

Epithelial activators of type 2 inflammation: Elevation of thymic stromal lymphopoietin, but not IL-25 or IL-33, in chronic rhinosinusitis with nasal polyps in Chicago, Illinois

To the Editor,

It is well recognized that chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by type 2 inflammation with eosinophilia and high levels of IL-5 and IL-13 in Western countries.¹ In addition, several studies suggest that the inflammatory endotypes of CRSwNP in Asian countries are shifting toward type 2 inflammation from noneosinophilic inflammation and that 50% or more of nasal polyps (NPs) currently show tissue eosinophilia in these countries.¹ However, the mechanisms of induction of type 2 inflammation in NPs are still largely unclear. Recent evidence suggests that the epithelial-derived cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) may be important initiators of innate type 2 immunity and amplifiers of adaptive type 2 inflammatory responses in many type 2 inflammatory diseases.¹ In the case of CRS, recent studies, including our own, found that TSLP is upregulated in NPs (Table S1).^{1,2} However, our initial studies suggested that IL-25 and IL-33 were not elevated in NP tissues compared to control sinus tissues.^{3,4} Although we concluded that TSLP may significantly contribute to type 2 inflammation in NPs, the role of IL-25 and IL-33 remains uncertain. In contrast to our study, other recent studies have shown that IL-25 is significantly elevated in NPs (Table S2). In the case of IL-33, published studies showed mixed results in NP tissue (Table S3). Therefore, the importance of IL-25 and IL-33 in CRSwNP is still largely unclear.

To revisit the validity of our earlier studies of IL-25 and IL-33, we collected control ethmoid sinus tissues and NPs from patients with aspirin-tolerant CRSwNP and aspirin-exacerbated respiratory disease (AERD, which has a triad of symptoms; CRSwNP, asthma and aspirin [or other NSAID] sensitivity) from a new cohort at Northwestern (Tables S4 and S5) and carefully determined the presence of IL-25 and IL-33 by 4 different methods: real-time RT-PCR, Luminex, Western blot, and immunohistochemistry. Detailed methods are given in the Data S1. First, we analyzed the expression of IL-25. To ensure the validity of our results, we purchased primer/probe sets for IL-25 from two different companies and performed real-time RT-PCR. We found that the levels of mRNA for IL-25 were near or below the detection limit of PCR and there was no significant difference between controls and NPs (Figure 1AB). As IL-25 is known to be expressed in the epithelial cells and the amount of epithelium might be much diluted in RNA from tissue biopsy

samples, we also performed real-time RT-PCR on epithelial scrapings. However, IL-25 mRNA was still almost undetectable and there was no significant difference between control sinus tissues and NPs (Figure S1). In contrast, we observed that total TSLP mRNA (both short and long variants) was significantly higher in NP tissue than in control tissue (Figure 1C), which confirmed our previous study.² We also found that mRNA for a long variant of TSLP which has cytokine activity was also significantly elevated in NPs (Figure 1D). The levels of TSLP mRNA were similar between NPs from CRSwNP and AERD (Figure 1CD). We next analyzed IL-25 protein in tissue homogenates by Luminex from 26 controls and 37 NPs and found that IL-25 was below the detection limit in all samples (not shown).

Although several groups recently published that IL-25 is highly upregulated in NP tissue, our data clearly showed that the level of IL-25 in our cohort at Chicago, Illinois, was very low and was not elevated in NPs (Figure 1). To identify potential reasons for this discrepancy, we searched for IL-25-related manuscripts. We found 11 manuscripts, of which 8 showed that IL-25 was elevated in NPs (Table S2). Interestingly, most of the positive results came from Asian countries including Korea, China, and Japan, except for one manuscript from Australia. In contrast, the three published negative results for IL-25 were studies from the United States, Australia, and Turkey. This suggests that the mechanisms of type 2 inflammation may be different between Asian and Western countries and that IL-25 may only be involved in Asian NPs. Future studies will be required to determine whether genetic and/or environmental factors elevate expression of IL-25 in NPs in Asian countries.

We next evaluated the presence of IL-33. IL-33 is a nuclear protein that is released from the nucleus as a cytokine during cell death and by other unknown mechanisms. Among the splice variants of IL-33 identified in airway epithelium by Gordon et al.,⁵ two variants, which had deletions of exons 3 and 4 (IL-33 Δ exon3,4) or IL-33 Δ exon3,4 with partial deletion of exon 5 (IL-33 Δ exon3,4s5), were able to move into the cytosol and be secreted as active cytokines without cell death. Interestingly, they also found that the expression of IL-33 Δ exon3,4 was strongly associated with type 2 airway inflammation, although full-length IL-33 was not.⁵ We therefore tested samples for the presence of mRNAs of full-length IL-33, IL-33 Δ exon3,4, and IL-33 Δ exon3,4s5 by RNase H-dependent quantitative RT-PCR using the published protocol.⁵ We found that mRNAs for full-length IL-33, IL-33 Δ exon3,4, and IL-33 Δ exon3,4s5 were present, but there were no differences between controls and NPs (Figure 2A-C). We next evaluated the presence of IL-33 protein in tissue extracts by Luminex and again found that IL-33 protein was present

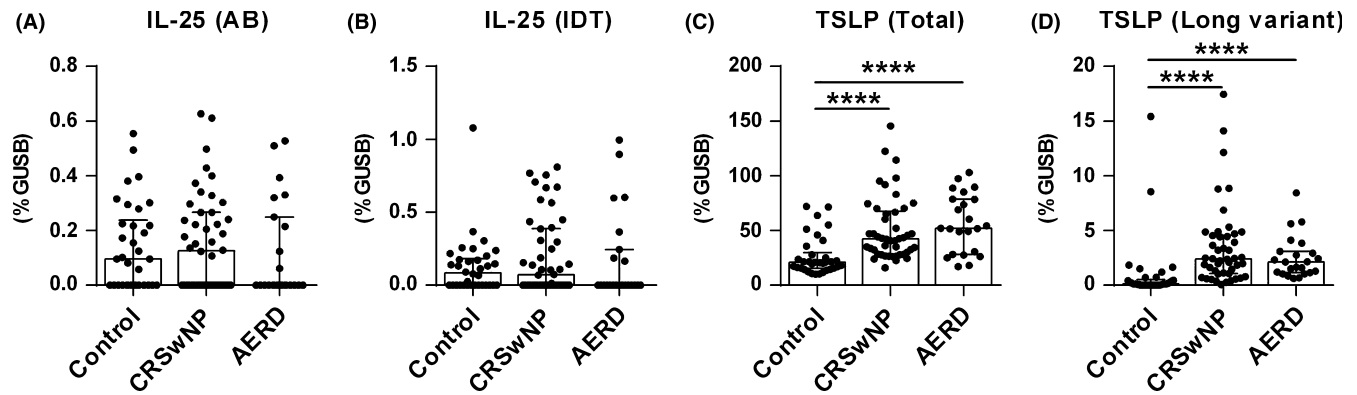


FIGURE 1 IL-25 was not elevated in nasal polyps (NPs). Total RNA was extracted from whole control ethmoid sinus tissue (Control; $n = 34$) and NP tissue from patients with chronic rhinosinusitis with nasal polyps (CRSwNP) ($n = 46$) and aspirin-exacerbated respiratory disease (AERD) ($n = 23$). We obtained primer/probe sets for IL-25 from two different companies, Applied Biosystems (AB) and Integrated DNA Technologies (IDT), and expression of mRNAs for IL-25 and thymic stromal lymphopoietin (TSLP) (total and a long variant) was analyzed by real-time RT-PCR (A-D). Gene expression was normalized to a housekeeping gene, β -glucuronidase (GUSB), and expression levels are shown as % expression of GUSB. Results are shown as median with interquartile range. **** $P < 0.0001$ by the 1-way ANOVA

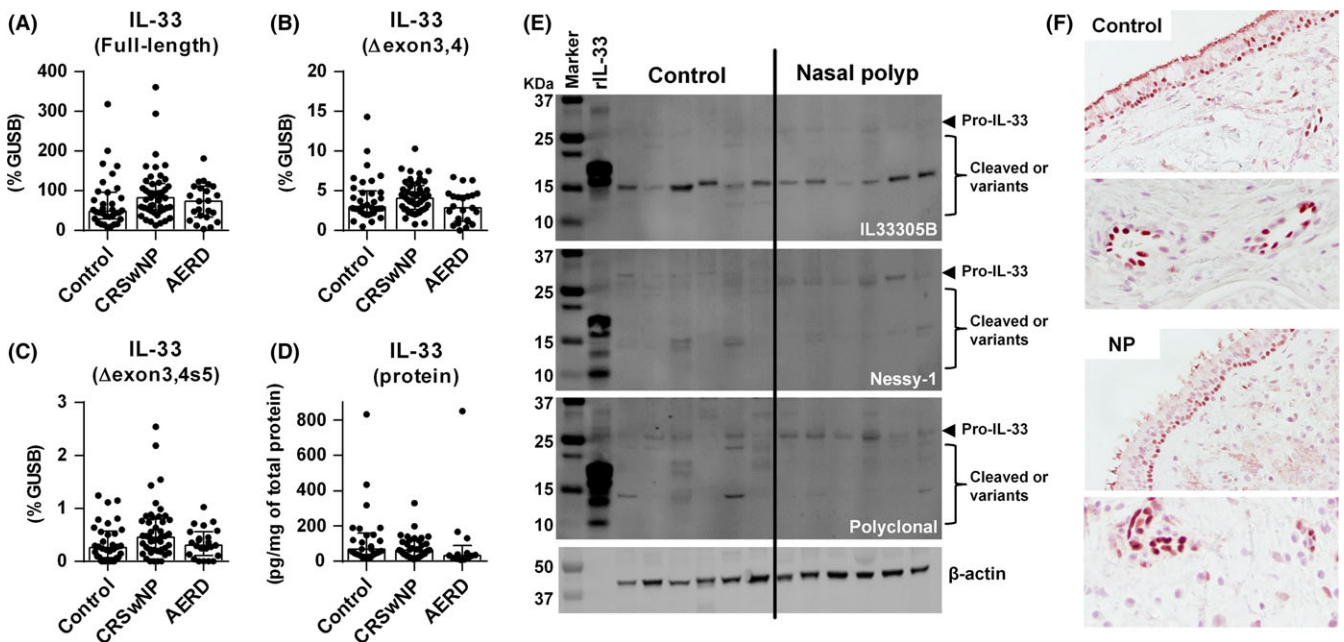


FIGURE 2 IL-33 was not elevated in nasal polyps (NPs). Expression of mRNAs for full-length IL-33 (A), IL-33 Δ exon3,4 (B), and IL-33 Δ exon3,4s5 (C) was determined by RNase H-dependent quantitative RT-PCR in control ethmoid sinus tissue (Control; $n = 34$) and NPs from CRSwNP ($n = 46$) and aspirin-exacerbated respiratory disease (AERD) ($n = 23$). IL-33 protein in tissue extracts was determined by Luminex (D, control ($n = 26$), chronic rhinosinusitis with nasal polyps (CRSwNP) NP [$n = 37$], and AERD NP [$n = 13$]) and Western blot (E). IL-33 protein concentration was normalized to the concentration of total protein (D). The results are representative of 2 separate experiments with separate donors (control [$n = 12$] and NP [$n = 11$]) (E). Representative immunostaining for IL-33 is shown in a control ethmoid tissue and an NP tissue (F). Magnification: $\times 400$ (upper) and $\times 1200$ (lower). Results are shown as median with interquartile range

but not elevated in NP tissues, even in NPs from patients with AERD (Figure 2D). It has been established that IL-33 is cleaved by proteases and cleaved IL-33 shows higher activity than pro-IL-33.⁶ However, it is not clear whether Luminex and commercial ELISA kits are able to detect both cleaved and splice variants of IL-33 proteins, and this may lead to inconsistent results. To determine the presence of pro (full-length), cleaved, and splice variants of IL-33 proteins in

tissue, we performed Western blots of IL-33 with three commonly used antibodies. However, we still could not detect major differences between control sinus tissues and NP tissues (Figure 2E). We also quantified the IL-33 bands by densitometry and found that there was no significant difference between controls ($n = 12$) and NPs ($n = 11$) (not shown). Finally, we evaluated the presence of IL-33 protein by immunohistochemistry and found that IL-33 was

localized mainly in the nuclei of basal epithelial cells and endothelial cells, but there was no significant difference between controls ($n = 10$) and NPs ($n = 10$) (Figure 2F and not shown).

Thus, in contrast to IL-25, we detected mRNA and protein for IL-33, but IL-33 was not elevated in NPs in our cohort (Figure 2).⁴ Although 19 manuscripts have evaluated the presence of IL-33 in NPs, these studies have reported mixed results. Eleven showed elevation of IL-33 in NPs, and 8 showed negative results (Table S3). Interestingly, unlike IL-25, the study locations did not clearly correlate with the findings (Table S3). In the case of the United States, Liu et al⁷ reported that IL-33 was elevated in NPs from patients with AERD compared to NPs from patients with aspirin-tolerant CRSwNP. However, we could not find a difference in IL-33 between AERD NP and non-AERD NP (Figure 2), which confirmed our previous finding.⁴ Although possible, we think that it is unlikely that the presence and degree of transcriptional regulators of IL-33 in NPs differ between Chicago, Illinois, and Boston, Massachusetts, leaving this disparity unexplained. Shaw et al⁸ found that IL-33 was not elevated in NP tissue but reported that epithelial cells from patients with CRSwNP expressed more IL-33 than cells from patients with nonpolypoid CRS after stimulation with *Aspergillus fumigatus*. Colonization by *Staphylococcus aureus* is known to occur in some cases of CRSwNP, and the presence of *Staphylococcus aureus* enterotoxin B-specific IgE is associated with severe type 2 inflammation in NPs.^{1,9} *Staphylococcus aureus*-derived products are known to induce IL-33 in mice.⁹ However, the presence of *Aspergillus fumigatus* and *Staphylococcus aureus* enterotoxin B-specific IgE in NPs in our cohort in Chicago was rare (not shown). These results suggest that induction of IL-33 in NPs might be strongly controlled by geographically variable environmental factors, including infectious conditions, and IL-33 might not be consistently elevated in NP around the world.

As several patients received corticosteroids before surgery which may affect the expression of IL-25 and IL-33 and may induce discrepancies in the study, we evaluated the effect of corticosteroid. However, we found no significant difference in the levels of IL-25 and IL-33 in NPs based on the status of steroid treatment (Figure S2 and not shown).

In conclusion, we report that IL-25 is almost absent, and IL-33 is present but not elevated in NP tissue in our US-based population. We have confirmed earlier reports by our group and others that TSLP is elevated in CRSwNP. We suggest that these findings primarily implicate TSLP as the major epithelial-derived cytokine that skews adaptive immune responses toward type 2 in CRSwNP. Although IL-25 and IL-33 have been considered to be therapeutic targets for treatment of CRSwNP, this strategy may not always fit in all international regions and may require careful investigation in geographic area before starting clinical trials.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Dietary assessment and the development of asthma in Korean adolescents and adults

To the Editor,

Changes in dietary habits, such as decreased intake of fresh fruits and vegetables and increased intake of processed foods, had been linked with the increasing prevalence of asthma in the past half-century.¹⁻³ However, dietary habits in different parts of the globe vary considerably according to cultural background, religious beliefs and socioeconomic background. For example, the traditional Korean diet is characterised by a relatively high consumption level of vegetables, moderate to high consumption of legumes and fish and low consumption of red meat.⁴ The dietary pattern may also differ among ages, particularly in a rapidly changing society such as Korea. Therefore, the role of a specific diet in the development of specific diseases can vary according to the dietary pattern of the population of interest.

To examine the relationship between diet and the development of asthma in different age groups in Korea, we used two nationwide survey data: Korea Youth Risk Behavior Web-based Survey 2012 (KYRBWS) and Korean National Health and Nutrition Survey 2011 (KNHANES). The development of asthma, described as recent asthma, was assessed through the response to the following question: "During the last 12 months, have you been diagnosed with asthma by a physician?" Consumption of specific foods was assessed using a qualitative food frequency questionnaire for the past 7 days. Subjects were categorised into high intake (defined as ≥ 5 per week) and low intake (< 5 per week) groups except for vegetable intake in adults. High intake of vegetables among adults was defined as twice per day or more, and low intake was defined as less than twice per day. Foods included in the analyses were vegetables (excluding kimchi, which is a traditional Korean pickled vegetable dish), fruits (excluding fruit juice), milk, fast food and instant noodles. Intake of processed meat was also included in the analysis of KNHANES. Univariate and multivariate logistic regression analyses were used to investigate the influence of each variable on the development of asthma. Covariates were age, sex, body mass index, exercise, region of residence, smoking status, income and education. Model 1 was adjusted for age and sex. Model 2 was adjusted for all covariates

that were significantly related to recent asthma in univariate analyses. All statistical tests were two-sided with a significance level of 0.05. The detailed methods and results can be found in the Appendix.

A total of 76 980 subjects representing 3 832 799 Korean middle and high school students were enrolled in KYRBWS 2012. The prevalence of recent asthma was 2.4%, and among the subjects with recent asthma, 5.8% and 3.7% consumed a large amount of fast food and instant noodle, respectively (Table S1). The consumption frequencies of these foods in subjects with recent asthma were significantly higher (odds ratio [OR] 2.62, 95% confidence interval [CI] 2.11-3.27 for fast foods; OR 1.63, 95% CI 1.34-1.98 for instant noodle) than those in subjects without recent asthma. After adjusting for covariates, high intake of fast food was consistently related to recent asthma in all regression models (OR 2.18, 95% CI 1.72-2.76 in model 1; OR 2.05, 95% CI 1.54-2.75 in model 2), whereas instant noodle intake was not associated with recent asthma. No associations were observed between the consumption of fruits, vegetables or milk and recent asthma, and no significant interactions were observed among the different food items (Figure 1A; Table 1).

There were 5811 subjects (2521 men and 3290 women) representing 33 629 698 Korean people aged 30 years and older in the KNHANES 2011. The prevalence of recent asthma was 0.5%. Among the subjects with recent asthma, 14.6% and 21.1% consumed a large amount of processed meat and instant noodle, respectively (Table S3). In univariate analyses, the amount of processed meat consumed was significantly higher in subjects who developed asthma recently than in those who did not (OR 21.45, 95% CI 2.54-180.8). After adjusting for covariates, processed meat intake was significantly associated with recent asthma in all regression models (OR 12.86, 95% CI 2.6-63.67 in model 1; OR 13.85, 95% CI 3.3-58.09 in model 2). In addition, instant noodle consumption was significantly related to recent asthma (OR 12.33, 95% CI 2.55-59.56) in univariate analyses and in all regression models (OR 11.74, 95% CI 3.81-36.22 in model 1; OR 9.49, 95% CI 3.32-27.13 in model 2). No associations





Supporting Information

Epithelial activators of type 2 inflammation; elevation of TSLP, but not IL-25 or IL-33, in chronic rhinosinusitis with nasal polyps in Chicago, Illinois

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Methods

Patients and tissue collection

Patients with CRSwNP were recruited from the Otolaryngology clinic and the Northwestern Sinus Center of Northwestern Medicine. All patients met the criteria for CRSwNP as defined by the European Position Paper on Rhinosinusitis and Nasal Polyps 2012.^{S1} Patients with an established immunodeficiency, pregnancy, coagulation disorder or diagnosis of Churg-Strauss syndrome or cystic fibrosis were excluded from the study. NP tissue was collected during endoscopic sinus surgery. Disease-free ethmoid sinus tissues from normal control patients without a history of CRS were obtained during procedures for conditions other than CRS (septoplasty for nasal obstruction, transnasal endoscopy skull base procedures, repairs of facial fractures, treatment of nasal disorders in obstructive sleep apnea, etc.). Several patients were taking a variety of medications, including corticosteroids. Asthma was diagnosed by documentation of diagnosis of asthma based on ICD9 or 10 and was confirmed in the medical records by treating allergist or pulmonologist. Atopy was identified by documentation of positive skin test in the medical record. All patients with AERD had physician-diagnosed asthma, CRSwNP, and documentation of at least 1 respiratory reaction to a non-steroidal anti-inflammatory drug (COX-1 inhibitor). Details of patients' characteristics are included in Table S4 and S5. All subjects signed informed consent and the study was approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine (IRB Project Number: STU00016917).

Real-time RT-PCR

A portion of nasal tissue for isolation of RNA was transferred in RNAlater (Ambion, Austin,

TX) and stored at -20°C. Total RNA from sinus tissue was extracted using QIAzol (Qiagen, Valencia, CA) and was cleaned and treated with DNase I using NucleoSpin RNA (Clontech Laboratories, Mountain View, CA) according to the manufacturer's instructions. The quality of total RNA from sinus tissue was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) using a RNA 6000 Nano LabChip (Agilent Technologies). RNA in which RIN was greater than 7.0 was used for cDNA synthesis. Single-strand cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and random primers. Real-time RT-PCR was performed with a TaqMan method using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) in 20 µl reactions (10 µl 2x TaqMan Fast Advanced Master Mix (Applied Biosystems), 1 µl 20x primer and probe mixture for target gene, 1 µl 20x primer and probe mixture for β -glucuronidase (GUSB) plus the cDNA equivalent of 10 ng of total RNA). We used primer and probe sets for IL-25 (Hs00224471_m1, Applied Biosystems), IL-25 (Hs.PT.56a.39225615, Integrated DNA Technologies, Coralville, IA), TSLP (total) (sense, 5'-CCCAGGCTATTCGGAAACTCA-3'; antisense, 5'-ACGCCACAATCCTTGTAATTGTG-3'; FAM/MGB probe, 5'-AAGGAAAGTCACAACCAATAA-3'), TSLP (a long variant) (Hs01572933_m1, Applied Biosystems) and GUSB (Human β -glucuronidase endogenous control, PN; 4326320E, Applied Biosystems) in this study. We also determined mRNAs for full-length IL-33, IL-33 Δ exon3,4 and IL-33 Δ exon3,4s5 by RNase H-Dependent quantitative RT-PCR using the published protocol by Gordon et al.^{S2} The mRNA expression levels were normalized to the expression of a housekeeping gene, GUSB. Expression of GUSB was not significantly different between control tissues and NPs (data not shown).

Tissue homogenates and protein assays

Freshly obtained tissue specimens were weighed, and 1 ml of PBS supplemented with 0.05 % Tween 20 (Sigma-Aldrich, St. Louis, MO) and 1% protease inhibitor cocktail (PIC, PN; P8340, Sigma-Aldrich) was added for every 100 mg of tissue. The tissue was then homogenized with a Bullet Blender Blue (Next Advance, Averill Park, NY) at setting 7 for 8 min at 4°C. After homogenization, the suspension was centrifuged at 4000 rpm for 20 min at 4°C and the supernatants were stored at -80°C. Before analysis, tissue homogenates were centrifuged at 16,000g for 15 min at 4°C and we used those supernatants for each assay.

The concentrations of IL-25 and IL-33 in cell free supernatants were measured using a MILLIPLEX MAP Human TH17 Magnetic Bead Panel from EMD Millipore (Billerica, MA). The minimal detection limits for IL-25 and IL-33 are 120 and 5 pg/ml, respectively. The concentration of cytokines in tissue homogenates was normalized to the concentration of total protein as detected by BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL).

Western blot analysis

Samples for Western blotting were dissolved in Bolt LDL sample buffer (Invitrogen). 15 µg of reduced tissue extracts was resolved on a Bolt 12% Bis-Tris Gel (Invitrogen) in a Bolt MES buffer system and then proteins were transferred onto a PVDF membrane (Bio-Rad, Hercules, CA). The membranes were blocked in blocking buffer for fluorescent western blotting (Rockland, Gilbertsville, PA) for more than 2 hours and subsequently incubated overnight at 4°C with 0.25 µg/ml mouse anti-human IL-33 mAb (clone IL33305B, AdipoGen, San Diego, CA), 1 µg/ml mouse anti-human IL-33 mAb (clone Nussy-1, Enzo Life Sciences, Farmingdale, NY), 0.5 µg/ml goat anti-human IL-33 polyclonal antibody (R&D systems, Minneapolis, MN) or 0.1 µg/ml mouse anti-beta actin mAb (clone C4, MP Biomedicals, Solon, OH). Following primary

antibody incubation, the membranes were washed repeatedly in 0.1% Tween 20/PBS and then labeled by 45 minutes incubation at room temperature with Alexa Fluor 680 conjugated goat anti-mouse IgG antibody (1:10,000, Invitrogen) or IRDye 680 conjugated donkey anti-goat IgG antibody (1:10,000, Rockland Immunochemicals). Protein was detected on an Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE).

Immunohistochemistry

Nasal tissue was dehydrated, infiltrated and embedded with paraffin, and tissue was sectioned at 3 μ m using a Leica RM2240 Cryostat. Sections were rehydrated and boiled in a citrate buffer (Dako, Carpinteria, CA) for 15 min to induce antigen retrieval. Tissue sections were then blocked for endogenous peroxidase activity with 3% H₂O₂/methanol. After washing, tissue sections were blocked for non-specific binding with 5% horse serum/0.3% Tween-20/PBS. Tissue sections were further blocked by an Avidin/Biotin Blocking Kit (Vector Laboratories, Burlingame, CA). The slides were then incubated with 2 μ g/ml mouse anti-human IL-33 mAb (clone Nussy-1, Enzo Life Sciences) in blocking buffer at 4°C overnight. After treatment with the primary antibody, tissue sections were incubated with biotinylated horse anti-mouse IgG for 1 hour and then ABC reagent (avidin-biotin-HRP complex; Vector Laboratories,) for 1 hour at room temperature. Sections were rinsed and incubated in ImmPACT NovaRED reagent (Invitrogen) for 80 seconds at room temperature. They were then rinsed in deionized H₂O, counterstained with hematoxylin, dehydrated, cleaned, mounted and coverslipped using Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI) in preparation for microscopic analysis.

Statistics

108 All data are reported as the median with interquartile range. Differences between groups
109 were analyzed using the 1-way ANOVA Kruskal-Wallis Dunn's multiple comparison test or the
110 Mann-Whitney U-test. A p value of less than 0.05 was considered significant.

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Table S1 Presence of TSLP in CRSwNP

Patient population	Elevation of TSLP in NP	Technique	Reference
China	Y	PCR, Western blot, ELISA	Liu et al. 2011 ^{S3}
China	Y (in ECRS)	PCR, IHC	Ouyang et al. 2013 ^{S4}
China	Y	PCR	Shi et al. 2014 ^{S5}
China	Y	PCR, IHC	Liao et al. 2015 ^{S6}
China	Y	PCR	Hong et al. 2018 ^{S7}
Japan	Y	PCR, IHC	Kimura et al. 2011 ^{S8}
Japan	Y	PCR, IHC	Kouzaki et al. 2016 ^{S9}
Netherlands	Y	PCR	Bal et al. 2016 ^{S10}
Italy	Y	PCR	Boita M et al. 2015 ^{S11}
Canada	Y	Bioassay	Allakhverdi et al. 2009 ^{S12}
USA	Y	PCR, Bioassay	Nagarkar et al. 2013 ^{S13}
USA	Y (in AERD)	Western blot	Buchheit et al. 2016 ^{S14}
China	N	Luminex	Chen et al. 2017 ^{S15}
Japan	N	RNA-seq	Kato et al. 2018 ^{S16}
Australia	N	PCR	Miljkovic et al. 2014 ^{S17}
Australia	N	PCR	Lam et al. 2015 ^{S18}

Y; yes, N; No, ECRS; eosinophilic CRS, IHC; immunohistochemistry, AERD; Aspirin-exacerbated respiratory disease.

117 **Table S2 Presence of IL-25 in CRSwNP**

118

Patient population	Elevation of IL-25 in NP	Technique	Reference
Korea	Y	ELISA, IHC, PCR	Shin et al. 2015 ^{S19}
Korea	Y	IHC, PCR	Xu et al. 2016 ^{S20}
China	Y	IHC , PCR	Liao et al. 2015 ^{S6}
China	Y	Luminex, PCR	Chen et al. 2017 ^{S15}
China	Y	Luminex, IHC, PCR	Hong et al. 2018 ^{S7}
Japan	Y	ELISA, IHC	Iinuma et al. 2015 ^{S21}
Japan	Y (only in ECRS)	ELISA, IHC, PCR	Kouzaki et al. 2016 ^{S9}
Australia	Y	IHC, PCR	Lam et al. 2015 ^{S18}
USA	N	ELISA	Peters et al. 2010 ^{S22}
Australia	N	PCR	Miljkovic et al. 2014 ^{S17}
Turkey	N	ELISA	Ozturan et al. 2017 ^{S23}

119 Y; yes, N; No, ECRS; eosinophilic CRS, IHC; immunohistochemistry.

120 **Table S3 Presence of IL-33 in CRSwNP**

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Patient population	Elevation of IL-33 in NP	Technique	Reference
China	Y (only epithelium)	IHC , PCR	Liao et al. 2015 ^{S6}
China	Y	IHC, PCR	Song et al. 2017 ^{S24}
China	Y	Luminex, IHC, PCR	Hong et al. 2018 ^{S7}
Japan	Y (in ECRS)	IHC	Endo et al. 2015 ^{S25}
Japan	Y (in CD45 ⁻ PECAM1 ⁺ cells)	PCR	Shinoda et al. 2016 ^{S26}
Japan	Y (only protein in ECRS)	ELISA, IHC, PCR	Kouzaki et al. 2016 ^{S9}
Japan	Y	RNA-seq	Kato et al. 2018 ^{S16}
Korea	Y	Luminex	Kim et al. 2017 ^{S27}
USA	Y (in AERD)	Western blot	Liu et al. 2015 ^{S28}
USA	Y	IHC	Paris et al. 2014 ^{S29}
Australia	Y	IHC, PCR	Lam et al. 2015 ^{S18}
China	N	Luminex, PCR	Chen et al. 2017 ^{S15}
Japan	N	ELISA, PCR	Baba et al. 2014 ^{S30}
Korea	N	IHC, PCR	Xu et al. 2016 ^{S20}
USA	N	PCR	Shaw et al. 2013 ^{S31}
USA	N	Luminex	Stevens et al. 2015 ^{S32}

Australia	N	PCR	Miljkovic et al. 2014 ^{S17}
Turkey	N	ELISA	Ozturan et al. 2017 ^{S23}
Netherlands	N	PCR	Bal et al. 2006 ^{S10}

122 Y; yes, N; No, ECRS; eosinophilic CRS, IHC; immunohistochemistry, AERD; Aspirin-
 123 exacerbated respiratory disease.

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Table S4 Subject characteristics in RNA study

	Control (n=34) n (%)	CRSwNP (n=46) n (%)	AERD (n=23) n (%)
Female	20 (59)	14 (30) †	9 (39)
Atopy	5 (15)	33 (72) †	17 (74) †
Asthma	1 (3)	24 (52) †	23 (100) †‡
Aspirin sensitivity	0 (0)	0 (0)	23 (100) †‡
Nasal steroid	2 (6)	13 (25) †	9 (39) †
Inhaled steroid	0 (0)	12 (26) †	5 (22) †
Oral steroid	0 (0)	11 (24) †	11 (48) †
Smoking status (current smoker)	6 (18)	3 (7)	1 (4)
Age (y), median (range)	58* (25-73)#	44 (23-76) †	44 (24-66) †

*median, #(range), †p < 0.05, vs Control, ‡p < 0.05, vs CRSwNP. The p value was determined by Fisher's exact test.

Table S5 Subject characteristics in protein study

	Control (n=26) n (%)	CRSwNP (n=37) n (%)	AERD (n=13) n (%)
Female	15 (58)	10 (27) †	4 (31)
Atopy	3 (12)	25 (68) †	10 (77) †
Asthma	0 (0)	15 (41) †	13 (100) †‡
Aspirin sensitivity	0 (0)	0 (0)	13 (100) †‡
Nasal steroid	3 (12)	12 (32)	4 (31)
Inhaled steroid	0 (0)	8 (22) †	5 (39) †
Oral steroid	0 (0)	12 (32) †	7 (54) †
Smoking status (current smoker)	3 (12)	2 (5)	1 (8)
Age (y), median (range)	58* (22-70)#	52 (25-70)	51 (28-80)

*median, #(range) ,†p < 0.05, vs Control, ‡p < 0.05, vs CRSwNP. The p value was determined by Fisher's exact test.

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Supplemental figure legend**Figure S1. Expression of IL-25 in epithelial scraping cells.**

Total RNA was extracted from epithelial scraping cells taken from uncinate process tissue of control subjects (n = 13) and NP tissues from patients with CRSwNP (n = 24). Expression of mRNAs for IL-25 was analyzed using real-time RT-PCR. Gene expression was normalized to a housekeeping gene, β -glucuronidase (GUSB), and expression levels were shown as % expression of GUSB. Results are shown as the median with interquartile range. Differences between groups were analyzed using the Mann-Whitney U-test and the p value was over 0.05.

Figure S2. Steroid treatment did not affect levels of IL-25 and IL-33 in NPs.

Total RNA was extracted from whole NP tissue from patients with CRSwNP and AERD, and expression of mRNAs for IL-25 and IL-33 was analyzed using real-time RT-PCR. We then compared levels of IL-25 and IL-33 in steroid untreated patients (n = 27) with steroid treated patients (**A**, n = 42), oral steroid treated patients (**B**, n = 22) or nasal steroid treated patients (**C**, n = 21). Differences between groups were analyzed using the Mann-Whitney U-test and p values were over 0.05 for all comparisons.