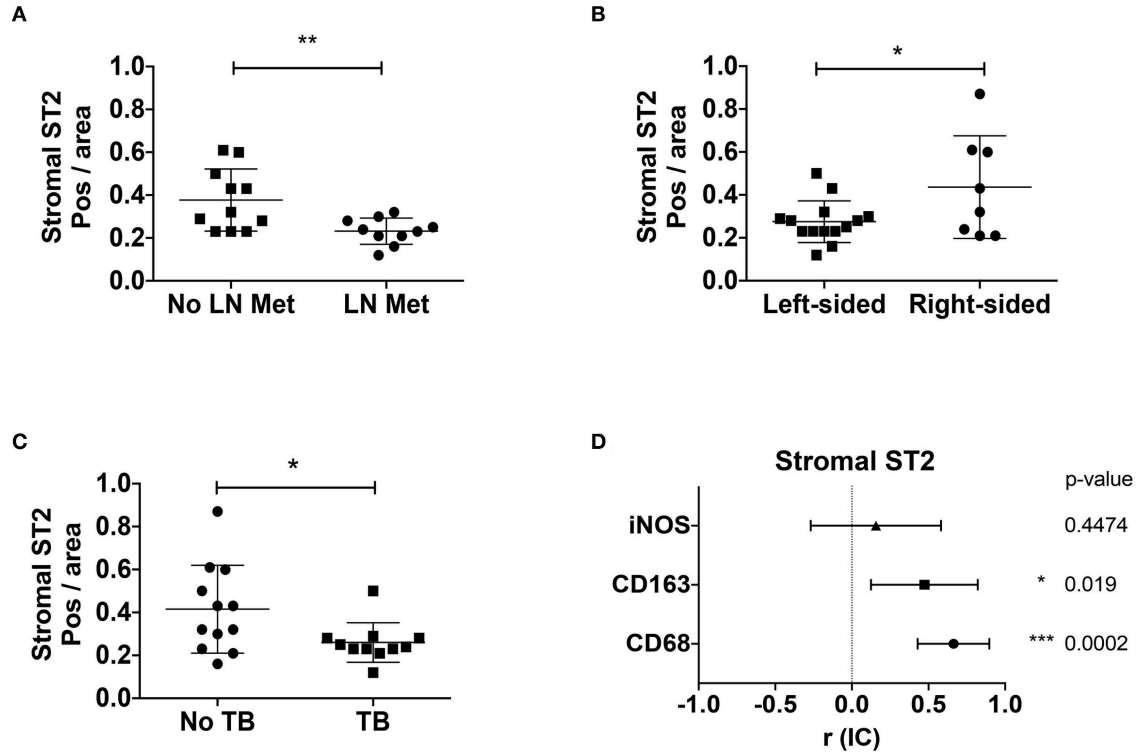


FIGURE 4 | Stromal ST2 is associated to poor prognosis factors and M2 macrophages in CRC patients. Positivity per area index (AU, arbitrary units) were evaluated from stromal ST2 immunostaining in tumor samples from CRC patients by presence of LN metastasis **(A)**, localization of tumor **(B)**, tumor budding **(C)**, histological positivity per area index from stromal ST2 correlated with macrophage markers **(D)**. Mann Whitney and Spearman correlation analyses were performed. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

supplied sera, stool, and tissue approximations by qPCR and immunohistochemistry (IHC) (34, 38, 39, 44–46). Most studies attribute a protumorigenic role to the IL-33/ST2 axis in human, murine and *in vitro* models, with increased levels of IL-33 and ST2 transcripts in tumor vs. healthy tissue from CRC patients (34, 36, 39). According to these antecedents, IL-33 increase was observed mainly in adenomas (low grade adenocarcinoma and in tumors of stages I–III), decreasing later in stage IV. In our study, we did not observe differences in IL-33 content according to the tumor stage. However, we must consider that we did not have a representative number of patients in stage IV (in part because ∼20% of patients are diagnosed at this advanced stage), and of these, a low percentage is a candidate for surgery as a curative option (47). Additionally, the similarity in IL-33 protein levels between tumor and non-tumor tissue might be showing a low-grade inflammation in healthy tissue, that is undetected by histological analysis.

The immunoreactivity of IL-33 was not associated with the TNM stage or the degree of differentiation, however, recent studies show an association between IL-33 expression in the tumor epithelium of metastatic CRC and a shorter survival, suggesting that the tumor IL-33 expression is clinically important in CRC progression (37). ST2 immunoreactivity in tumor epithelium was not associated with the stage or differentiation degree, however, stromal ST2 was inversely associated with stage. Reduced ST2 might be related to the immunosuppressive milieu favored by the tumor microenvironment (48), since *in vitro* studies have shown that sST2 expression is induced by pro-inflammatory cytokines, such as IL1β and TNFα (49). Consistent with our observations, one study reported a decrease in ST2L and sST2 transcript levels in tumor with respect to adjacent normal tissue, also continuing to decrease during progression to later stages (38, 44). The discrepancies described in the papers on the role of IL-33/ST2 axis in intestinal tumorigenesis may be associated to variations in experimental design, models utilized (37, 39, 44, 45), use of chemotherapy in patients (44), as well as differences in their expression in the tumor epithelial cells, tumor microenvironment or tumor location (50). However, there are some studies that propose an antitumor role of IL-33/ST2 which might depend on the tumor microenvironment and in specific stages of tumor development and progression, as some recent comprehensive reviews have described (51–55). Therefore, protumor or antitumor role of IL-33/ST2 axis remains controversial.

In relation to IL-33/ST2 distribution, epithelial IL-33 was directly associated with a higher degree of desmoplasia while stromal ST2 was inversely associated. The presence of desmoplasia is an exacerbated reaction of myofibroblast activation (given by the content of α-SMA) in the tumor

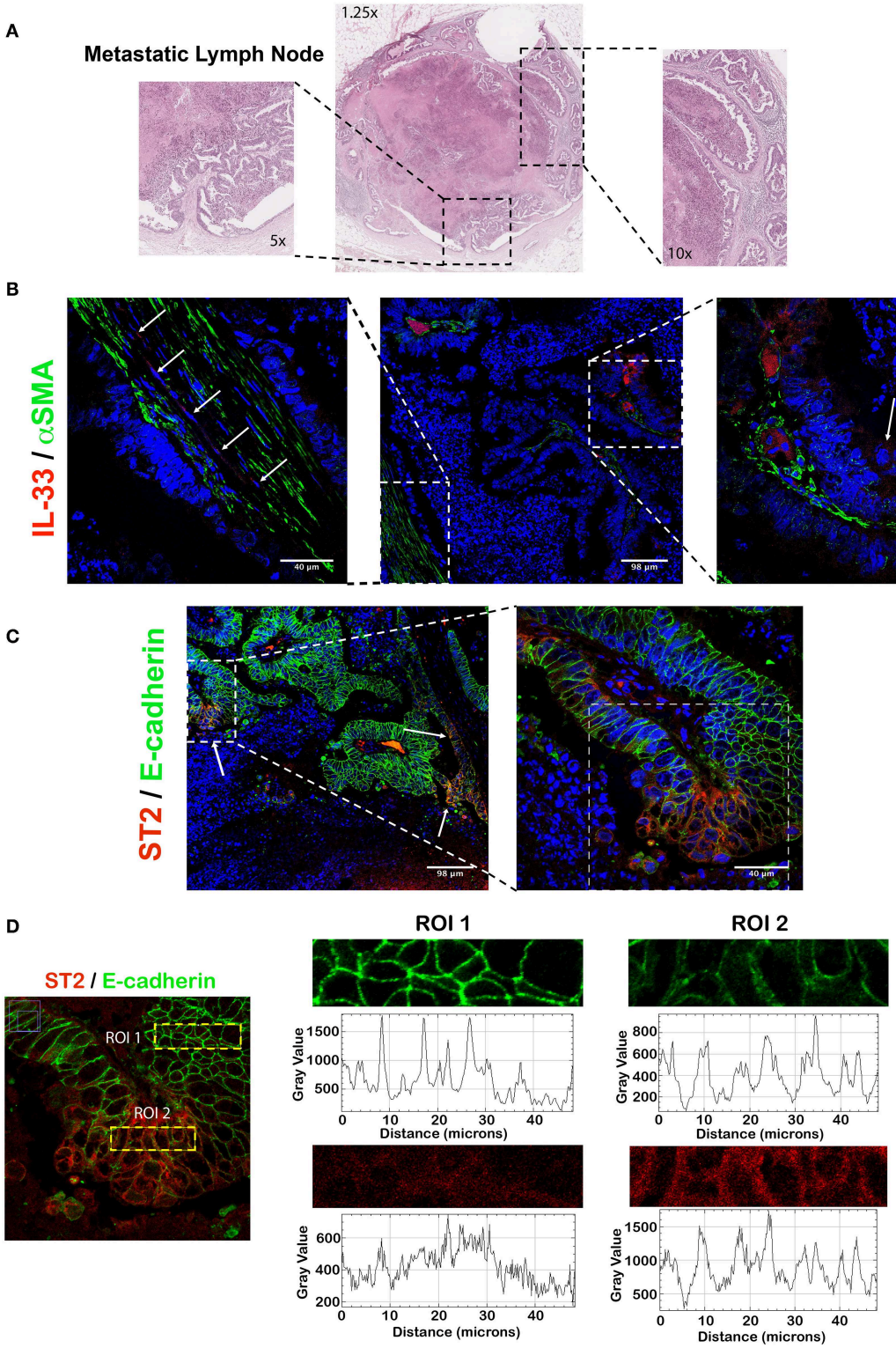


FIGURE 5 | IL-33 and ST2 from metastatic LNs show similar cellular distribution than primary tumor in CRC patients. Representative image of IL-33 and ST2 localization evaluated by IFI in a CRC metastatic LN. In **(A)**, H&E staining showing high necrosis and abundant desmoplasia. In **(B)**, IL-33 (red) is expressed by both metastatic tumor cells and α-SMA^{+} cells in desmoplastic areas (green). In **(C)**, ST2 (red) is expressed in patches by metastatic tumor cells, with an intensity inversely correlated to E-cadherin membrane expression (green) **(D)**.

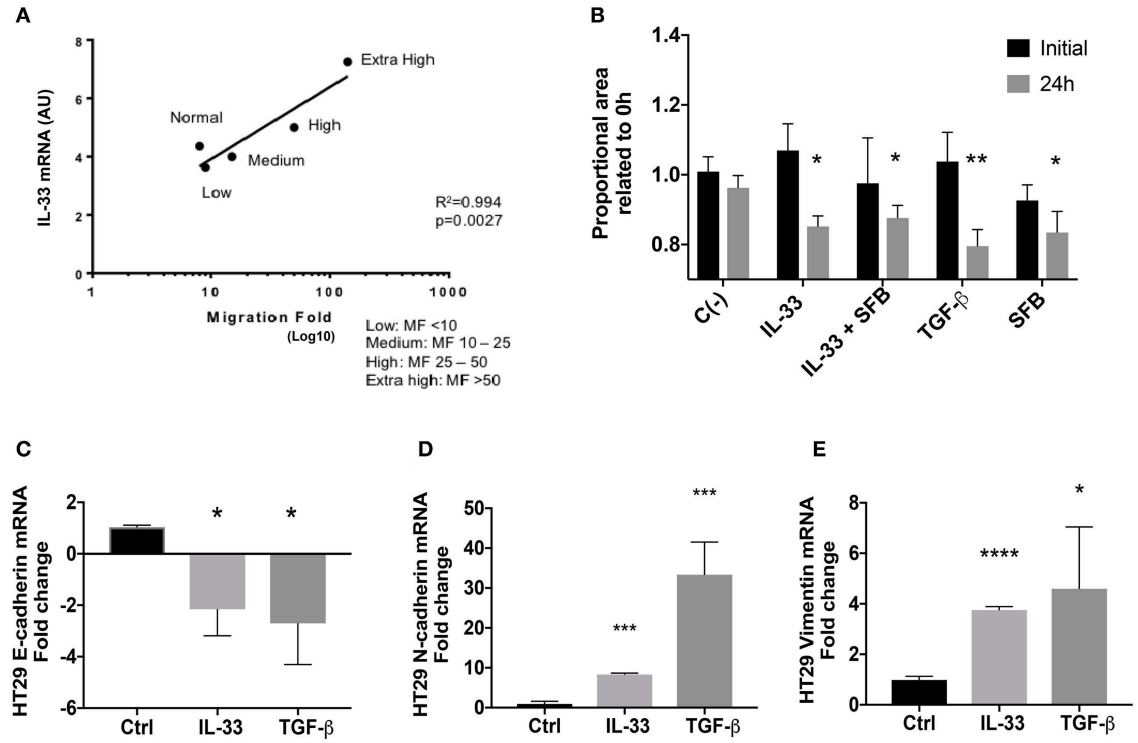


FIGURE 6 | IL-33 transcript correlates with a mesenchymal phenotype in HT29 cells. In **(A)**, IL-33 transcript levels from CRC patients-derived CAFs are associated with cell migration induced by CAFs conditioned media (Lineal regression test, $R^2 = 0.99$, $p = 0.0027$). In **(B)**, IL-33 induces HT29 cell migration at 24 h and decreases mRNA transcripts from E-cadherin **(C)** and increases N-cadherin **(D)** and vimentin **(E)** transcript levels. Student *t*- test was performed for these analyses, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

microenvironment, and it is a poor prognostic factor for CRC recurrence (17). In addition, desmoplasia exerts the modification of the extracellular matrix to favor tumor metastasis (13). The mechanism by which epithelial cells increase IL-33 expression is unclear (56), however, the effect of IL-33 on intestinal myofibroblasts was demonstrated in the CCD18Co cell line, activating the transcription of pathways related to a profibrotic response (34). Interestingly, it has been reported that IL-33 can enhance the recruitment and functions of different types of innate cells such as mast cells, Th2, regulatory T cells (Tregs), and innate lymphoid cells type 2 (ILC2s) (49, 57, 58). Of these, IL-33-mediated Treg infiltration has been described to contribute to an immunosuppressive milieu and poor prognosis not only in cancer, as seen in CRC and non-small cell lung cancer (NSCLC) models (58, 59), but also in murine models of chronic inflammatory diseases, such as colitis-induced colorectal cancer and allergic contact dermatitis-skin tumorigenesis, which in turn, might be prone to develop cancer (60). Also, ILC2s are of great interest due to their reported role in promoting fibrosis and desmoplasia after activation by IL-33 through the release of the profibrotic cytokine IL-13 (61). Thus, IL-33 could contribute to the development of desmoplasia by acting directly on myofibroblasts or indirectly through the recruitment and activation of immune cells like ILC2s.

The principal component analysis and cluster hierarchization allowed to separate patients with scarce desmoplasia from abundant desmoplasia by the pattern of epithelial and stromal IL-33, ST2, and α-SMA. This suggests that a certain distribution or pattern in the content of these molecules could be useful in discriminating the degree of desmoplasia especially in those intermediate cases, benefiting the patient's diagnosis and possibly the clinical management of this patients. However, a larger sample size of patients is necessary to validate this finding.

Another factor associated with tumor progression is the presence of tumor budding, which has been linked to a malignant phenotype on the invasive front, together with greater desmoplasia and higher risk of developing metastasis (62). Our findings show that the presence of TB correlates with tumors with greater desmoplasia, therefore, with an increase in stromal α-SMA immunoreactivity. The stromal ST2 immunoreactivity is diminished in tumors that present TB, also with decreased epithelial E-cadherin immunoreactivity. In the literature an association between TB and partial activation of EMT has been reported (63), with a decreased expression of E-cadherin or a modified membrane localization (64), probably due to alternative mechanisms activating canonical EMT transcription factors (Snail, Twist, Slug). These transcription factors have been observed at higher levels in the stroma, suggesting the participation of cells with mesenchymal phenotype (CAFs or

completely dedifferentiated tumor cells) (64). In CRC, the activation of EMT and the formation of TB could be exerted by CAFs (desmoplastic reaction) by activating the IL-33/ST2 axis, as was observed in head and neck cancer cell lines (33), favoring metastasis to LNs. Additionally, other cells that respond to IL-33 could contribute to the activation of EMT during colorectal cancer progress, as is the case of Tregs, which were described by *Xiong and cols* as promoters of EMT in a context of radiation-induced pulmonary fibrosis (65). These findings highlight the capacity of IL-33 to exert its EMT inducing effects through the action of different types of cells, a characteristic that was also seen in the case of desmoplasia.

Systematic reviews show that patients with left colon tumors are different than patients with right colon tumors in terms of mutation profile, gene expression profiles and consensus molecular subtypes (41, 43, 66). In addition, patients with CRC with normal KRAS (67) may respond differently to immunological therapies according to the location of the tumor, which suggests that the immune responses and tumor evolution in the right and left colon may be different. Therefore, we were interested in evaluating the protein levels and distribution of IL-33/ST2 according to tumor location. In CRC left colon tissue in early stages, the IL-33 content is decreased vs. normal tissue but increased when lymphatic metastasis occurs (stage TNM 3). However, the IL-33 immunoreactivity in epithelium or stroma did not show differences in the location or association with any variable of progression. The ST2 immunoreactivity decreases in the stroma of left colon tumors and in those patients with lymphatic metastasis. According to this data together with antecedents that attribute an anti-tumorigenic role to ST2 in murine models (38, 44), we suggest that ST2 could have a protective role on early stages of tumor progression, particularly in left colon and specially soluble ST2, which might neutralize IL-33. However, further studies are needed where these variables are analyzed directly, mostly because the antibody used in IHC and ELISA does not distinguish between soluble and membrane ST2 variants. In right colon tumors no association between the IL-33/ST2 content with the variables evaluated was observed, unfortunately, sample size of right colon CRC patients was not enough to statistically validate. In left-colon tumors it has been described higher levels of EGFR ligands Epiregulin (EREG) and Amphiregulin (AREG). Also, activation of EGFR has been associated to increased IL-33 expression in murine models, which might explain the increased levels of IL-33 in left- vs. right-sided tumors (41, 56, 68). Alternatively, the relationship between stromal ST2 and macrophage M2 markers suggest that this cell population can be a source of ST2L, inducing early profibrotic events or activating desmoplasia. There is evidence that M2 macrophages and CAFs favor tumor progression collaboratively in various types of cancer (18, 19, 69). However, we cannot rule out other ST2-positive cells that might contribute to CRC pathogenesis, such as mast cells and ILC2 cells (70, 71).

The content of IL-33 in tumor was greater than in healthy tissue of the left colon of CRC patients with lymphatic metastasis, so we evaluated IL-33 and ST2 distribution (determined by co-staining by IFI of IL-33/α-SMA and ST2/Ecad) and found nuclear and cytoplasmic IL-33 localized in tumor epithelium (similar to the primary tumor) with cytoplasmic in fibroblastic cells (areas with desmoplasia, α-SMA$^+$). Alternatively, those areas with greater ST2 immunoreactivity coincided with a decreased immunoreactivity of membrane E-cadherin in tumor cells. Given that IL-33 is localized in both tumor and fibroblastic cells (unlike the healthy LN), and additionally that IL-33 content is increased in the tumor of patients with lymphatic metastases, it is suggested that IL-33 could participate in mechanisms of tumor progression, either in an autocrine or paracrine fashion. In addition, ST2 immunoreactivity in tumor with decreased E-cadherin could reflect the induction of a mesenchymal phenotype leading to tumor progression. An association between IL-33 immunoreactivity in the tumor epithelium of patients with metastatic CRC and shorter survival has been reported, suggesting that tumor expression of IL-33 would be clinically important in its progression (37). In murine models of CRC, increased IL-33 levels in tumor cells induce greater hepatic metastasis, increase tumor size and, therefore, lower survival rate (36, 39). In breast and cervical cancer, increased expression of IL-33 has been observed in tumor cells from patients with LN metastases (72, 73), suggesting that IL-33/ST2 might participate in invasion and metastasis by remodeling primary and metastatic tumor microenvironment.

The effect of IL-33 on stromal activation was observed in a subepithelial fibroblast cell line (34), increasing the transcription of pathways mainly associated with TGF-β and extracellular matrix remodeling. In addition, the activation of fibroblasts according to the degree of invasion is particularly important in tumor progression, as it modifies the organization and composition of the extracellular matrix, forming pathways facilitating the migration and invasion of tumor cells (13). In patients with CRC, abundant desmoplasia is a predisposing factor to a shorter survival (74) and is also a risk factor for LN metastases (62). This suggests that one of the direct mechanisms by which IL-33 could be favoring tumor progression in CRC is through desmoplasia activation. But also, IL-33 from stromal fibroblasts can promote macrophage polarization to an M2 profile, secretion of MMP-9 and metastasis, as seen in an pancreatic adenocarcinoma model (75), and indirectly, IL-33 can also induce chemokine secretion by the tumor, recruiting M2 type macrophages (38) and contributing to greater desmoplasia.

As IL-33 content increases in patients with lymphatic metastasis, we evaluated the effect of IL-33 on the migratory capacities of the CRC cell line HT29 using the wound closure test as an approximation of the migration phenomenon (44). IL-33 induced greater migration observed at 24 h with respect to the control 0.5% FBS. There were no evaluations after 24 h since cell proliferation interferes in the measurement, while IL-33 stimulation (in the presence of FBS 10%) also increases proliferation at 24 h, again interfering with the wound test. Additionally, and since ST2 immunoreactivity coincides with a decrease of E-cadherin in metastatic lymphoid tumor cell membrane, we evaluated the effect of IL-33 on the transcript levels of classical EMT markers (E-cadherin, N-cadherin and vimentin). We have determined that IL-33 decreases E-cadherin transcript and increases vimentin and N-cadherin transcripts, which indicate the induction of a mesenchymal phenotype in HT29 cells. These results, coupled with the wound closure assay, suggest that IL-33 could induce a change toward a mesenchymal

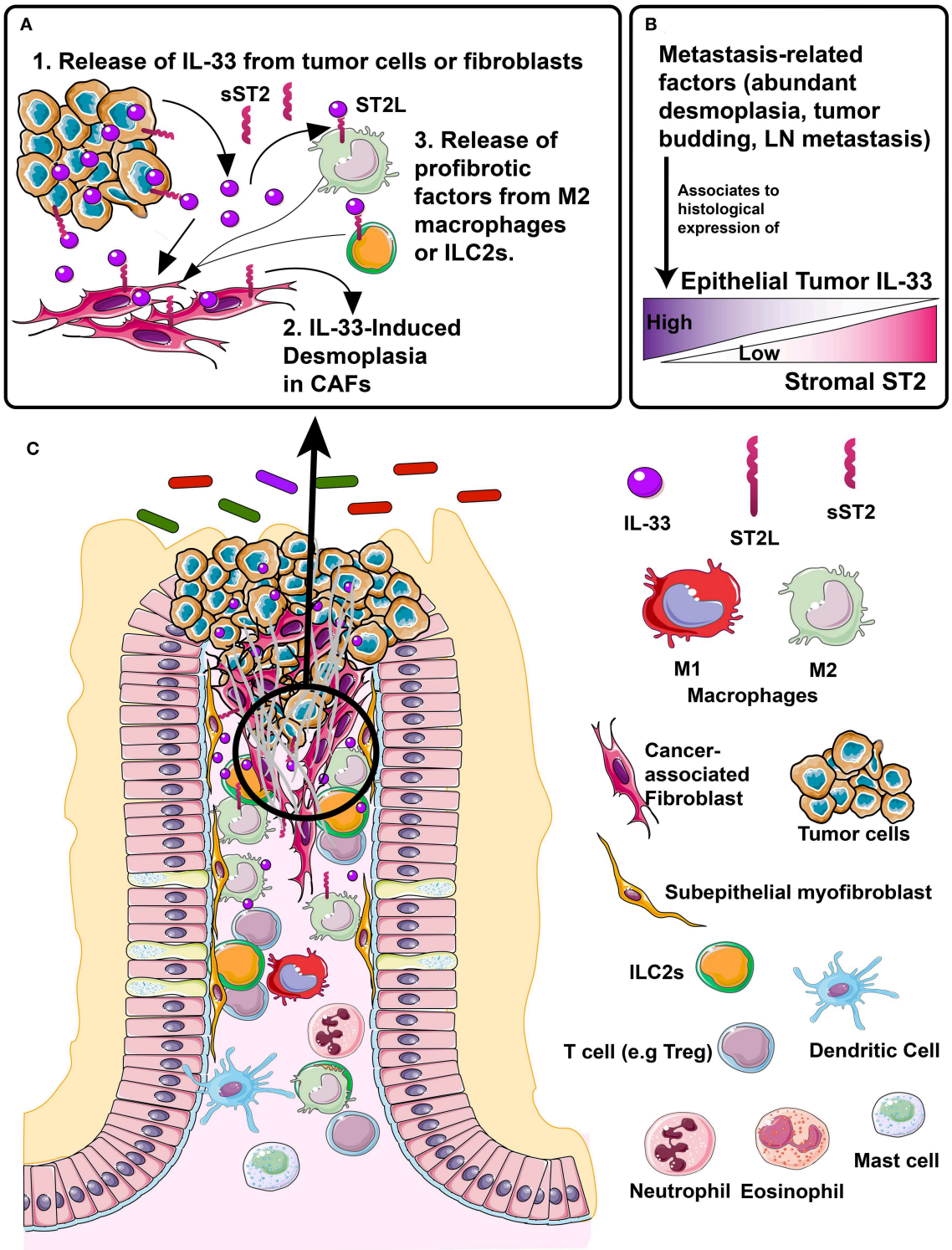


FIGURE 7 | Proposed model of IL-33/ST2 axis association to desmoplasia, a metastasis-related process in colorectal cancer patients. **(A)** In left-colon tumors, different events, e.g., necrosis, inflammation, mechanical strain, can induce the release of IL-33 in the tumor microenvironment, from both the tumor epithelium and fibroblasts, (A.1). IL-33 binds to ST2 receptor located in fibroblasts, directly inducing desmoplasia (A.2). The M2 macrophage or ILC2s located in the stroma can express both sST2 (which in early stages may neutralize IL-33 effects) and membrane bound ST2L which may activate desmoplasia indirectly by secreting profibrotic factors, such as IL-13 and TGFβ (A.3). **(B)** Metastasis-related factors such as abundant desmoplasia, tumor budding and LN metastasis are histologically associated with increased/high tumor cell IL-33 expression and decreased/low stromal ST2 expression, which if validated, could become a histological signature of tumor progression. **(C)** Representation of CRC tumor microenvironment (left) with distinctive molecules and cells (right) signaling through IL-33/ST2 axis and involved in tumor progression and metastasis.

phenotype in HT29 cells through the activation of EMT as was described in a head and neck cancer model (33); nevertheless, the analysis of additional EMT markers is needed to confirm the involvement of this process. The effect of IL-33 was also studied on HCT116 cells (**Supplementary Figures 6B–D**), where we observed a downregulation of E-cadherin and increase of vimentin transcript levels in response to IL-33, but no changes in migration or in N-cadherin transcript levels, which may be due to ST2L decrease, therefore truncating the process of mesenchymal activation. Alternatively, phenotypic and molecular differences of HT29 and HCT116 lines suggest the HCT116 line needs an additional stimulus to complete the mesenchymal phenotype of EMT.

Therefore, the IL-33/ST2 axis may participate in the interaction of the tumor microenvironment, mediating processes associated with metastasis in CRC primarily in the left colon (**Figure 7**).

CONCLUSION

In conclusion, tumor IL-33 increase and stromal ST2 decrease are associated with greater desmoplasia in left colon tumors, which in turn might contribute to the development of lymphatic metastasis. The inflammatory content of the microenvironment increases IL-33 transcript in CAFs, whose levels are associated with increased cell migration, whilst activating the onset of a migrating mesenchymal phenotype in an adenocarcinoma cell line. Controlling IL-33/ST2 axis expression represents a potential target to improve a personalized left-sided CRC therapy.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Review Boards and Local Ethical Committees from Universidad de Chile Clinical Hospital, Tissue Biobank of Universidad de Chile and Clinica Las Condes, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki and were identified only by codification established by their respective Center to keep anonymity. The protocol was approved by the Local Ethical Committees from Universidad de Chile Clinical Hospital, Tissue Biobank of Universidad de Chile and Clinica Las Condes.

AUTHOR CONTRIBUTIONS

GL designed and performed most of the experiments, analyses of results and manuscript drafting. MD, KD-C, DP-V, and DD-J contributed to design, discussion of results and performed IL-33 detection in human samples. MD also contributed with macrophages IHQ analyses. DR and SS performed the immunohistochemistry staining of TMA, JR validated Aperio algorithms and performed histological characterization of TMAs. CS contributed in statistical analyses. CP contributed with CAF microarray data. RQ, FL-K, UK, MA, and DS enrolled CRC patients. IG selected colorectal tissue from Tissue Biobank. M-JG and OO-S participated in the analysis and discussion of results. HC contributed with qPCR equipment and EMT expertise. GD-A and MH contributed to study design and supervised work. All the authors contributed to drafting and discussion of the manuscript.

FUNDING

Clinica Las Condes Academic Project PI2013-B002 (RQ), National Fund for Scientific and Technological Development No. 1170648 and Support of International Networking Between Research Centers REDES180134 (MH), National Fund for Scientific and Technological Development No. 1151214 (HC), National Fund for Scientific and Technological Development No. 3150328 (MD), National Commission for Scientific and Technological Research Scholarship No. 21140837 (GL), MECESUP UCH1304 travel grant (GL) and MECESUP Scholarship No. UCH 0714 (KD-C), National Commission for Scientific and Technological Research Scholarship No. 21150264 (DD-J) and National Commission for Scientific and Technological Research Scholarship No. 21150517 (DP-V).

ACKNOWLEDGMENTS

We wish to thank to CP Laboratory, Madrid, Spain for their support to this research. Also, we thank to the Tissue Biobank from Universidad de Chile for obtaining colorectal samples and the Histopathology Nucleus from Pontifical Catholic University of Chile for their histological assistance. We thank to Dr. Katherine Marcelain (Universidad de Chile) for facilitating Cytation 3 equipment for wound closure assay. The figures were produced using Servier Medical Art from www.servier.com. We would also like to thank David Cox for his editing contribution.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01394/full#supplementary-material

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2018) 68:394–424. doi: 10.3322/caac.21492
2. Zarate AJ, Alonso FT, Garmendia ML, López-Köstner F. Increasing crude and adjusted mortality rates for colorectal cancer in a developing South American country. *Colorectal Dis.* (2013) 15:47–51. doi: 10.1111/j.1463-1318.2012.03110.x
3. Rao CV, Yamada HY. Genomic instability and colon carcinogenesis: from the perspective of genes. *Front Oncol.* (2013) 3:130. doi: 10.3389/fonc.2013.00130

4. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. *Nat Publ Gr.* (2015) 1:1–25. doi: 10.1038/nrdp.2015.65
5. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. *Oncogene.* (2008) 27:5904–12. doi: 10.1038/onc.2008.271
6. Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. *Exp Cell Res.* (2010) 316:1324–31. doi: 10.1016/j.yexcr.2010.02.045
7. Swartz MA, Iida N, Roberts EW, Sangaletti S, Wong MH, Yull FE, et al. Tumor microenvironment complexity: emerging roles in cancer therapy. *Cancer Res.* (2012) 72:2473–80. doi: 10.1158/0008-5472.CAN-12-0122
8. Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic Inflammation and cytokines in the tumor microenvironment. *J Immunol Res.* (2014) 2014:149185. doi: 10.1155/2014/149185
9. Rybinski B, Franco-Barraza J, Cukierman E. The wound healing, chronic fibrosis, and cancer progression triad. *Physiol Genom.* (2014) 46:223–44. doi: 10.1152/physiolgenomics.00158.2013
10. Turley SJ, Cremasco V, Astarita JL. Immunological hallmarks of stromal cells in the tumour microenvironment. *Nat Publ Gr.* (2015) 15:669–82. doi: 10.1038/nri3902
11. Kobayashi H, Enomoto A, Woods SL, Burt AD, Takahashi M, Worthley DL. Cancer-associated fibroblasts in gastrointestinal cancer. *Nat Rev Gastroenterol Hepatol.* (2019) 16:282–95. doi: 10.1038/s41575-019-0115-0
12. Karagiannis GS, Petraki C, Prassas I, Saraon P, Musrap N, Dimitromanolakis A, et al. Proteomic Signatures of the Desmoplastic Invasion Front Reveal Collagen Type XII as a Marker of Myofibroblastic Differentiation During Colorectal Cancer Metastasis. *Oncotarget.* (2012) 3:267–85. doi: 10.18632/oncotarget.451
13. Malik R, Lelkes PI, Cukierman E. Biomechanical and biochemical remodeling of stromal extracellular matrix in cancer. *Trends Biotechnol.* (2015) 33:230–6. doi: 10.1016/j.tibtech.2015.01.004
14. Ueno H, Shinto E, Shimazaki H, Kajiwara Y, Sueyama T, Yamamoto J, et al. Histologic categorization of desmoplastic reaction: its relevance to the colorectal cancer microenvironment and prognosis. *Ann Surg Oncol.* (2015) 22:1504–12. doi: 10.1245/s10434-014-4149-9
15. Ueno H, Kanemitsu Y, Sekine S, Ishiguro M, Ito E, Hashiguchi Y, et al. Desmoplastic pattern at the tumor front defines poor-prognosis subtypes of colorectal cancer. *Am J Surg Pathol.* (2017) 41:1506–12. doi: 10.1097/PAS.0000000000000946
16. Ueno H, Shinto E, Kajiwara Y, Fukazawa S, Shimazaki H, Yamamoto J, et al. Prognostic impact of histological categorisation of epithelial-mesenchymal transition in colorectal cancer. *Br J Cancer.* (2014) 111:2082–90. doi: 10.1038/bjc.2014.509
17. Tsujino T, Seshimo I, Yamamoto H, Chew YN, Ezumi K, Takemasa I, et al. Stromal myofibroblasts predict disease recurrence for colorectal cancer. *Clin Cancer Res.* (2007) 13:2082–90. doi: 10.1158/1078-0432.CCR-06-2191
18. Herrera M, Herrera A, Domínguez G, Silva J, García V, García JM, et al. Cancer-associated fibroblast and M2 macrophage markers together predict outcome in colorectal cancer patients. *Cancer Sci.* (2013) 104:437–44. doi: 10.1111/cas.12096
19. Chiarugi P. Cancer-associated fibroblasts and macrophages: friendly conspirators for malignancy. *Oncoimmunology.* (2013) 2:e25563. doi: 10.4161/onci.25563
20. Liao D, Luo Y, Markowitz D, Xiang R, Reisfeld RA. Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. *PLoS ONE.* (2009) 4:e7965. doi: 10.1371/journal.pone.0007965
21. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015
22. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. *Biochem Biophys Res Commun.* (2009) 384:105–9. doi: 10.1016/j.bbrc.2009.04.081
23. Moussion C, Ortega N, Girard JP. 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:e3331. doi: 10.1371/journal.pone.0003331
24. De la Fuente M, MacDonald TT, Hermoso MA. The IL-33/ST2 axis: Role in health and disease. *Cytokine Growth Factor Rev.* (2015) 26:615–23. doi: 10.1016/j.cytogfr.2015.07.017
25. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TKA, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature.* (2010) 464:1367–70. doi: 10.1038/nature08900
26. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. *J Immunol.* (2010) 185:3472–80. doi: 10.4049/jimmunol.1000730
27. Beltrán CJ, Núñez LE, Díaz-Jiménez D, Farfan N, Candia E, Heine C, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1097–107. doi: 10.1002/ibd.21175
28. Jovanovic IP, Pejnovic NN, Radosavljevic GD, Pantic JM, Milovanovic MZ, Arsenijevic NN, et al. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. *Int J Cancer.* (2014) 134:1669–82. doi: 10.1002/ijc.28481
29. Schmieder A, Multhoff G, Radons J. Interleukin-33 acts as a pro-inflammatory cytokine and modulates its receptor gene expression in highly metastatic human pancreatic carcinoma cells. *Cytokine.* (2012) 60:514–21. doi: 10.1016/j.cyto.2012.06.286
30. Chen XJ, Huang Y, De Li N, Chen M, Liu F, Pu D, et al. Correlations between serum IL-33 and tumor development: a meta-analysis. *Asian Pacific J Cancer Prev.* (2014) 15:3503–5. doi: 10.7314/APJCP.2014.15.8.3503
31. Villarreal DO, Wise MC, Walters JN, Reuschel EL, Choi MJ, Obeng-Adjei N, et al. Alarmin IL-33 Acts as an immunoadjuvant to enhance antigen-specific tumor immunity. *Cancer Res.* (2014) 74:1789–800. doi: 10.1158/0008-5472.CAN-13-2729
32. Gao K, Li X, Zhang L, Bai L, Dong W, Gao K, et al. Transgenic expression of IL-33 activates CD8 + T cells and NK cells and inhibits tumor growth and metastasis in mice. *Cancer Lett.* (2013) 335:463–71. doi: 10.1016/j.canlet.2013.03.002
33. Chen S, Nieh S, Jao S, Wu MZ, Liu C, Chang YC, et al. The paracrine effect of cancer-associated fibroblast-induced interleukin-33 regulates the invasiveness of head and neck squamous cell carcinoma. *J Pathol.* (2013) 231:180–9. doi: 10.1002/path.4226
34. Maywald RL, Doerner SK, Pastorelli L, Salvo C, De Benton SM, Dawson EP, et al. IL-33 activates tumor stroma to promote intestinal polyposis. *Proc Natl Acad Sci USA.* (2015) 112:E2487–96. doi: 10.1073/pnas.1422445112
35. Cui G, Qi H, Gundersen MD, Yang H, Christiansen I, Sørbye SW, et al. Dynamics of the IL-33/ST2 network in the progression of human colorectal adenoma to sporadic colorectal cancer. *Cancer Immunol Immunother.* (2015) 64:181–90. doi: 10.1007/s00262-014-1624-x
36. Liu X, Zhu L, Lu X, Bian H, Wu X, Yang W, et al. IL-33/ST2 pathway contributes to metastasis of human colorectal cancer. *Biochem Biophys Res Commun.* (2014) 453:486–92. doi: 10.1016/j.bbrc.2014.09.106
37. Fang M, Li Y, Huang K, Qi S, Zhang J, Zgodzinski W, et al. IL-33 promotes colon cancer cell stemness via JNK activation and macrophage recruitment. *Cancer Res.* (2017) 77:2735–45. doi: 10.1158/0008-5472.CAN-16-1602
38. Akimoto M, Maruyama R, Takamaru H, Ochiya T, Takenaga K. Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by modifying the tumour microenvironment. *Nat Commun.* (2016) 7:1–15. doi: 10.1038/ncomms13589
39. Zhang Y, Davis C, Shah S, Hughes D, Ryan JC, Altomare D, et al. IL-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis. *Mol Carcinog.* (2017) 56:272–87. doi: 10.1002/mc.22491
40. Gaspodarowicz M, Wittekind C. *TNM Classification of Malignant Tumors*. 8th edn. John Wiley & Sons (2017). p. 1–15.
41. Lee MS, Menter DG, Kopetz S. Right versus left colon cancer biology: Integrating the consensus molecular subtypes. *JNCCN J Natl Compr Cancer Netw.* (2017) 15:411–9. doi: 10.6004/jnccn.2017.0038
42. Herrera M, Islam ABMMK, Herrera A, Martín P, García V, Silva J, et al. Functional heterogeneity of cancer-associated fibroblasts from human colon tumors shows specific prognostic gene expression signature. *Clin Cancer Res.* (2013) 19:5914–26. doi: 10.1158/1078-0432.CCR-13-0694

43. Lee GH, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer? - A systematic review. *Eur J Surg Oncol.* (2015) 41:300–8. doi: 10.1016/j.ejso.2014.11.001
44. O'Donnell C, Mahmoud A, Keane J, Murphy C, White D, Carey S, et al. An antitumorigenic role for the IL-33 receptor, ST2L, in colon cancer. *Br J Cancer.* (2016) 114:37–43. doi: 10.1038/bjc.2015.433
45. Mertz KD, Mager LF, Wasmer M-H, Thiesler T, Koelzer VH, Ruzzante G, et al. The IL-33/ST2 pathway contributes to intestinal tumorigenesis in humans and mice. *Oncoimmunology.* (2015) 5:e1062966. doi: 10.1080/2162402X.2015.1062966
46. Maric V, Jovanovic M, Zdravkovic N, Jovanovic M, Gajovic N, Jurisevic M, et al. Increased IL-33 and IL-17 in colorectal carcinoma patients with severe disease. *Serbian J Exp Clin Res.* (2018) 15:29–32. doi: 10.2478/sjecr-2018-0034
47. Bannura CG, Barrera EA, Cumsille GMA, Contreras PJ, Melo LC, Soto CD. Role of palliative excision of stage IV colon and rectal cancer. Analysis of 177 patients | Rol de la resección paliativa en cáncer colorrectal estadio iv: Análisis de 177 pacientes. *Rev Chil Cir.* (2009) 61:266–74. doi: 10.4067/S0718-40262009000300009.
48. Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weissleder R, von Boehmer H, et al. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF- signals *in vivo*. *Proc Natl Acad Sci USA*. (2005) 102:419–24. doi: 10.1073/pnas.0408197102
49. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front Immunol.* (2017) 8:475. doi: 10.3389/fimmu.2017.00475
50. Wasmer MH, Krebs P. The role of IL-33-dependent inflammation in the tumor microenvironment. *Front Immunol.* (2017) 7:682. doi: 10.3389/fimmu.2016.00682
51. Larsen KM, Minaya MK, Vaish V, Peña MMO. The role of IL-33/ST2 pathway in tumorigenesis. *Int J Mol Sci.* (2018) 19:1–28. doi: 10.3390/ijms19092676
52. Akimoto M, Takenaga K. Role of the IL-33/ST2L axis in colorectal cancer progression. *Cell Immunol.* (2018). doi: 10.1016/j.cellimm.2017.12.014. [Epub ahead of print].
53. Fournié JJ, Poupot M. The pro-tumorigenic IL-33 involved in antitumor immunity: a yin and yang cytokine. *Front Immunol.* (2018) 9:2506. doi: 10.3389/fimmu.2018.02506
54. Cui G, Yuan A, Pang Z, Zheng W, Li Z, Goll R. Contribution of IL-33 to the pathogenesis of colorectal cancer. *Front Oncol.* (2018) 8:561. doi: 10.3389/fonc.2018.00561
55. Shen JX, Liu J, Zhang GJ. Interleukin-33 in malignancies: friends or Foes? *Front Immunol.* (2018) 9:3051. doi: 10.3389/fimmu.2018.03051
56. Islam MS, Horiguchi K, Iino S, Kaji N, Mikawa S, Hori M, et al. EGF is a critical regulator of the interleukin-33 in intestinal epithelial cells. *Br J Pharmacol.* (2016) 173:2532–42. doi: 10.1111/bph.13535
57. Lu J, Kang J, Zhang C, Zhang X. The role of IL-33/ST2L signals in the immune cells. *Immunol Lett.* (2015) 164:11–7. doi: 10.1016/j.imlet.2015.01.008
58. Li A, Herbst RH, Canner D, Schenkel JM, Smith OC, Koch DH. Longitudinal single cell profiling of regulatory T cells identifies IL-33 as a driver of tumor immunosuppression. *BioRXiv.* (2019) 2:7. doi: 10.1101/512905
59. Zhou Y, Ji Y, Wang H, Zhang H, Zhou H. IL-33 Promotes the development of colorectal cancer through inducing tumor-infiltrating ST2L+ regulatory T cells in mice. *Technol Cancer Res Treat.* (2018) 17:1–11. doi: 10.1177/1533033818780091
60. Lee RT, Zaalberg A, Demehri S, Park JH, Lopez E, Li T, et al. IL-33/regulatory T cell axis triggers the development of a tumor-promoting immune environment in chronic inflammation. *Proc Natl Acad Sci USA.* (2019) 116:201815016. doi: 10.1073/pnas.1815016116
61. Mattner J, Wirtz S. Friend or foe? the ambiguous role of innate lymphoid cells in cancer development. *Trends Immunol.* (2017) 38:29–38. doi: 10.1016/j.it.2016.10.004
62. Jayasinghe C, Simiantonaki N, Kirkpatrick CJ. Histopathological features predict metastatic potential in locally advanced colon carcinomas. *BMC Cancer.* (2015) 15:14. doi: 10.1186/s12885-015-1013-7
63. Grigore A, Jolly M, Jia D, Farach-Carson M, Levine H. Tumor budding: the name is EMT. Partial EMT. *J Clin Med.* (2016) 5:51. doi: 10.3390/jcm5050051
64. Yamada N, Sugai T, Eizuka M, Tsuchida K, Sugimoto R, Mue Y, et al. Tumor budding at the invasive front of colorectal cancer may not be associated with the epithelial-mesenchymal transition. *Hum Pathol.* (2017) 60:151–9. doi: 10.1016/j.humpath.2016.10.007
65. Xiong S, Pan X, Xu L, Yang Z, Guo R, Gu Y, et al. Regulatory T cells promote β-catenin-mediated epithelium-to-mesenchyme transition during radiation-induced pulmonary fibrosis. *Int J Radiat Oncol Biol Phys.* (2015) 93:425–35. doi: 10.1016/j.ijrobp.2015.05.043
66. Petrelli F, Tomasello G, Borgonovo K. Prognostic survival associated with left-sided vs right-sided colon cancer: a systematic review and meta-analysis. *JAMA Oncol.* (2017) 3:211–9. doi: 10.1001/jamaoncol.2016.4227
67. Venook AP, Niedzwiecki D, Innocenti F, Fruth B, Greene C, O'Neil BH, et al. Impact of primary (1°) tumor location on overall survival (OS) and progression-free survival (PFS) in patients (pts) with metastatic colorectal cancer (mCRC): analysis of CALGB/SWOG 80405 (Alliance). *J Clin Oncol.* (2016) 34:Abstr 3504. doi: 10.1200/JCO.2016.34.15_suppl.3504
68. Xu Q, Chiao P, Sun Y. Amphiregulin in cancer: new insights for translational medicine. *Trends Cancer.* (2016) 2:111–3. doi: 10.1016/j.trecan.2016.02.002
69. Comito G, Giannoni E, Segura CP, Barcellos-de-Souza P, Raspollini MR, Baroni G, et al. Cancer-associated fibroblasts and M2-polarized macrophages synergize during prostate carcinoma progression. *Oncogene.* (2013) 33:2423–31. doi: 10.1038/onc.2013.191
70. Blatner NR, Bonertz A, Beckhove P, Cheon EC, Krantz SB, Strouch M, et al. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. *Proc Natl Acad Sci USA.* (2010) 107:6430–5. doi: 10.1073/pnas.0913683107
71. Tian Z, van Velkinburgh JC, Wu Y, Ni B. Innate lymphoid cells involve in tumorigenesis. *Int J Cancer.* (2016) 138:22–9. doi: 10.1002/ijc.29443
72. Liu J, Shen JX, Hu JL, Huang WH, Zhang GJ. Significance of interleukin-33 and its related cytokines in patients with breast cancers. *Front Immunol.* (2014) 5:141. doi: 10.3389/fimmu.2014.00141
73. Wu Z, Zhang H. Prognostic value of interleukin-33 in cervical cancer patients who underwent radical hysterectomy. *Int J Clin Exp Pathol.* (2016) 9:5466–72. Available online at: http://www.ijcep.com/V9_No5.html
74. Sis B, Sarioglu S, Sokmen S, Sakar M, Kupelioglu A, Fuzun M. Desmoplasia measured by computer assisted image analysis: an independent prognostic marker in colorectal carcinoma. *J Clin Pathol.* (2005) 58:32–8. doi: 10.1136/jcp.2004.018705
75. Andersson P, Yang Y, Hosaka K, Zhang Y, Fischer C, Braun H, et al. Molecular mechanisms of IL-33-mediated stromal interactions in cancer metastasis. *JCI Insight.* (2018) 3:e122375. doi: 10.1172/jci.insight.122375

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Landskron, De la Fuente López, Dubois-Camacho, Díaz-Jiménez, Orellana-Serradell, Romero, Sepúlveda, Salazar, Parada-Venegas, Quera, Simian, González, López-Köstner, Kronberg, Abedrapo, Gallegos, Contreras, Peña, Díaz-Araya, Roa and Hermoso. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

CORRECTION
published: 24 September 2019
doi: 10.3389/fimmu.2019.02149

Corrigendum: Interleukin 33/ST2 Axis Components Are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer

*Glauben Landskron[1], Marjorie De la Fuente López[1,2], Karen Dubois-Camacho[1], David Díaz-Jiménez[1], Octavio Orellana-Serradell[1], Diego Romero[3], Santiago A. Sepúlveda[3], Christian Salazar[1], Daniela Parada-Venegas[1], Rodrigo Quera[4], Daniela Simian[2], María-Julieta González[5], Francisco López-Köstner[6], Udo Kronberg[6], Mario Abedrapo[6,7], Iván Gallegos[8], Héctor R. Contreras[9], Cristina Peña[10], Guillermo Díaz-Araya[11], Juan Carlos Roa[3] and Marcela A. Hermoso[1]**

[1] Immunology Program, Innate Immunity Laboratory, Faculty of Medicine, Biomedical Sciences Institute, Universidad de Chile, Santiago, Chile, [2] Research Sub-direction, Academic Direction, Clinica Las Condes, Santiago, Chile, [3] Pathology Department, Faculty of Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile, [4] Inflammatory Bowel Disease Program, Gastroenterology Department, Clinica Las Condes, Santiago, Chile, [5] Cell and Molecular Biology Program, Faculty of Medicine, Institute of Biomedical Sciences, Universidad de Chile, Santiago, Chile, [6] Coloproctology Department, Clinica Las Condes, Santiago, Chile, [7] Coloproctology Surgery Department, Hospital Clinico Universidad de Chile, Santiago, Chile, [8] Pathology Department, Hospital Clinico Universidad de Chile, Santiago, Chile, [9] Department of Basic and Clinic Oncology, Faculty of Medicine, Universidad de Chile, Santiago, Chile, [10] Medical Oncology Department, Ramon y Cajal University Hospital, IRYCIS, CIBERONC, Madrid, Spain, [11] Molecular Pharmacology Laboratory, Faculty of Chemical Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile

Keywords: colorectal cancer, cancer associated fibroblasts, interleukin 33, desmoplasia, epithelial-mesenchymal transition

OPEN ACCESS

Approved by:
Frontiers Editorial Office, Frontiers Media SA, Switzerland

***Correspondence:**
Marcela A. Hermoso
mhermoso@med.uchile.cl

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *30 July 2019*
Accepted: *27 August 2019*
Published: *24 September 2019*

Citation:
Landskron G, De la Fuente López M, Dubois-Camacho K, Díaz-Jiménez D, Orellana-Serradell O, Romero D, Sepúlveda SA, Salazar C, Parada-Venegas D, Quera R, Simian D, González M-J, López-Köstner F, Kronberg U, Abedrapo M, Gallegos I, Contreras HR, Peña C, Díaz-Araya G, Roa JC and Hermoso MA (2019) Corrigendum: Interleukin 33/ST2 Axis Components Are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer. Front. Immunol. 10:2149. doi: 10.3389/fimmu.2019.02149

A Corrigendum on

Interleukin 33/ST2 Axis Components Are Associated to Desmoplasia, a Metastasis-Related Factor in Colorectal Cancer

by Landskron, G., De la Fuente López, M., Dubois-Camacho, K., Díaz-Jiménez, D., Orellana-Serradell, O., Romero, D., et al. (2019). Front. Immunol. 10:1394. doi: 10.3389/fimmu.2019.01394

"Francisco López-Köstner" was not included as an author in the published article. The corrected Author Contributions Statement appears below.

"GL designed and performed most of the experiments, analyses of results and manuscript drafting. MD, KD-C, DP-V, and DD-J contributed to design, discussion of results and performed IL-33 detection in human samples. MD also contributed with macrophages IHQ analyses. DR and SS performed the immunohistochemistry staining of TMA, JR validated Aperio algorithms and performed histological characterization of TMAs. CS contributed in statistical analyses. CP contributed with CAF microarray data. RQ, FL-K,

UK, MA, and DS enrolled CRC patients. IG selected colorectal tissue from Tissue Biobank. M-JG and OO-S participated in the analysis and discussion of results. HC contributed with qPCR equipment and EMT expertise. GD-A and MH contributed to study design and supervised work. All the authors contributed to drafting and discussion of the manuscript."

"There was also an error regarding the affiliations for Mario Abedrapo. As well as having affiliation 7, they should also have affiliation 6."

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Copyright © 2019 Landskron, De la Fuente López, Dubois-Camacho, Díaz-Jiménez, Orellana-Serradell, Romero, Sepúlveda, Salazar, Parada-Venegas, Quera, Simian, González, López-Köstner, Kronberg, Abedrapo, Gallegos, Contreras, Peña, Díaz-Araya, Roa and Hermoso. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership