West Road, Yuexiu District, 510120 Guangzhou, China.
Email: tianpeng3@mail.sysu.edu.cn
Hua Zhong, Department of Otorhinolaryngology, First Affiliated Hospital of Sun Yat-sen University, Sun Yat-sen University, Zhongshan Er Lu, No. 58, Yuexiu District, 510080 Guangzhou, China.
Email: zhongh7@mail.sysu.edu.cn

Yun Ma and Yi Wei should be considered joint first authors.

ORCID

Yun Ma https://orcid.org/0000-0002-4672-3584
Yi Wei https://orcid.org/0000-0002-7310-5634
Hua Zhong https://orcid.org/0000-0002-9120-2323

REFERENCES

1. Fokkens WJ, Lund VJ, Hopkins C, et al. European position paper on rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020;58(Suppl S29):1-464.
2. Lou H, Zhang N, Bachert C, Zhang L. Highlights of eosinophilic chronic rhinosinusitis with nasal polyps in definition, prognosis, and advancement. *Int Forum Allergy Rhinol*. 2018;8(11):1218-1225.
3. Xie S, Zhang H, Liu Y, et al. The role of serum metabolomics in distinguishing chronic rhinosinusitis with nasal polyp phenotypes. *Front Mol Biosci*. 2020;7:593976.
4. Miyata J, Fukunaga K, Kawashima Y, et al. Dysregulated fatty acid metabolism in nasal polyp-derived eosinophils from patients with chronic rhinosinusitis. *Allergy*. 2019;74(6):1113-1124.
5. Xu X, Li J, Zhang Y, Zhang L. Arachidonic acid 15-lipoxygenase: effects of its expression, metabolites, and genetic and epigenetic variations on airway inflammation. *Allergy Asthma Immunol Res*. 2021;13(5):684-696.
6. Archambault AS, Tinto F, Dumais É, et al. Biosynthesis of the novel endogenous 15-lipoxygenase metabolites N-13-hydroxy-octodecadienoyl-ethanolamine and 13-hydroxy-octodecad ienoyl-glycerol by human neutrophils and eosinophils. *Cells*. 2021;10(9):2322.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

DOI: 10.1111/all.15127

Tissue eosinophils express the IL-33 receptor ST2 and type 2 cytokines in patients with eosinophilic esophagitis

To the Editor,

Eosinophilic esophagitis (EoE) is a type 2 inflammatory disease of the esophagus largely driven by food antigens and is characterized by esophageal eosinophilia, fibrosis, and clinically by dysphagia and food impactions [1]. The alarmin interleukin (IL)-33 is elevated in the esophageal epithelium and endothelium of patients with EoE [2,3]. Exogenous IL-33 causes esophageal eosinophilia and basal hyperplasia in mice [3], implicating IL-33 in EoE pathogenesis. The IL-33 receptor, suppression of tumorigenicity 2 (IL1RL1/ST2), induces type 2 cytokines IL-4, IL-5, and IL-13. As blockade of IL-4/IL-13 improves both histologic and clinical EoE [4], and trials of anti-IL-5R are ongoing, we aimed to identify cells that can respond to IL-33 and the cellular sources of pathogenic Type 2 cytokines in EoE. T-helper 2 (Th2) CD4 lymphocytes are known producers of IL-4, IL-5, and IL-13, as are group 2 innate lymphoid cells (ILC2), but whether tissue eosinophils, mast cells, or basophils contribute type 2 cytokines in active EoE is under explored [5,6].

We examined biopsies and blood samples from patients undergoing esophagogastroduodenoscopy at our institution (Table S1). We found eosinophils, mast cells, basophils, and Th2 cells; however, ILC2s were not reliably detected in esophageal tissue and were withheld from further analyses (Figure 1A-B). ST2 was robustly detected on tissue eosinophils from patients with active EoE (Figure 1C, 1E), whereas blood eosinophils from healthy control, remission, and active EoE were uniformly low for ST2 (Figure 1D, 1F-G; Figure S1). Similarly, serum levels of soluble ST2 (sST2), which is shed as a decoy receptor, were not significantly different among healthy controls, patients with EoE in remission or active disease (Figure 1H, 1I).

We next measured IL-4, IL-5, and IL-13 by flow cytometry and immunofluorescence from esophageal tissue in patients with active EoE (Figure 2A-H). Eosinophils (which unlike T cells were not stimulated with PMA/ionomycin) were frequent expressors of these cytokines (Figure 2A-E). IL-4 was detected in most unstimulated eosinophils (60%) compared to mast cells (8.0%) and basophils 3.8% (Figure 2C). We found IL-13 in 74% of eosinophils compared to 17% of stimulated CRTH2+ Th2 cells, 3.5% of mast cells, and 3.4% of basophils (Figure 2D-F). Although the proportion

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2021 The Authors. *Allergy* published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.

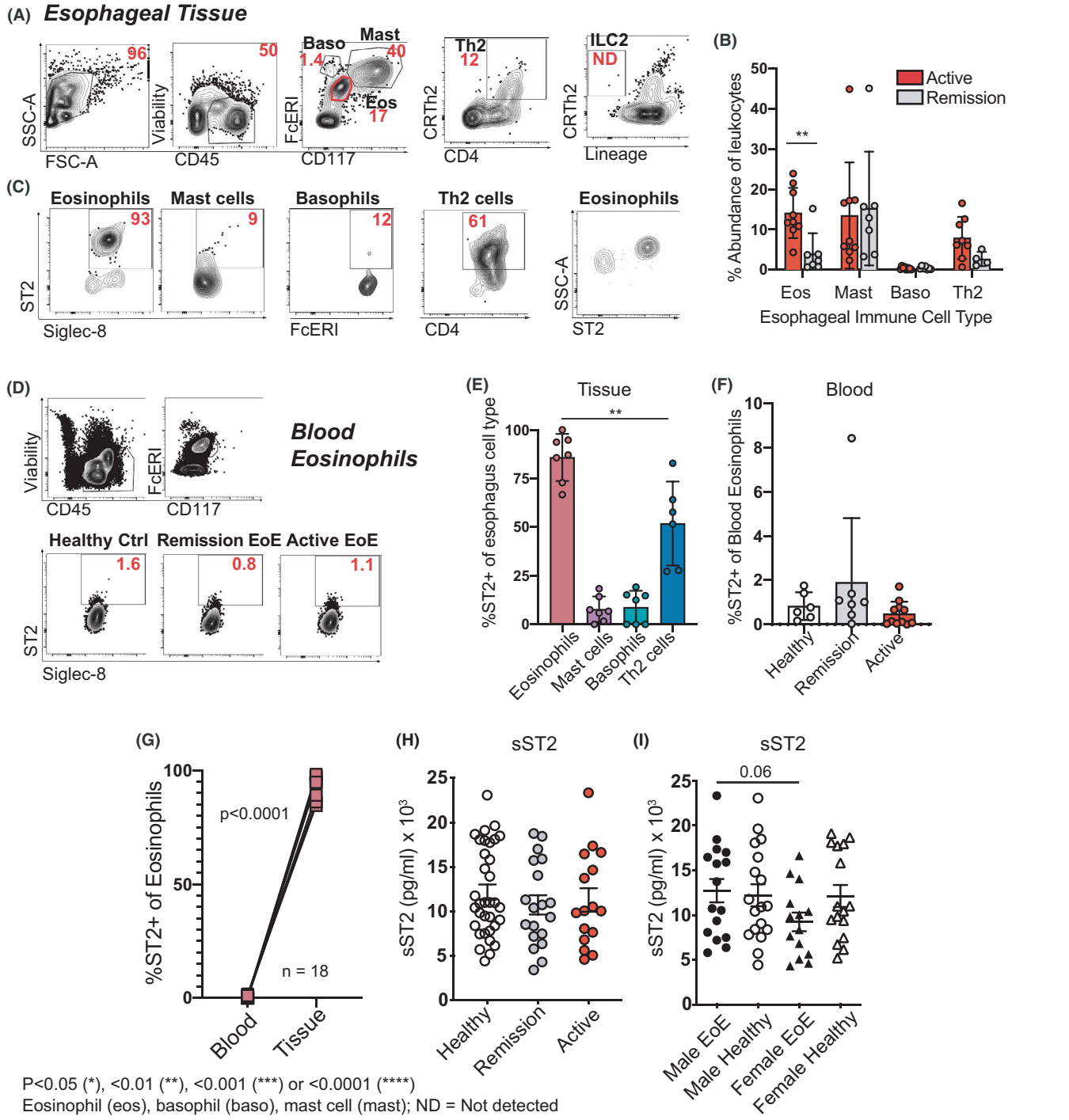

FIGURE 1 Tissue eosinophils express ST2. (A) Flow cytometry of esophageal biopsies. (B) Cell type as percent of $CD45^+$ leukocytes in patients with active (n=8–9) and remission (n=4–7) EoE. Flow cytometry showing ST2 expression across esophageal immune cells (C) and blood eosinophils (D). Quantification of ST2 in tissue (E) and blood eosinophils (F). (G) ST2 on blood versus tissue eosinophils. (H) Concentration of sST2 in healthy non-EoE, remission, and active EoE patients shown overall (H) and by sex (I). N=34 EoE (16 active, 16 remission), n=34 controls

of unstimulated mast cells positive for type 2 cytokines was relatively low, mast cells may be pathogenic in EoE and warrant further investigation. Tissue eosinophil expression of type 2 cytokine protein was further verified by immunofluorescent staining for IL-4, IL-13, and eosinophil peroxidase (EPX). A majority of the $IL\text{-}4^+$ cells in the esophageal epithelium were eosinophils (80.7%); this was similar for $IL\text{-}13^+$ cells (82.7%) (Figure 2G-H; Figure S2). To assess whether eosinophils were necessary for type 2 cytokine induction, we administered IL-33 to eosinophil-sufficient (BALB/c WT) and deficient (BALB/c ΔdblGATA-1) mice using a previously established model of EoE [3] (Figure 2I). Immunofluorescence revealed eosinophil infiltration in IL-33-treated WT esophagi but not PBS-treated

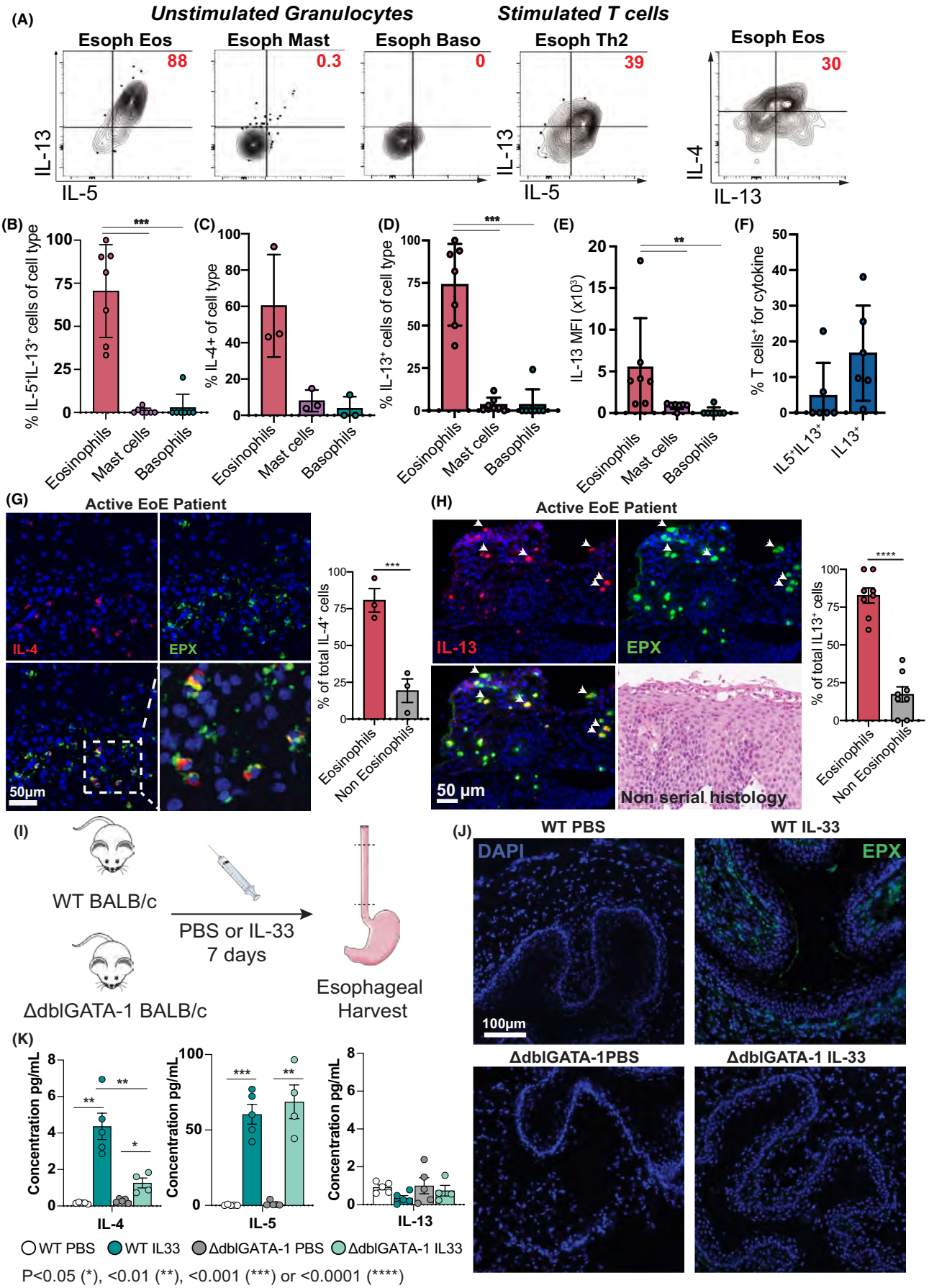
(A)
Unstimulated Granulocytes
Stimulated T cells
Esoph Eos
Esoph Mast
Esoph Baso
Esoph Th2
Esoph Eos
88
0.3
0
39
30
IL-13
IL-5
IL-4
(B)
% IL-5+IL-13+ cells of cell type
(C)
% IL-4+ of cell type
(D)
% IL-13+ cells of cell type
(E)
IL-13 MFI (x10³)
(F)
% T cells+ for cytokine
Eosinophils
Mast cells
Basophils
IL5+IL13+
IL13+
(G)
Active EoE Patient
IL-4
EPX
50µm
% of total IL-4+ cells
Eosinophils
Non Eosinophils
(H)
Active EoE Patient
IL-13
EPX
50 µm
Non serial histology
% of total IL13+ cells
(I)
WT BALB/c
PBS or IL-33
7 days
ΔdblGATA-1 BALB/c
Esophageal
Harvest
(J)
WT PBS
WT IL-33
DAPI
EPX
100µm
ΔdblGATA-1PBS
ΔdblGATA-1 IL-33
(K)
Concentration pg/mL
IL-4
IL-5
IL-13
WT PBS
WT IL33
ΔdblGATA-1 PBS
ΔdblGATA-1 IL33
P<0.05 (*), <0.01 (**), <0.001 (***) or <0.0001 (****)

FIGURE 2 Human esophageal eosinophils express type 2 cytokines IL-4, IL-5, and IL-13 by flow cytometry and immunofluorescence. (A) Flow cytometry of intracellular cytokine IL-4, IL-5, and IL-13 on esophageal immune cells, quantification shown in (B) with IL-5 and IL-13 co-expression, (C) IL-4, (D) IL-13, and (E) IL-13 MFI. T-cell cytokines shown in (F). (G-H) Immunofluorescence of human esophageal tissue identifies eosinophils by eosinophil peroxidase (EPX), IL-4- and IL-13-expressing cells with quantification shown to the right. (I) IL-33 EoE mouse model; n=5/group. (J) Immunofluorescence of mouse esophagi. (K) Esophageal cytokine concentrations for IL-4, IL-5, and IL-13 from various treatment groups

WT or ΔdblGATA-1 mice, as expected (Figure 2J). Although IL-13 was below the limit of detection in this model, multiplex cytokine analysis of esophagi showed IL-33-induced IL-4 was critically dependent on the presence of eosinophils (Figure 2K).

Here, we report frequent expression of the IL-33 receptor ST2 on esophageal-infiltrating eosinophils compared to blood eosinophils, other granulocytes, and Th2 cells. Noting eosinophils lack antigen-specific T-cell receptors, their ability to respond to IL-33 may explain continued inflammation observed in patients after removal of dietary antigen. Future studies are warranted to identify factors influencing ST2 and IL-33 expression and determine whether modulation of this pathway may be a novel therapeutic approach to this increasingly diagnosed yet poorly understood allergic disease.

KEYWORDS

clinical immunology, eosinophils, flow cytometry, interleukins, mucosal immunity

FUNDING INFORMATION

National Institutes of Health; American Partnership for Eosinophilic Disorders; Massachusetts General Hospital; Harvard Medical School; Bristol-Myers Squibb; American Gastroenterological Association

ACKNOWLEDGMENTS

We would like to acknowledge the contributions from the Harvard Stem Cell Institute-Center for Regenerative Medicine Flow Cytometry Core and the Program for Membrane Biology Microscopy Core at Simches Research Center, Massachusetts General Hospital. The authors also thank Paula Montero Llopis and Timothy James Ross-Elliott of the Harvard Medical School MicRoN core facility for microscopy assistance and Diane Bracket, MD for capturing non-serial histology image.

CONFLICT OF INTEREST

AMU, PJL, PV, KCM, MVY, LQ, LAC, and LRA have no conflicts of interest to disclose. MD receives research support from Novartis and Eli Lilly, consulting fees from Partner Therapeutics, Tillotts Pharma, ORIC Pharmaceuticals, AzurRx, SQZ, Moderna, WebMD, and Experts at Your Fingertips, and Genentech, and is a member of the Scientific Advisory Board for Neoleukin Therapeutics. JJG reports no conflicts of interest related to this study but has received research support from Takeda and the American Partnership for Eosinophilic Disorders. SKD reports no conflicts of interest related to this study, but has received research support from Novartis, BMS, and Eli Lilly, is a co-founder and SAB member for Kojin, and received consulting fees from GSK.

AUTHOR CONTRIBUTIONS

AMU performed and analyzed flow cytometry, immunofluorescence, and animal experiments. PV, PJL, MVY, LQ, and LRA assisted with acquiring data and conducting experiments. KCM and LAC screened and recruited subjects from the patient cohort and obtained samples for analysis. MD provided patient samples and also managed patient cohort, in addition to providing intellectual guidance. JJG provided patient samples, managed patient cohort, supervised experiments, and analyzed all data providing intellectual guidance of the project. SKD supervised experiments, analyzed all data, and provided intellectual guidance of the project. All authors approved the final version of the manuscript.

FUNDING INFORMATION

AMU received funding through NIH T32DK007191 grant. PJL was funded by NIH T32CA207021. JJG has received research award support from the American Partnership for Eosinophilic Disorders (APfED). LQ was funded by a SITC-Bristol-Myers Squibb Postdoctoral Cancer Immunotherapy Translational Fellowship. SKD was funded by the Emerson Collective Cancer Research Fund, NIH U01 CA224146, the Ludwig Center at Harvard and is a Pew-Stewart Scholar in Cancer Research. MD was funded by a Mentored Clinical Scientist Development Award 1K08DK114563, the MGH Center for the Study of Inflammatory Bowel Disease DK043351, and an American Gastroenterology Association Research Scholars Award.

Amiko M Uchida[1,2,3]
Patrick J Lenehan[2,3]
Praveen Vimalathas[1]
Kaia C Miller[1]
Mabel Valencia-Yang[1]
Li Qiang[3,4]
Lauren A Canha[1]
Lestat R Ali[2,3]
Michael Dougan[1,2,3,4]
John J Garber[1,2]
Stephanie K Dougan[3,4]

[1]*Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA*
[2]*Department of Medicine, Harvard Medical School, Boston, MA, USA*
[3]*Cancer Immunology and Virology, Dana Farber Cancer Institute, Boston, MA, USA*
[4]*Department of Immunology, Harvard Medical School, Boston, MA, USA*

Correspondence
Amiko M. Uchida, Gastroenterology Unit, Massachusetts General Hospital, Cancer Immunology and Virology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.
Email: amuchida@mgh.harvard.edu

John J Garber and Stephanie K Dougan contributed equally to this study as senior authors.

ORCID
Amiko M Uchida https://orcid.org/0000-0003-2171-4769

REFERENCES

1. Dellon ES, Liacouras CA, Molina-Infante J, Furuta GT, Spergel JM, Zevit N, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterol*. 2018;155(4):1022-33 e10.
2. Travers J, Rochman M, Caldwell JM, Besse JA, Miracle CE, Rothenberg ME. IL-33 is induced in undifferentiated, non-dividing esophageal epithelial cells in eosinophilic esophagitis. *Sci Rep*. 2017;7(1):17563.
3. Judd LM, Heine RG, Menheniott TR, et al. Elevated IL-33 expression is associated with pediatric eosinophilic esophagitis, and exogenous IL-33 promotes eosinophilic esophagitis development in mice. *Am J Physiol Gastrointest Liver Physiol*. 2016;310(1):G13-25.
4. Hirano I, Dellon ES, Hamilton JD, et al. Efficacy of Dupilumab in a Phase 2 Randomized Trial of Adults With Active Eosinophilic Esophagitis. *Gastroenterology*. 2020;158(1):111-22 e10.
5. Doyle AD, Masuda MY, Kita H, Wright BL. Eosinophils in Eosinophilic Esophagitis: The Road to Fibrostenosis is Paved With Good Intentions. *Front Immunol*. 2020;11:603295.
6. Straumann A, Kristl J, Conus S, et al. Cytokine expression in healthy and inflamed mucosa: probing the role of eosinophils in the digestive tract. *Inflamm Bowel Dis*. 2005;11(8):720-726.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

DOI: 10.1111/all.15128

Allergic eye disease: Blocking LTB4/C5 in vivo suppressed disease and Th2 & Th9 cells

To the Editor,

Vernal keratoconjunctivitis (VKC) affects children and can impair vision if the cornea becomes involved. Immunosuppressives (steroids and cyclosporin A) are required but can have side effects, and novel immunotherapeutic approaches are needed.[1] The aim of this study was to investigate the contributions of LTB4 and complement C5 in a model of allergic eye disease (experimental allergy conjunctivitis, EAC).[2] Previous studies have demonstrated that nomacopan, a bifunctional recombinant biologic derived from blood-feeding ticks, has anti-inflammatory properties by capturing LTB4 and preventing it from interacting with its two known G protein-coupled cell surface receptors (GPCR BLT1 and BLT2). Simultaneously, nomacopan inhibited C5 end terminal complement activation, thereby preventing formation of C5b-9 and C5a.[3] These two pathways have evolutionary connections as phylogenetic analysis showed that tick saliva started as an LTB4 inhibitor, and subsequently acquired the ability to inhibit C5. It has been shown in a mouse model of inflammatory arthritis that C5a production resulted in the release of LTB4 to promote further neutrophil migration to the interstitium.[4]

EAC is a model of allergic eye disease mainly driven by effector Th2 cells and mast cells (MC). During EAC, conjunctival inflammation can be detected after 5 days, scored non-invasively[2] (Figure S1A–C), with elevated levels of conjunctival IL-9-expressing $CD4^+$T cells and MC detected in tissues and cells expanded from conjunctival explants (Figure S1D–J). Significantly increased levels of $tryptase^+$ conjunctival MC were observed, co-expressing intracellular IL-9 in the sub-epithelial area within the fornix of conjunctival EAC tissues (Figure S1G).

In this study, we investigated the effects of topically administered nomacopan in EAC and observed a significant suppression of disease in treated mice, and a decrease in IL-9-expressing $CD4^+$T cells (Figure 1A–C; Figure S2A). Recent studies have demonstrated that IL-9 can be produced by Th9 and Th2 cells.[5,6] To determine which $CD4^+$T cell subset was producing IL-9, transcription factor expression was investigated. IL-9-producing Th2 cells express GATA3, but not PU-1, whereas IL-9-producing Th9 cells express PU.1, but not GATA3. Within the infiltrating $CD4^+$T cells in EAC, Th9 cells ($IL\text{-}9^+PU.1^+$) and IL-9-expressing Th2 cells

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2021 The Authors. Allergy published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.