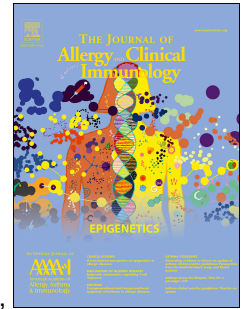


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TNF induces the production of type 2 cytokines in human group 2 innate lymphoid cells

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24 Phone: 312-503-0086, FAX: 312-503-0078, E-Mail: a-kato@northwestern.edu

25 **Capsule summary:**

26 TNF receptor II is expressed on ILC2s and TNF is able to induce production of type 2 cytokines
27 in human ILC2s. TNF may play a role in causing or amplifying type 2 immunity contributing to
28 health and disease.

29

30 **Key words**

31 ILC2; TNF; TNFR2; TSLP; Type 2 cytokines.

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To the Editor:

It is well recognized that group 2 innate lymphoid cells (ILC2s) play a key role in both innate immunity to protect from parasitic pathogens and pathological processes for many type 2 inflammatory diseases including asthma and chronic rhinosinusitis with nasal polyps (CRSwNP).^{1, 2} ILC2-mediated inflammation is mainly triggered via the production of type 2 cytokines including IL-5 and IL-13. Although the epithelial derived cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) are well appreciated to contribute to ILC2-mediated production of type 2 cytokines, recent studies found that members of the TNF superfamily (TNFSF), including TNFSF11 (receptor activator of NF- κ B ligand [RANK-L]), TNFSF15 (TNF-like cytokine 1A [TL1A]) and TNFSF18 (glucocorticoid-induced TNFR-related protein ligand [GITR-L]) are also able to induce IL-5 and IL-13 in ILC2s by activation of their respective receptors: TNF receptor superfamily 11A (TNFRSF11A [RANK]), TNFRSF25 (death receptor 3 [DR3]) and TNFRSF18 (GITR).¹⁻⁵ Nonetheless, whether TNF itself (also known as TNF α) is able to activate ILC2s and whether TNF receptors, TNFRSF1A (TNF receptor I [TNFRI]) and TNFRSF1B (TNFRII), are expressed on ILC2s has not been investigated. Many TNFRSFs, including RANK, GITR and DR3, share the NF- κ B signaling pathway via the activation of TNF receptor associated factor family members, and TNFRII has similar signaling pathways to them.² The aim of this study was to examine whether TNFRII is expressed on ILC2s and whether TNF induces production of type 2 cytokines in ILC2s.

We first measured mRNA for TNFRII in human ILC2s. We sorted human ILC2s from peripheral blood from healthy subjects and from nasal polyps (NPs - a type 2 inflammatory manifestation) collected from CRSwNP patients during sinus surgery. We also used human peripheral blood monocytes and the Jurkat cell line as positive and negative controls of TNFRII,

respectively. Real-time RT-qPCR was performed using a TaqMan method and detailed methods are given in this article's Online Repository. We found that TNFR2 mRNA was similarly expressed on both blood and NP ILC2s at about 14% of the level on monocytes and was significantly higher than the Jurkat cell line (Fig 1A). We then determined cell surface expression of TNFR2 on ILC2s by flow cytometry. Gating strategies identifying human ILC2s are shown in Supplemental Figure E1. We found that TNFR2 was expressed on ILC2s from both blood and NPs and levels were significantly higher than Jurkat cells (Fig 1B and C).

We next cultured human ILC2s sorted from blood and NP tissue in RPMI-1640 medium supplemented with 25 IU/mL IL-2 and 10% FBS and investigated the effect of TNF. We found that TNF time- and dose-dependently induced production of IL-5 and IL-13, though IL-5 protein was only detected at 96 hour after stimulation in human blood ILC2s (Fig 2A and E2A). We previously found that ILC2s are activated in NP tissue *in vivo* and therefore ILC2s sorted from NP tissue release IL-5 and IL-13 without additional stimulation.⁶ We found that TNF further enhanced this spontaneous production of IL-5 and IL-13 in NP ILC2s (Fig 2B). Since TSLP acts in synergy with common ILC2 activators including IL-33, we examined whether TSLP synergistically acts with TNF on blood ILC2s. We found that TSLP significantly and synergistically enhanced TNF-mediated production of type 2 cytokines in blood ILC2s (Fig 2C and E2). To further examine the role of NF- κ B in the TNF-mediated production of type 2 cytokines, we stimulated blood ILC2s with TNF and TSLP, and examined the effect of glucocorticoid (dexamethasone, an inhibitor of the NF- κ B pathway) and IMD-0354 (an inhibitor of I κ B kinase- β that blocks NF- κ B nuclear translocation) on IL-5 and IL-13 production. We found that dexamethasone and 10 μ M IMD-0354, but not dimethyl sulfoxide (DMSO, vehicle control) completely suppressed TNF-mediated production of IL-5 and IL-13 and partially

induced cell death in blood ILC2s (Fig 2D and E3). We also found that 1 μ M IMD-0354 partially suppressed cytokine production without enhancement of cell death in ILC2s (Fig E3B and not shown). These results suggest that NF- κ B is one of the key transcription factors controlling TNF-mediated induction of type 2 cytokines in human ILC2s. We further found that a STAT6 inhibitor significantly suppressed TNF-mediated production of IL-5 and IL-13 (Fig E4). Since TNF does not directly activate STAT6, the autocrine/paracrine loop of IL-13 may influence TNF-mediated production of type 2 cytokines in ILC2s.

While TNF induced production of both IL-5 and IL-13 in human blood ILC2s, it induced 15-fold greater quantities of IL-13 than IL-5 (Fig 2C). In contrast, IL-33 induced IL-5 and IL-13 equally in ILC2s (Fig 2E). We also found that the combination of TNF and IL-33 showed additive and/or synergistic effects on the production of type 2 cytokines in human blood ILC2s (Fig 2E). These results suggest that there are different signaling pathways mediating TNF- and IL-33-induced activation of ILC2s. To test for differences between TNF- and IL-33-mediated signaling, we investigated the effects of known inhibitors for IL-33-mediated ILC2 activation (Fig E5)² and found that dexamethasone, TGF- β , IFN- β , IFN- γ and IL-27 but not IL-10 significantly suppressed TNF plus TSLP-mediated production of IL-13 in blood ILC2s (Fig 2D and 2F). These results suggest that signaling pathways that can be suppressed by IL-10 (potentially via STAT3) are present in IL-33-mediated but not in TNF-mediated pathways and that this IL-33-specific pathway may be key to signaling strong induction of IL-5 in ILC2s. Future studies are required to characterize TNF and IL-33 specific signaling pathways in ILC2s.

During helminth infection, epithelial cells release IL-25 and IL-33 and then induce ILC2-mediated production of IL-13 to clear invading worms.¹ Helminth infection also induces production of pro-inflammatory cytokines, including TNF, in immune cells such as macrophages

and structural cells such as epithelial cells, via innate pattern recognition receptors such as toll-like receptors.⁷ These findings suggest that TNF, together with other innate cytokines including IL-33, may play a role in the protection from helminth infection by the activation of ILC2s. In the case of human diseases, TNF is known to be elevated in type 2 inflammatory diseases including asthma, however, treatment with TNF blockers did not show clear efficacy in asthmatics.⁸ Asthma is a heterogeneous disease that is characterized by numerous inflammatory endotypes (e.g. eosinophilic or neutrophilic forms).⁹ It is possible that distinct ILC2 activators drive disease in patients with eosinophilic asthma, and further clinical study using endotype analysis based on type 2 markers and ILC2 activators will be required to examine the role of the TNF-ILC2 cascade in asthma. In addition, elevation of TSLP in type 2 inflammatory diseases is expected to produce a synergistic response via TNF and IL-1 family molecules, including TNF and IL-33. Inhibition of TSLP may have greater benefit in type 2 inflammatory diseases than targeting individual ILC2 activators including TNF and IL-33. In conclusion, TNF is able to induce and greatly enhance production of type 2 cytokines in ILC2s and it may play a role in causing or amplifying type 2 immunity contributing to health and disease.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest as to the interpretation and presentation of this manuscript.

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Figure Legends**Figure 1. TNFRII is expressed on human ILC2s.**

Total RNA was extracted from blood monocytes (n=4), blood ILC2s (n=5), NP ILC2s (n=5) and Jurkat cells (n=4). Expression of TNFRII mRNA was analyzed by real-time RT-qPCR using a TaqMan RNA-to-CT 1-Step Kit (A). Gene expression was normalized to a housekeeping gene, β -actin, and expression levels were shown as Δ Ct. Representative histograms of flow cytometric plots for cell surface expression of TNFRII on ILC2s are shown. Levels of cell surface expression of TNFRII on ILC2s from blood from healthy subjects (n=8) and NPs (n=6), Jurkat cells (n=5) and blood monocytes (n=9) are shown by geometric mean fluorescence intensity (gMFI) (B). Comparisons of gMFI ratio of TNFRII to isotype IgG2a are shown in C. Results are shown as mean with SEM. * $p < 0.05$ and ** $p < 0.01$ by the Mann-Whitney test (A and C) and the Ratio paired t test (B).

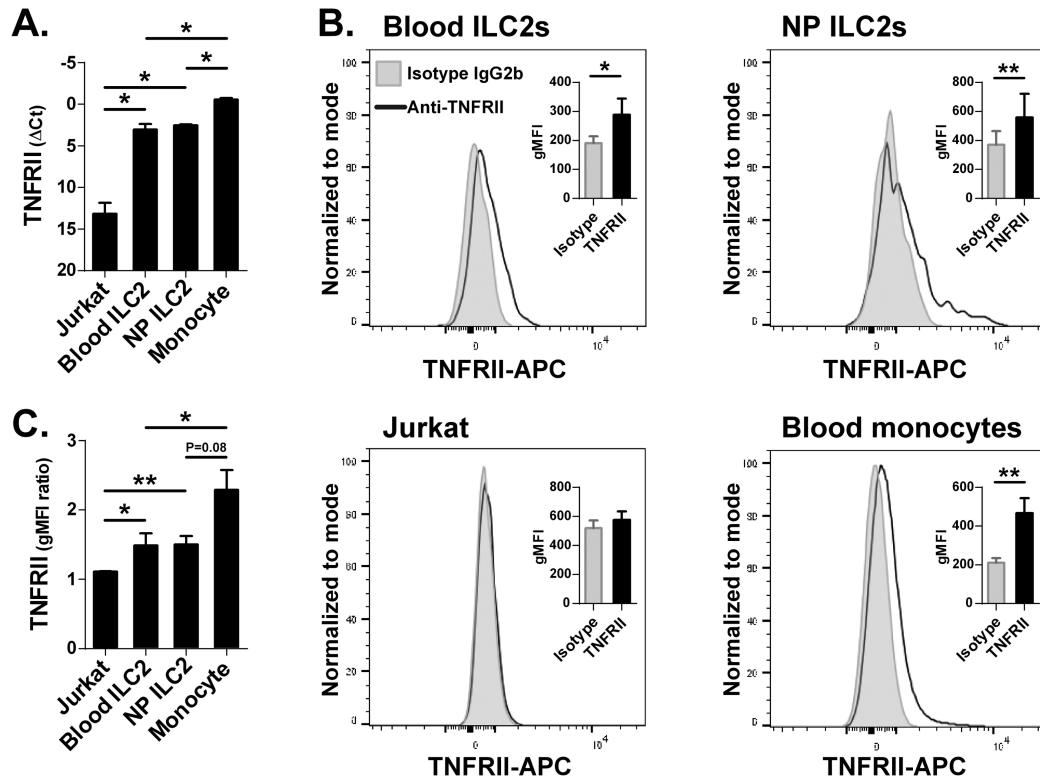
Figure 2. TNF induces production of IL-5 and IL-13 in human ILC2s

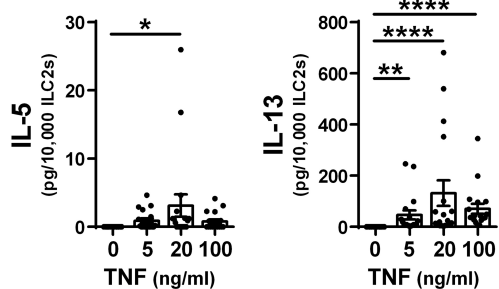
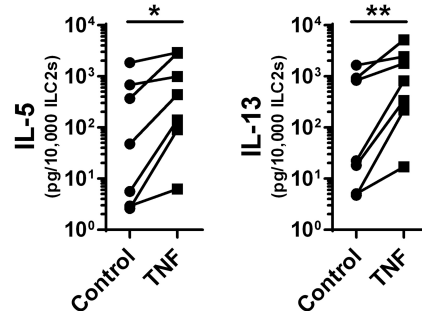
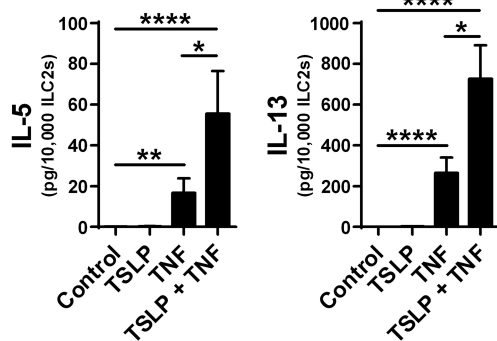
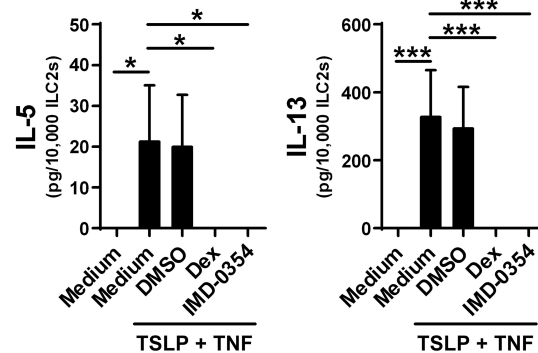
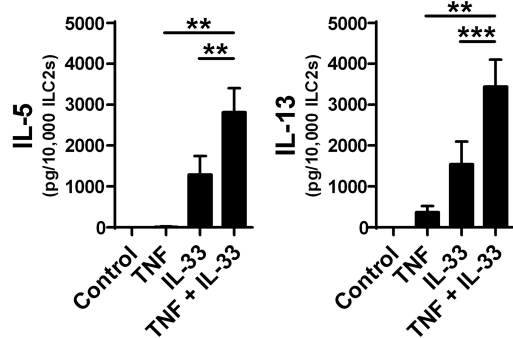
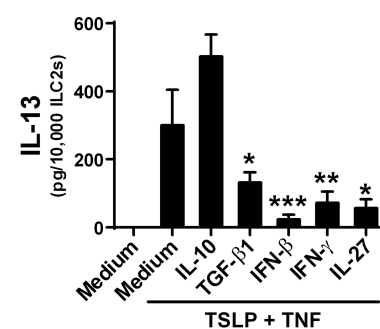
Sorted ILC2s from blood of healthy subjects (A, C-F) and NPs (B) were suspended in RPMI-1640 medium supplemented with 25 IU/ml IL-2, 10% FBS, 100 U/ml penicillin, and 100 μ g/ml streptomycin and were stimulated with 5-100 ng/ml TNF (A. n=9) or 20 ng/ml TNF (B. n=7) for 4 days. Blood ILC2s were stimulated with 20 ng/ml TNF, 10 ng/ml TSLP, 10 ng/ml IL-33 or their combination for 4 days (C. n=14, E. n=5). Blood ILC2s were cultured with 20 ng/ml TNF and 10 ng/ml TSLP in the presence or absence of 0.01% DMSO (vehicle control), 100 nM dexamethasone (Dex), 10 μ M IMD-0354 (an inhibitor of IKK β that blocks NF- κ B nuclear translocation), 10 ng/ml IL-10, 20 ng/ml TGF- β 1, 1000 IU/ml IFN- β , 10 ng/ml IFN- γ or 50

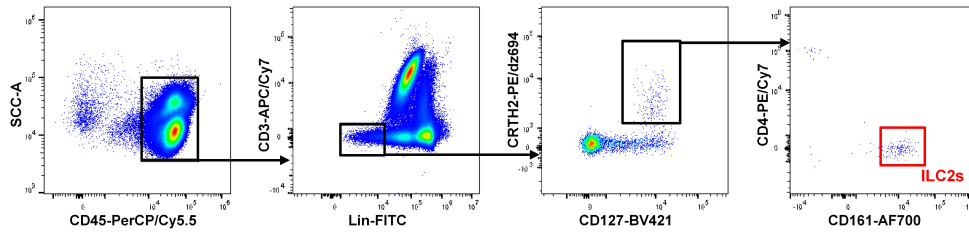
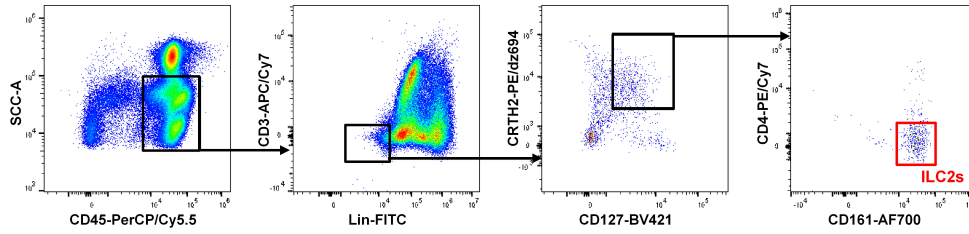
211 ng/ml IL-27 for 4 days (**D**, n=6, **F**, n=8). Each experiment was performed in duplicate with
212 independent donors. The concentrations of IL-5 and IL-13 were measured by Luminex assay.
213 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ were calculated by the Ratio paired t test (**B**)
214 and the one-way ANOVA Friedman test (**A**, **C-F**).

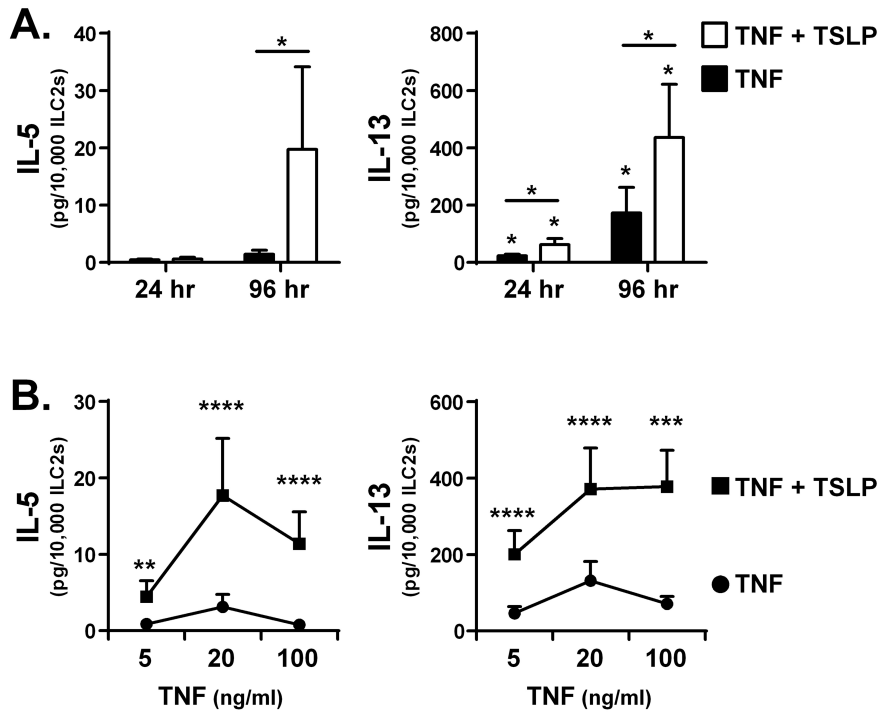
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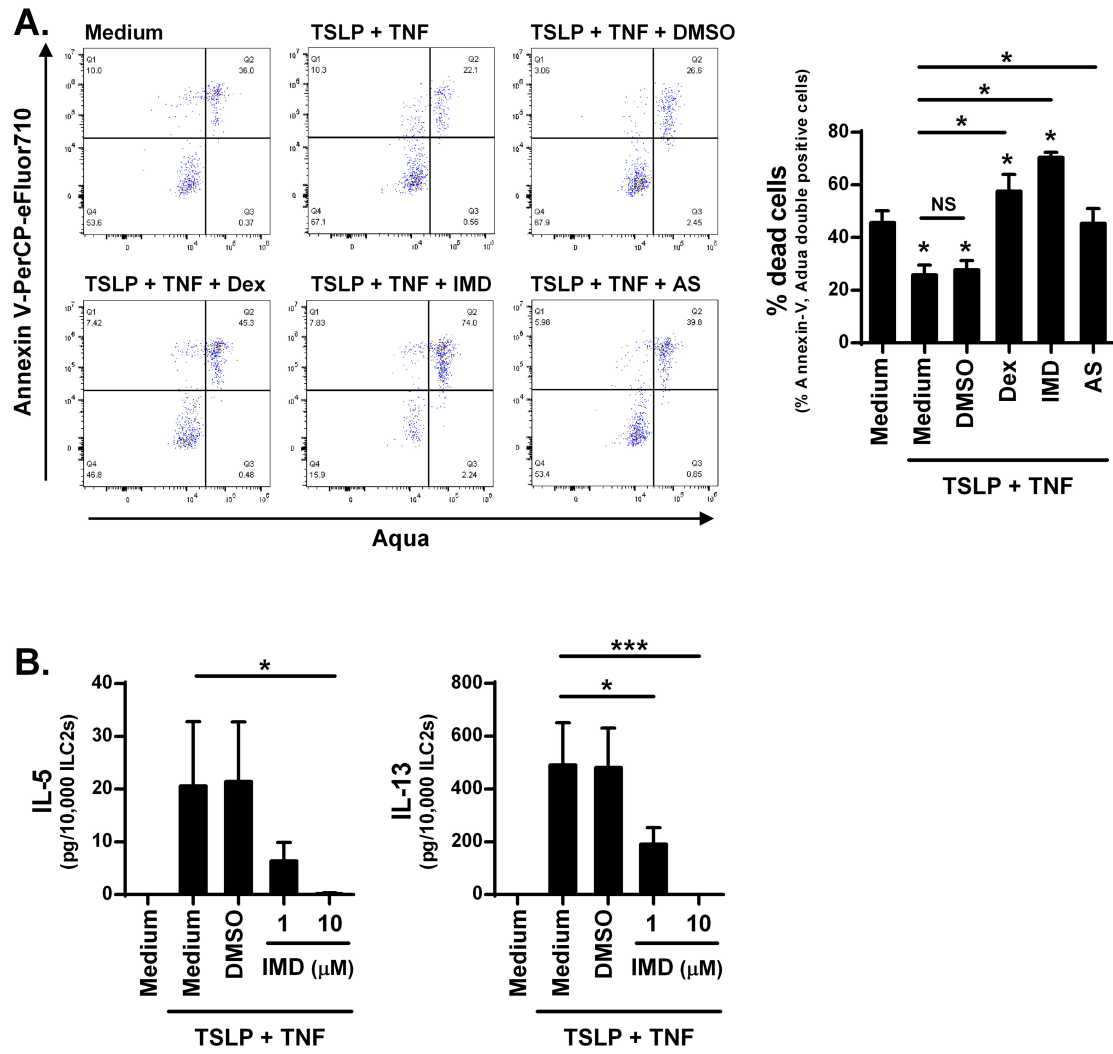
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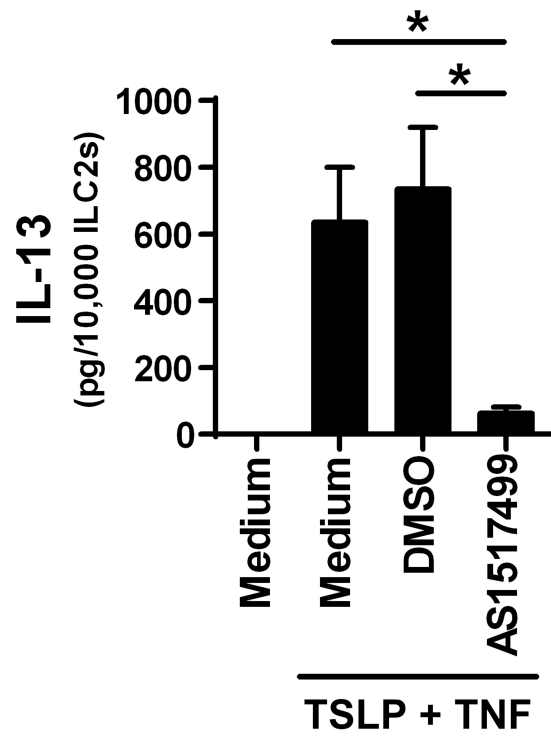


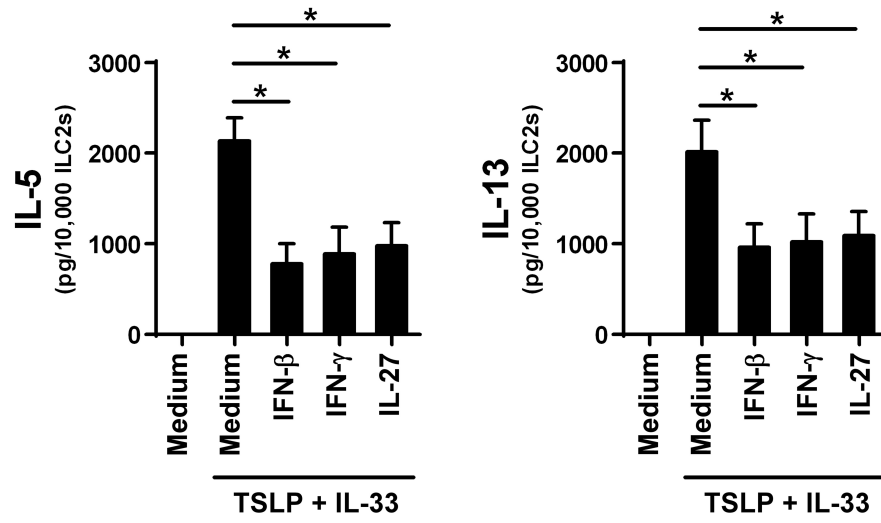
A. Blood ILC2s**B. NP ILC2s****C. Blood ILC2s****D. Blood ILC2s****E. Blood ILC2s****F. Blood ILC2s**

A. Blood ILC2s**B. NP ILC2s**









Online Supplement

TNF induces the production of type 2 cytokines in human group 2 innate lymphoid cells

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METHODS

Patients and tissue collection

All patients with chronic rhinosinusitis with nasal polyps (CRSwNP) were recruited from the Otolaryngology clinic and the Northwestern Sinus Center of Northwestern Medicine. All CRS patients met the criteria for CRSwNP as defined by the International Consensus Statement on Allergy and Rhinology: Rhinosinusitis.^{E1} Patients with an established immunodeficiency, pregnancy, coagulation disorder or diagnosis of eosinophilic granulomatosis with polyangiitis (eGPA or Churg-Strauss syndrome) or cystic fibrosis were excluded from the study. All patients signed informed consent and the study was approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine (IRB Project Number: STU00080917). Human peripheral blood leuko paks (STEMCELL Technologies, Vancouver, British Columbia, Canada) were obtained from healthy subjects for isolation of blood ILC2s and monocytes.

Cell isolation and cell sorting

Human blood leukocytes were isolated from a human peripheral blood leuko pak (STEMCELL Technologies) by centrifugation at 500 g for 10 minutes with an equal volume of Dulbecco's phosphate buffered saline (dPBS) with 2% fetal bovine serum (FBS) at room temperature. After aspiration of the supernatant, red blood cells were lysed by ammonium chloride solution (STEMCELL Technologies) and supernatant was removed after centrifugation at 300 g for 5 minutes. To remove platelets, cells were resuspended in dPBS + 2% FBS and centrifuged at 200 g for 10 minutes at room temperature.

Blood ILC2s were enriched from human blood leukocytes by EasySep™ Human ILC2 Enrichment Cocktail kit (STEMCELL Technologies) according to the manufacturer's instructions. After enrichment, cells were blocked by Fc Block reagent (Miltenyi Biotec, Auburn, CA) and then incubated with FITC anti-Lineage Cocktail (CD3, CD14, CD16, CD19, CD20, CD56), FITC anti-FcεRIα, FITC anti-CD11c, FITC anti-CD303, BUV395 anti-CD4, PE anti-CD5, Brilliant Violet 421 anti-CD45, Alexa Fluor 647 anti-CRTH2, PE/Cy7 anti-CD127 and APC/Cy7 anti-CD161. All antibodies were purchased from Biolegend (San Diego, CA), BD Biosciences (San Jose, CA) or R&D systems (Minneapolis, MN) and are listed in Table E1. We then sorted blood ILC2s as CD45⁺, Lin (CD3, CD11c, CD14, CD16, CD19, CD20, CD56, CD303, FcεRIα)⁻, CD4⁻, CD5⁻, CD127⁺, CRTH2⁺, CD161⁺ cells with a BD FACSARIA SORP cell sorter (BD Biosciences, San Jose, CA) at the Robert H Lurie Comprehensive Cancer Center at Northwestern University. The purity of blood ILC2s was greater than 98% (not shown). We also isolated monocytes from human blood leukocytes by positive selection using human CD14 MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions. The purity of blood monocytes was between 90-96% (not shown). The Jurkat cell line (Clone E6-1) was obtained from ATCC (Manassas, VA).

Cells were isolated from NP tissues using a previously described method.^{E2, 3} Briefly, tissue samples were weighed, washed, fragmented and then incubated with media containing 30 µg/ml DNase I and 1 mg/ml type I collagenase at 4°C overnight. Following this, tissues were minced using a gentleMACS dissociator (Miltenyi Biotec,) and the cells were filtered through 70 µm nylon mesh (BD Biosciences). Cells were then treated with red blood cell lysis solution (Miltenyi Biotec) and washed with dPBS before counting and staining for flow cytometric analysis or cell sorting.

We sorted NP ILC2s as previously described.^{E4} Briefly, after the isolation of cells from whole NP tissue, cells were first treated with Aqua dead cell staining reagent (Invitrogen, Carlsbad, CA) at room temperature in the dark. After washing, cells were blocked by Fc Block reagent and then incubated with FITC anti-human Lineage Cocktail, FITC anti-CD11c, FITC anti-CD303, PE/Cy7 anti-FcεRIa, BV395 anti-CD4, PE/Dazzle 594 anti-CD5, Alexa Fluor 700 anti-CD45, Alexa Fluor 647 anti-CRTH2, BV421 anti-CD127 and APC/Cy7 anti-CD161, PE anti-CD117 at 4°C in the dark. We sorted NP ILC2s (Aqua-, CD45+, Lin (CD3, CD11c, CD14, CD16, CD19, CD20, CD56, CD303)-, CD4-, CD5-, FcεRIa-, CD127+, CRTH2+ and CD161+ cells) with a BD FACS Aria SORP cell sorter. The purity of blood and NP ILC2s was greater than 98% (not shown).

Flow cytometric analysis

Cells were first treated with Aqua LIVE/DEAD fixable dead cell staining reagent as a live/dead discriminator. Cells were then incubated with an Fc Block reagent for 10 minutes at 4°C in the dark. Cells were stained with FITC anti-human Lineage Cocktail, FITC anti-CD11c, FITC anti-CD303, FITC anti-FcεRIa, APC/Cy7 anti-CD3, PE/Cy7 anti-CD4, PerCP/Cy5.5 anti-CD45, PE/Dazzle 594 anti-CRTH2, BV421 anti-CD127, Alexa Fluor 700 anti-CD161 and APC anti-TNFR II or isotype mouse IgG2a control for 30 minutes at 4°C in the dark and washed with MACS buffer (Miltenyi Biotech). After washing, cells were fixed with a BD Cytofix/Cytoperm Kit (BD Biosciences), resuspended in MACS buffer and stored at 4°C in the dark before analysis on a CytoFLEX flow cytometer (Beckman Coulter, Indianapolis, IN). All analysis was performed with FlowJo software, version 10.4 (TreeStar, Ashland, OR), and the experimental method was established and verified with the proper single-stained control beads (BD

Biosciences and eBiosciences) and fluorescence minus one (FMO) negative controls (data not shown).

For apoptosis study, cultured blood ILC2s were treated with Aqua LIVE/DEAD fixable dead cell staining reagent for 15 minutes at room temperature in the dark. Cells were resuspended in Annexin V binding buffer (eBioscience, Carlsbad, CA) and were stained with PerCP-eFluor 710 Annexin V (eBioscience) and Alexa Fluor 700 anti-CD45 (BioLegend) for 15 minutes at room temperature in the dark. After washing, cells were suspended in Annexin V binding buffer and were analyzed by a CytoFLEX flow cytometer within 3 hours of staining.

Quantitative polymerase chain reaction (qPCR)

Total RNAs were isolated from Jurkat cells, blood monocytes, blood ILC2s and NP ILC2s using PicoPure™ RNA Isolation Kit (Arcturus, Mountain View, CA) according to the manufacturer's instructions. The quality and concentration of total RNA was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) using a RNA 6000 Pico LabChip (Agilent Technologies). Real-time RT-qPCR was performed with a TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems, Foster City, CA) using a StepOnePlus Real-Time PCR System (Applied Biosystems) in 20 µl reactions (10 µl 2x TaqMan RT-PCR Mix, 0.5 µl 40x TaqMan RT Enzyme Mix, 1 µl 20x primer and probe mixture for TNFR2 (*TNFRSF1B*, Hs.PT.58.40638488, Integrated DNA Technologies, IA), 1 µl 20x primer and probe mixture for β -actin (*ACTB*, Forward primer: CTGGCCGGGACCTGACT, Reverse primer: GCAGCCGTGGCCATCTC, VIC-MGB TaqMan probe: CACCACCACGGCCGA) plus 2 ng of total RNA). The mRNA expression levels were normalized to a housekeeping gene, *ACTB*.

Cell culture and measurement of protein

Sorted blood and NP ILC2s (10,000 cells/ml) were suspended in RPMI-1640 medium supplemented with 25 IU/ml IL-2 (Prometheus, San Diego, CA), 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were stimulated with 5-100 ng/ml TNF (R&D systems), 10 ng/ml TSLP (R&D systems) and 10 ng/ml IL-33 (BioLegend) in the presence or absence of 0.001% DMSO (Sigma-Aldrich, St. Louis, MO), 100 nM dexamethasone (Sigma-Aldrich), 1-10 µM IMD-0354 (Selleckchem, Houston, TX), 100 nM AS1517499 (Selleckchem), 10 ng/ml IL-10 (R&D systems), 20 ng/ml TGF-β1 (R&D systems), 1000 U/ml IFN-β1a (R&D systems), 10 ng/ml IFN-γ (R&D systems) or 50 ng/ml IL-27 (R&D systems) for 4 days. Each experiment was performed in duplicate with independent donors.

The concentration of IL-5 and IL-13 in cell-free supernatants was measured by Luminex multiplex assay (MILLIPLEX MAP Human Cytokine/Chemokine Panel from EMD Millipore, Burlington, MA). The minimal detection limits for IL-5 and IL-13 are 3.2 pg/ml.

Statistics

All data were reported as the mean ± SEM. Normality tests were performed with the Shapiro-Wilk normality test (2 groups) and the Brown-Forsythe test (multiple groups). Comparisons of 2 groups were performed using the Ratio paired t test or the Wilcoxon matched-pairs signed rank test. Multiple groups were compared using the one-way ANOVA Friedman test followed by a Dunn's post-test. A p value of less than 0.05 was considered significant.

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Supplemental Table E1. Antibodies in flow cytometry

Antibody specificity	Format	Clone	Final concentration	Vendor
CD3	APC/Cy7	HIT3a	1.25 µg/ml	Biolegend
CD4	PE/Cy7	RPA-T4	2.5 µg/ml	Biolegend
CD4	BUV395	RPA-T4	1:20	BD Biosciences
CD5	PE	UCHT2	1 µg/ml	Biolegend
CD5	PE/Dazzle 594	UCHT2	1.25 µg/ml	Biolegend
CD11c	FITC	Bu15	4 µg/ml	Biolegend
CD45	Alexa Fluor 700	HI30	5 µg/ml	Biolegend
CD45	PerCP/Cy5.5	HI30	1.25 µg/ml	Biolegend
CD45	BV421	HI30	0.625 µg/ml	Biolegend
CD117	PE	104D2	5 µg/ml	Biolegend
CD127	BV421	A019D5	1-2.5 µg/ml	Biolegend
CD127	PE/Cy7	A019D5	2 µg/ml	Biolegend
CD161	APC/Cy7	HP-3G10	5 µg/ml	Biolegend
CD161	Alexa Fluor 700	HP-3G10	5 µg/ml	Biolegend
CD294 (CRTH2)	PE/Dazzle 594	BM16	7.5 µg/ml	Biolegend
CD294 (CRTH2)	Alexa Fluor 647	BM16	5 µg/ml	Biolegend
CD303	FITC	201A	4 µg/ml	Biolegend
Lineage Cocktail (CD3/CD14/CD16/C D19/CD20/CD56)	FITC	UCHT1, HCD14, 3G8, HIB19, 2H7, HCD56	1:20	Biolegend
FcεRIa	FITC	AER-37 (CRA1)	2 µg/ml	Biolegend

FcεRIa	PE/Cy7	AER-37 (CRA1)	1 µg/ml	Biolegend
TNFRII (CD120b)	APC	22235	4 µg/ml	R&D systems
Mouse IgG2a	APC	20102	4 µg/ml	R&D systems

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Figure Legends**Supplemental Figure E1. Gating strategy to identify human ILC2s.**

Representative flow cytometric plots for ILC2s in human peripheral blood mononuclear cells (**A**) and NP tissue (**B**). We gated on single (not shown), live (not shown) CD45-positive cells and excluded granulocytes (side scatter high). We then assessed ILC2s in the lineage-negative population (CD3, CD4, CD11c, CD14, CD16, CD19, CD20, CD56, FcεRIa and CD303 negative) as CD127, CRTH2, and CD161 triple-positive cells.

Supplemental Figure E2. TSLP synergistically enhances TNF-mediated production of IL-5 and IL-13 in human ILC2s.

Blood ILC2s were stimulated with 20 ng/ml TNF in the presence or absence of 10 ng/ml TSLP for 24 and 96 hours (**A**, n=3). * $p < 0.05$ was determined between medium control (undetectable, not shown) and stimulation and was calculated by the Wilcoxon matched-pairs signed rank test. Blood ILC2s were stimulated with 5-100 ng/ml TNF in the presence or absence of 10 ng/ml TSLP for 4 days (**B**, n=9). The concentrations of IL-5 and IL-13 were measured by Luminex assay. ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ were determined between with and without TSLP and were calculated by the Wilcoxon matched-pairs signed rank test.

Supplemental Figure E3. Dexamethasone and IMD0354 partially enhance cell death of human blood ILC2s.

Blood ILC2s were cultured with 20 ng/ml TNF and 10 ng/ml TSLP in the presence or absence of 0.01% DMSO (vehicle control), 100 nM dexamethasone (Dex), 10 μ M IMD-0354 (IMD), 1 μ M

IMD (**B**) or 100 nM AS1517499 (AS) for 4 days (**A**. n=3, **B**. n=4). Annexin V⁺ Aqua⁺ dead cells were detected within the CD45⁺ population by flow cytometry. The concentrations of IL-13 were measured by Luminex assay. *p<0.05 and ***p<0.001 were determined by comparison with medium control or between indicated conditions and were calculated by the paired t test (**A**) and the one-way ANOVA Friedman test (**B**). NS, not significant.

Supplemental Figure E4. A STAT6 inhibitor suppresses TNF plus TSLP-induced production of type 2 cytokines in human ILC2s.

Blood ILC2s were stimulated with 20 ng/ml TNF and 10 ng/ml TSLP in the presence or absence of 0.01% DMSO or 100 nM STAT6 inhibitor AS1517499 for 4 days (n=3). Each experiment was performed in triplicate with independent donors. The concentrations of IL-13 were measured by Luminex assay. *p<0.05 was calculated by the one-way ANOVA Friedman test.

Supplemental Figure E5. IFNs and IL-27 inhibit IL-33-mediated production of IL-5 and IL-13 in human ILC2s.

Blood ILC2s were cultured with 10 ng/ml IL-33 and 10 ng/ml TSLP in the presence or absence of 1000 U/ml IFN- β , 10 ng/ml IFN- γ or 50 ng/ml IL-27 for 4 days (n=4). Each experiment was performed in duplicate with independent donors. We have previously shown that 10 ng/ml IL-10 and 20 ng/ml TGF- β 1 significantly suppressed IL-33 + TSLP-mediated production of IL-5 and IL-13 in human blood ILC2s.^{E4} The concentrations of IL-5 and IL-13 were measured by Luminex assay. *p<0.05 was determined between with and without inhibitor and were calculated by the one-way ANOVA Friedman test.