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REFERENCES

1. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis. *Nat Immunol*. 2017;18:1076-1083.
2. Gupta J, Johansson E, Bernstein JA, et al. Resolving the etiology of atopic disorders by using genetic analysis of racial ancestry. *J Allergy Clin Immunol*. 2016;138:676-699.
3. Speidel L, Forest M, Shi S, Myers SR. A method for genome-wide genealogy estimation for thousands of samples. *Nat Genet*. 2019;51:1321-1329.
4. Raj T, Kuchroo M, Replogle JM, Raychaudhuri S, Stranger BE, de Jager PL. Common risk alleles for inflammatory diseases are targets of recent positive selection. *Am J Hum Genet*. 2013;92:517-529.
5. Deschamps M, Laval G, Fagny M, et al. Genomic signatures of selective pressures and introgression from archaic hominins at human innate immunity genes. *Am J Hum Genet*. 2016;98:5-21.
6. Kreiner E, Waage J, Standl M, et al. Shared genetic variants suggest common pathways in allergy and autoimmune diseases. *J Allergy Clin Immunol*. 2017;140:771-781.

SUPPORTING INFORMATION

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

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Soluble ST2 enhances IL-33–induced neutrophilic and pro-type 2 inflammation in the lungs

To the Editor,

Soluble suppression of tumorigenicity 2 (sST2) is a decoy receptor for interleukin 33 (IL-33). We previously reported that high serum levels of sST2 predict unfavorable outcomes in patients with pneumonia[1] or asthma,[2] although its function in the lungs remains poorly understood. We hypothesized that sST2 modifies IL-33–induced neutrophilic inflammation in the lungs. We measured levels of sST2, IL-33, and pro-neutrophilic or pro-Th17 chemokines in the sputum of patients with asthma, chronic obstructive pulmonary disease (COPD), or asthma–COPD overlap (ACO), and administered a recombinant soluble form of ST2 (ST2-Fc) and/or IL-33 to mice.

Both sST2 and IL-33 were high in patients with ACO, whereas only sST2 was high in patients with asthma and only IL-33 was high in patients with COPD (Figure 1A,B; Table S1). There was no correlation between sST2 and IL-33 levels (Figure 1C). IL-33 levels were positively correlated with chemokine (CXC motif) ligand 8 (CXCL8) and chemokine (CC motif) ligand 20 (CCL20) but were not correlated with CXCL1 levels (Figure 1D). We expected sST2 to negatively regulate IL-33–induced pro-neutrophilic inflammation, but this was not the case (Figure 1E). Conversely, elevated levels of both sST2 and IL-33 (characteristic of ACO patients) were associated with elevation of CXCL1 (as reported previously[3]) and CCL20 levels (Figure 1F). Cell counts were not conducted at the time of collection, so they could not be analyzed. These results do not support the hypothesis that sST2 works as a decoy receptor for IL-33 in human airways.

Based on the clinical data, we suspected that sST2 may be a carrier protein. To test this idea, we administered mice with mouse (m) IL-33 and/or mST2-Fc at 2.5 and 25 μg protein, respectively (molar ratio, 1:2.8), because the concentration of sST2 in the sputum of patients with ACO was approximately 10 times that of IL-33 (medians of 927 and 74.8 pg/m, respectively; Table S1). Surprisingly, mST2-Fc augmented the mIL-33–induced influx of neutrophils and dendritic cells into the alveolar spaces, as well as bronchial inflammation and secretion of CXCL1, matrix metalloproteinase 9 (MMP9), dsDNA, and CCL17 into the airspaces (Figure 2A–C). Also, mIL-33 promoted an influx of eosinophils (Figure 2A) and secretion of IL-5 (data not shown) into the airspaces, but ST2-Fc did not augment this process. Furthermore, we detected ST2-Fc–IL-33 complexes and enhanced recovery of mIL-33 and mST2-Fc in BAL fluid (Figure 2D). Finally, we confirmed that ST2-Fc–IL-33 complex formation did occur between mST2-Fc and mIL-33 in vitro (Figure 2E). Therefore, mST2-Fc augments mIL-33–induced airway neutrophilia and pro-type 2 inflammation via complex formation.

We thus demonstrated that ST2-Fc enhances IL-33 activity in vivo. Similar forms of augmentation, caused by several types of cytokines and their corresponding anti-cytokine monoclonal antibodies, have been reported. For instance, anti-IL-4 and anti-granulocyte-colony stimulating factor (G-CSF) monoclonal antibodies enhance IL-4 and G-CSF, respectively, in mice, via the formation of immune complexes in the circulation system.[4] These findings support our discovery that ST2-Fc augments IL-33 activity.

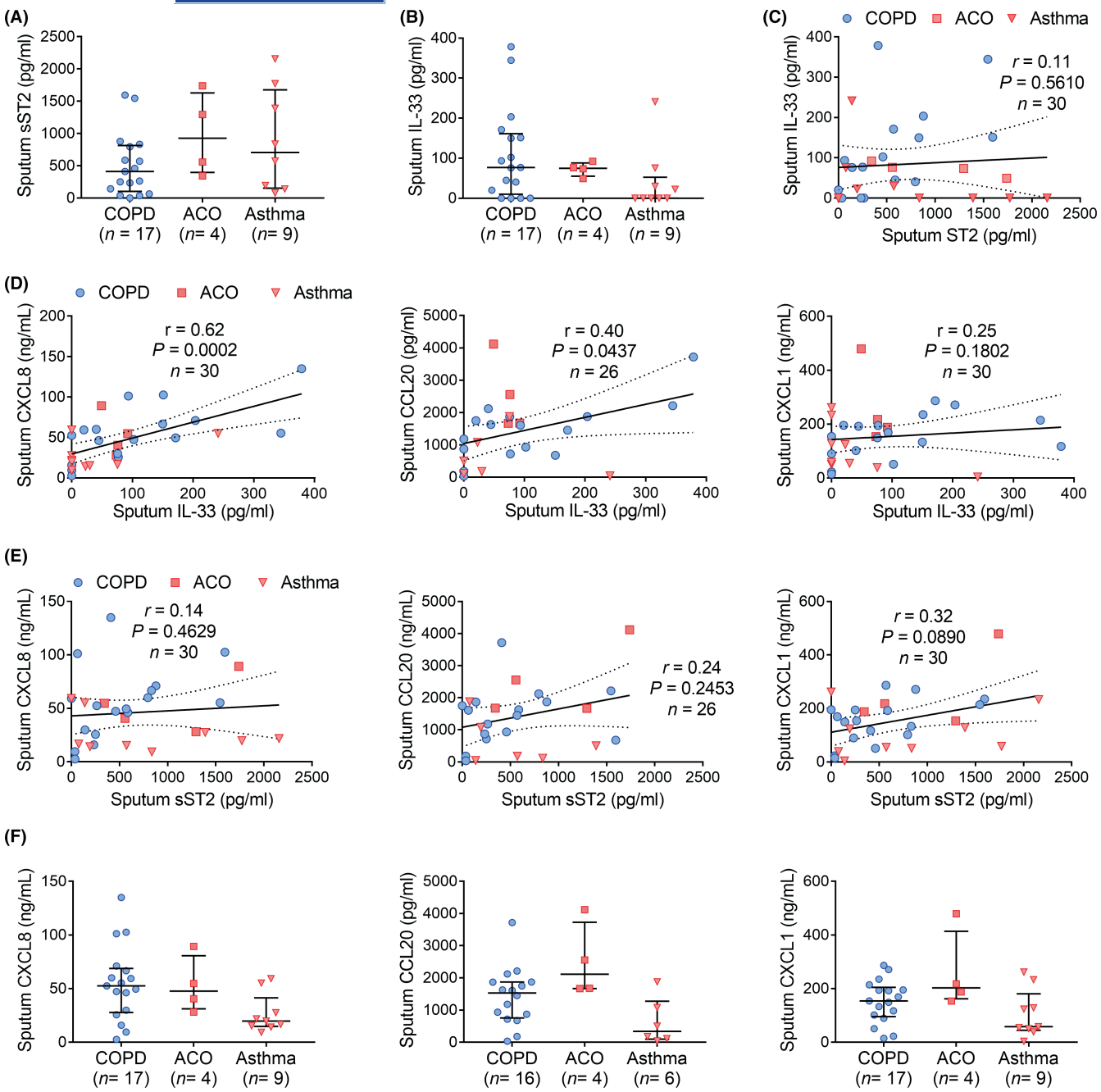

FIGURE 1 Levels of sST2, IL-33, and chemokines in the airways of patients with asthma, COPD, or ACO. (A, B) Levels of sST2 (A) or IL-33 (B) in sputum from patients with COPD, ACO, or asthma. Data are expressed as median and interquartile range. (C–E) Scatter plots of sST2 and IL-33 (C), IL-33 and chemokine (D), and sST2 and chemokine (E) levels in sputum from the same patients. (F) Levels of CXCL8, CCL20, and CXCL1 in sputum from the same patients. *p*-values were calculated using Spearman's rank correlations. ACO, asthma–COPD overlap; COPD, chronic obstructive pulmonary disease

Cell-surface IL-33 receptors are composed of cell-surface ST2 (ST2L) and IL-1RAcP, and a fusion protein of ST2 and IL-1RAcP, IL-33trap, binds to IL-33 thirty times more strongly than sST2.[5] These findings support our claim that sST2 carries IL-33 to cell-surface IL-33 receptors. Together, ST2-Fc and IL-33 induced greater neutrophilic inflammation with the release of dsDNA than IL-33 alone. Asthmatics with high levels of neutrophil-derived extracellular DNA in their sputum experience more asthma exacerbation and poorer lung function than those without.[6] Thus, sST2–IL-33 complexes may contribute to the pathogenesis of neutrophilic asthma. In conclusion, sST2 may augment IL-33 activity via the formation of sST2–IL-33 complexes.

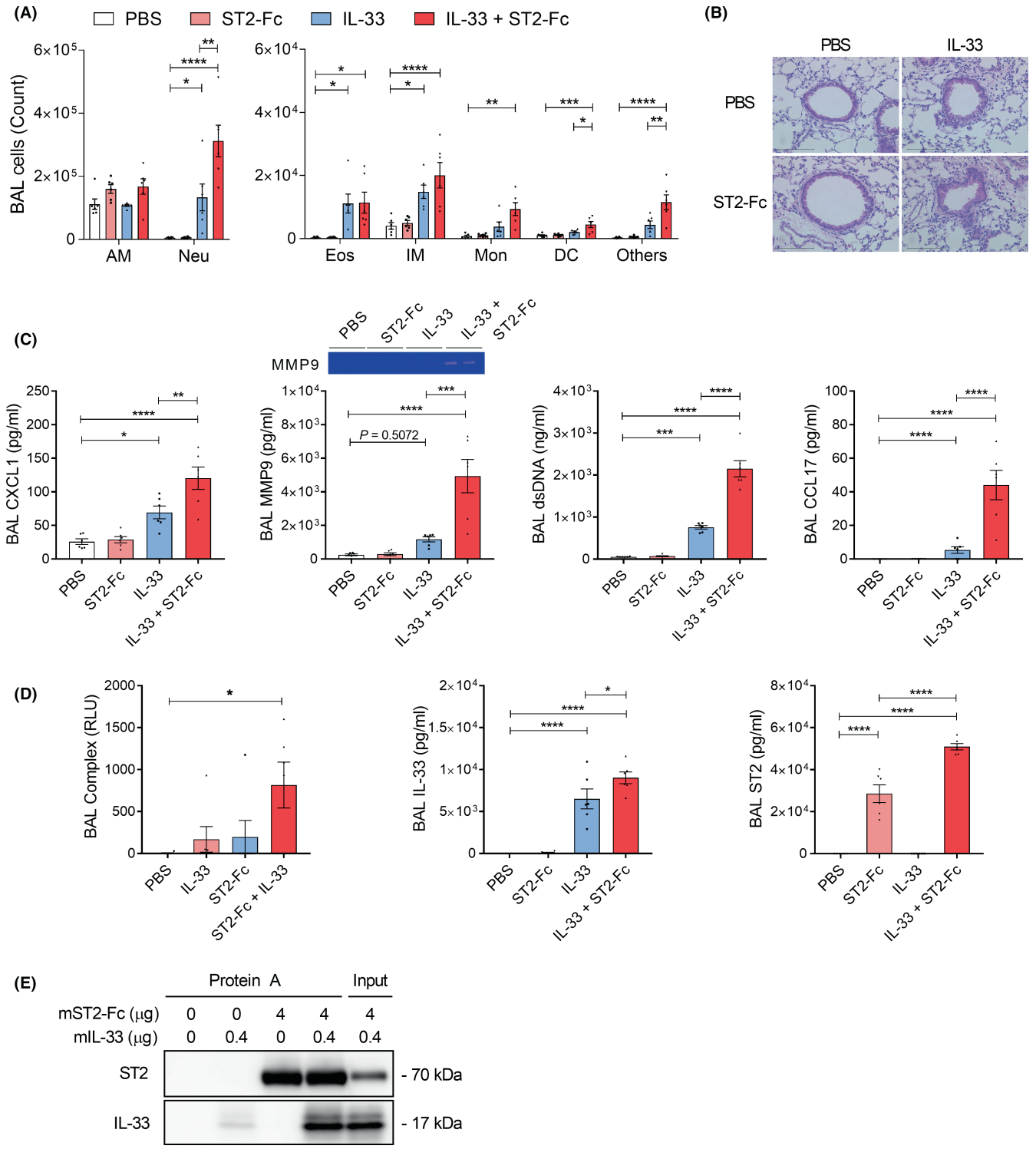

FIGURE 2 Soluble form of ST2 augments IL-33–induced inflammation in mouse lungs. Recombinant mIL-33 (2.5 μg), mST2-Fc (25 μg), both mIL-33 and mST2-Fc (2.5 and 25 μg, respectively), or PBS (50 μl) were administrated to female BALB/c mice via intratracheal spray. Differential cell counts (A), HE stains of lung tissue (B), secretion of CXCL1, MMP9 (with gelatin zymography of MMP9, upper panel), dsDNA, and CCL17 (C), and recovery of mST2-Fc–mIL-33 complexes, IL-33, and ST2 (D) in BAL fluid were assessed 24 h after administration. (E) Presence of mST2-Fc–mIL-33 complexes in a solution of mST2-Fc (4 μg) and mIL-33 (0.4 μg) in PBS (50 μl) after incubation for 1 h at 37°C. The complex (mIL-33 bound to mST2-Fc) was isolated via immunoprecipitation using protein A beads and detected via immunoblot assay. A mixture that was not immunoprecipitated (containing free mIL-33, free mST2-Fc, and mST2-Fc–mIL-33 complexes) was also assessed (input). Data are pooled from three experiments ($n = 6$, each group) and are expressed as mean ± SEM. ****$p < .0001$, ***$p < .001$, **$p < .01$, *$p < .05$. p-values were calculated using one-way ANOVA and post hoc Holm–Sidak tests (A, C, and D). BAL, bronchoalveolar lavage; PBS, Phosphate-buffered saline

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KEYWORDS

asthma, asthma–COPD overlap, COPD, neutrophil, sputum

CONFLICT OF INTEREST

None of the authors have any conflicts of interest to declare.

CONSENT FOR PUBLICATION

Not applicable.

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REFERENCES

1. Watanabe M, Takizawa H, Tamura M, et al. Soluble ST2 as a prognostic marker in community-acquired pneumonia. *J Infect*. 2015;70(5):474-482.
2. Watanabe M, Nakamoto K, Inui T, et al. Serum sST2 levels predict severe exacerbation of asthma. *Respir Res*. 2018;19(1):169.
3. Inui T, Watanabe M, Nakamoto K, et al. Bronchial epithelial cells produce CXCL1 in response to LPS and TNFalpha: a potential role in the pathogenesis of COPD. *Exp Lung Res*. 2018;44(7):323-331.
4. Woytschak J, Keller N, Krieg C, et al. Type 2 Interleukin-4 receptor signaling in neutrophils antagonizes their expansion and migration during infection and inflammation. *Immunity*. 2016;45(1):172-184.

5. Holgado A, Braun H, Van Nuffel E, et al. IL-33trap is a novel IL-33-neutralizing biologic that inhibits allergic airway inflammation. *J Allergy Clin Immunol*. 2019;144(1):204-215.
6. Lachowicz-Scroggins ME, Dunican EM, Charbit AR, et al. Extracellular DNA, neutrophil extracellular traps, and inflammasome activation in severe asthma. *Am J Respir Crit Care Med*. 2019;199(9):1076-1085.

SUPPORTING INFORMATION

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Impact of time-varying confounders on the association between early-life allergy sensitization and the risk of current asthma: A post hoc analysis of a birth cohort

To the Editor,

Existing literature on the relationship between early-life (first year of life) allergy sensitization and risk of childhood asthma is mixed.[1,2] This is in part due to the use of statistical analytic methods that ignore changes in both allergy sensitization status and asthma-related treatment exposure that may influence future asthma risk as a child grows older. Our recent disease transition models show that both childhood allergy sensitization and current asthma states (and plausibly related treatment) are time-varying, and the likelihood of a feedback loop cannot be ruled out[3]; this underscores the analytic challenges associated with evaluating to what extent early-life allergy sensitization may be causally related to childhood asthma development. Moreover, it is unclear if early-life allergen avoidance prevents or merely delays the onset of current asthma into adolescence or adulthood.[4]

In contrast to traditional discrete-time and longitudinal models (e.g., Generalized Estimating Equations—GEE) used in previous research,[1,2] Marginal Structural Models (MSM) can be used to adjust for time-varying confounding to produce consistent average causal effect estimands.[5] Previous simulation studies have demonstrated the superiority of the MSM over traditional longitudinal statistical methodology.[5] In this study, we hypothesize that among infants genetically predisposed to asthma, early-life allergy sensitization is associated with an increased risk of current asthma but this risk can be attenuated by allergen avoidance. We carried out a post hoc analysis using the MSM approach to estimate the average causal effect of early-life allergy sensitization and allergen avoidance on the risk of current asthma under the context of dynamic (changing) allergy sensitization-current asthma states in the Canadian Asthma Primary Prevention Study (CAPPS).[6] CAPPS was a multifaceted intervention designed to decrease exposure in the first year of infancy to indoor aeroallergens such as house dust mites and pets and to encourage prolonged breastfeeding and delayed introduction of milk and solid foods.[6] Current asthma and allergy sensitization were based on a pediatric allergist's clinical decision and skin prick test results, respectively, at age 1-, 2-, 7-, and 15-years. Briefly, MSMs are estimated using an inverse-probability-of-treatment (or exposure) weighted approach[5] to remove the effects of time-varying confounders (i.e., post-baseline allergy sensitization and asthma-related treatment states) in the pathway between early-life sensitization and subsequent risk of current asthma (see Appendix S1 for details).

The prevalence of current asthma and allergy sensitization varied during follow-up with and without CAPPS exposure (Figures 1, S1 and S2). The prevalence of current asthma did not differ between children sensitized to aeroallergens vs. food allergens in the first 2 years; however, during the 7th and 15th years, the odds of current asthma were six and four-fold higher among children sensitized (vs. un-sensitized) to food and aeroallergens, respectively (Figure S2). These results suggest different profiles of allergy sensitization (define by type and age) may differentially influence or modify the propensity of school-age asthma development. Our MSM model results (Table 1) showed that the odds of current asthma were higher among children with (vs. without) an early-life allergy sensitization (adjusted odds ratio [aOR]: 3.02; 95% CI: 1.51, 6.01) at age 7-years;

Abbreviations: aOR, adjusted Odds Ratio; CAPPS, Canadian Asthma Primary Prevention Study; CI, confidence intervals; GEE, Generalized Estimating Equations; IPTW, inverse-probability-of-treatment (or exposure) weighted approach; MSM, Marginal Structural Models; OR, Odds Ratio.