

Letter to the Editor

TNF- α /IL-17 synergy inhibits IL-13 bioactivity via IL-13R α 2 induction

To the Editor:

IL-13 is a pleiotropic cytokine that provokes diverse pathophysiological outcomes. Although its effect during gastrointestinal helminth infection is prototypic of a protective T_H2 response (increased peristalsis, goblet cell hyperplasia and mucus secretion, eosinophil recruitment, fibroblast activation, and wound repair), temporal or spatial dysregulation of this response is thought to underlie diseases such as asthma, allergic hyperreactivity, and organ fibrosis.¹ IL-13 signals via the IL-13R α 1/IL-4R α heterodimer to induce several genes specific to T_H2 inflammation including *CCL26*, *CCL11*, *POSTN*, and *MUC5AC*.² IL-13R α 2 binds IL-13 with significantly higher affinity, and the secreted form found in mice acts as a decoy receptor, protecting mice from IL-13-induced immunopathology. However, humans do not alternatively splice the *IL13RA2* transcript and therefore IL-13R α 2 is expressed only as a cell surface protein.³ The factors that regulate the expression of IL-13R α 2 are unclear, and the biological function of the endogenously expressed IL-13R α 2 remains controversial.^{4,5} In this study, we show that the inflammatory cytokines TNF- α and IL-17, often associated with severe asthma, synergize to induce IL-13R α 2 in primary human lung fibroblasts and mouse lungs, and evaluate its biological role using a novel IL-13R α 2-blocking antibody.

Primary human neonatal lung fibroblasts (NLFs) were grown to confluence and incubated with TNF- α , IL-4, and IL-17 for 72 hours, at which time cell lysates were analyzed by using quantitative PCR with primers specific for *IL13RA2* (see the

Methods section in this article's Online Repository at www.jacionline.org). Although TNF- α and IL-4 synergistically induced *IL13RA2* as described,⁶ a combination of TNF- α and IL-17 induced higher expression (Fig 1, A). In contrast, *IL13RA1* decreased in the presence of TNF- α (Fig 1, B). IL-13R α 2 mRNA progressively increased over time, peaking at 72 hours (Fig 1, C), and longer incubations did not consistently result in any further increase (data not shown); therefore, 72 hours was chosen as the ideal end point for subsequent experiments. To determine whether IL-13R α 2 expression was sustainable, NLFs were incubated with a combination of TNF/IL-17 for 72 hours, after which the supernatants were removed and the wells rinsed and replenished with fresh media without added cytokines. On removal of TNF- α and IL-17, IL-13R α 2 expression declined between 24 hours and 72 hours postwash, though it remained elevated (Fig 1, D). We confirmed that primary lung fibroblasts derived from healthy adult donors (adult lung fibroblasts) also manifested similar synergy to TNF- α and IL-17 (see Fig E1 in this article's Online Repository at www.jacionline.org). Using flow cytometry, we verified that the transcriptional induction of IL-13R α 2 was associated with augmented surface expression of protein (Fig 1, E and F). To ascertain whether this phenomenon can be recapitulated *in vivo*, we administered multiple doses of the cytokines into mouse airways. IL-13R α 2 transcripts were strikingly higher in the lungs of mice that received both TNF and IL-17, while IL-13R α 1 message changed negligibly (Fig 1, G).

Next, we examined whether TNF/IL-17-induced expression of IL-13R α 2 was capable of altering the biological effects of IL-13. NLFs were incubated with TNF/IL-17 for 72 hours to upregulate

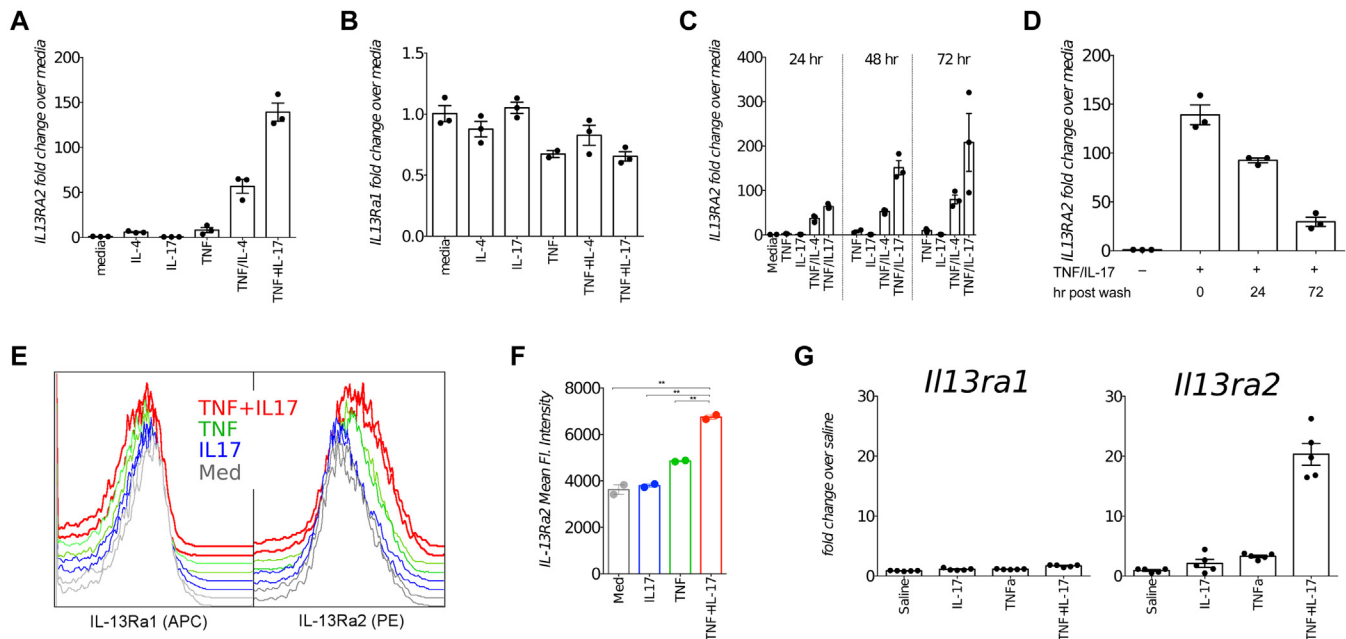


FIG 1. TNF- α and IL-17 synergistically induce IL-13R α 2. Primary NLFs (**A** and **B**) were incubated with specified cytokines for 72 hours. Kinetics of *IL13RA2* expression in NLFs, assayed at 24, 48, or 72 hours poststimulation (**C**) and decay postcytokine withdrawal (**D**). Flow cytometric measurement of IL-13R α 1 and IL-13R α 2 on ALF surface after cytokine stimulation (**E** and **F**). Expression of *Il13ra1* and *Il13ra2* transcripts in mouse lungs after TNF- α and IL-17 administration (**G**). ALF, Adult lung fibroblast; APC, Allophycocyanin; PE, phycoerythrin.

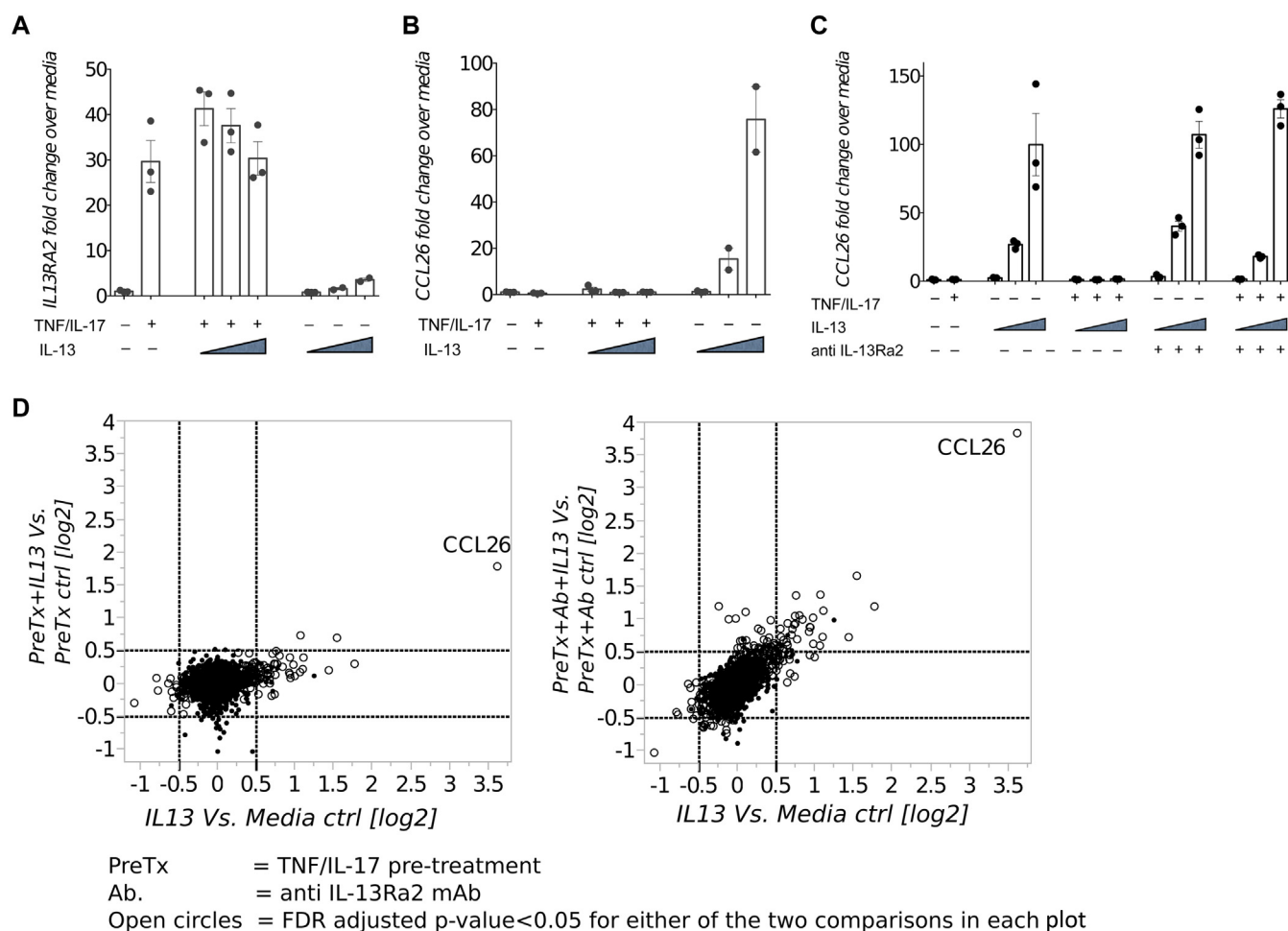


FIG 2. NLFs were treated with TNF- α and IL-17 for 72 hours, washed, and then treated with IL-13 in increasing concentrations (0.1-2.5 ng/mL) for 24 hours. Increased *IL13RA2* induction is associated with loss of IL-13-induced *CCL26* production (**A** and **B**). Recovery of IL-13-induced *CCL26* expression by blocking IL-13R α 2 with mAb (**C**). Pairwise analyses of transcriptome signature of NLFs treated with IL-13 $\pm$ TNF/IL-17 pretreatment (PreTx), $\pm$ anti-IL-13R α 2 mAb (**D**). Also refer to Fig E3.

IL-13R α 2. Subsequently, the wells were washed with media, and treated with IL-13 in increasing concentrations for 24 hours. Cellular RNA was assayed by using real-time quantitative PCR for *CCL26* (eotaxin-3) expression, as a marker for the canonical IL-13 signaling through IL-13R α 1. The expression of *CCL26* increased in a dose-dependent fashion on IL-13 stimulation in control wells in which IL-13R α 2 was at baseline levels, whereas the expression of *CCL26* was abrogated in conditions in which the expression of IL-13R α 2 was augmented (Fig 2, A and B). Similar data were obtained with adult lung fibroblasts (see Fig E2 in this article's Online Repository at www.jacionline.org).

Although the inhibition of IL-13 activity in the setting of increased IL-13R α 2 was likely due to the IL-13R α 2 receptor functioning as a decoy, a number of other alternative explanations, such as concomitant downregulation of IL-13R α 1 or dampening of intracellular signaling networks, could have also led to the attenuation of IL-13 bioactivity after TNF/IL-17 treatment. To investigate these possibilities, NLFs were first incubated with TNF/IL-17 to induce high IL-13R α 2 expression and before the addition of IL-13, some wells were treated with a highly selective neutralizing anti-human IL-13R α 2 mAb. Subsequently, wells were treated with IL-13 in increasing concentrations for 24 hours

and *CCL26* expression was assayed by using quantitative PCR. As before, in wells expressing basal levels of IL-13R α 2, *CCL26* was induced in a dose-dependent fashion after IL-13 stimulation. In wells in which IL-13R α 2 was induced with TNF/IL-17, *CCL26* expression was suppressed. However, in wells treated with anti-IL-13R α 2 antibody, addition of IL-13 resulted in the restoration of *CCL26* expression in a dose-dependent fashion, confirming that the diminution of IL-13 activity by TNF/IL-17 was due to the increased expression of IL-13R α 2 (Fig 2, C).

Although the above results confirm potent decoy activity for IL-13R α 2,⁷ they fail to explore its signaling activity, which has been proposed in some studies.⁵ To uncover the possible signaling potential for this ligand-receptor pair in fibroblasts, we performed a transcriptome-wide microarray analysis in which the effect of IL-13 stimulation in NLFs with or without TNF/IL-17 pretreatment, in the presence or absence of the anti-IL-13R α 2 mAb, was compared. With this approach, the effects of IL-13 signaling through IL-13R α 1 and IL-13R α 2 could be distinguished. *CCL26* was the most abundant IL-13-induced transcript in this genome-wide screen, upregulated 12.3-fold (ie, $2^{3.6}$, q value = 6×10^{-8}) over baseline (Fig 2, D; see Fig E3 in this article's Online Repository at www.jacionline.org). On TNF/IL-17 pretreatment, this

diminished to 3.2-fold ($2^{1.7}$), in accord with the above-described IL-13R α 2-mediated inhibition. Surprisingly, although IL-13 binding to IL-13R α 2 reduced the transcript abundance of CCL26, it did not result in any additional statistically significant changes in the transcriptome profile of fibroblasts (Fig 2, D, and Fig E3) that were distinguishable from the pretreatment controls. Blocking IL-13R α 2 with the antibody restored IL-13-induced CCL26 expression (Fig 2, D, and Fig E3). These data indicate that in NLFs, IL-13 binding to the high-affinity IL-13R α 2 serves to dampen canonical IL-13 signaling, as opposed to inducing other signaling pathways.

Asthma syndrome is a heterogeneous mixture of distinct phenotypic subtypes and only those cohorts that have a “IL-13 high” signature are indeed likely to benefit from therapeutic modalities that block IL-13/IL-13R α 1 interactions.⁸ Steroid-resistant and severe asthma subtypes are often associated with neutrophilia, TNF- α , and IL-17 activity⁹ and therefore may not benefit from the blockade of IL-13, and perhaps the mechanisms we described above already operate in these patients to quench endogenous IL-13 activity via IL-13R α 2. Indeed, due caution must be exercised because blocking IL-13/IL-13R α 1 interactions in these patients might actually lead to disease aggravation because IL-13 has been shown to downmodulate the inflammation induced by TNF- α and IL-17.¹⁰

We thank the Wynn lab members for helpful suggestions.

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This study was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases. T. Ort is an employee of Johnson and Johnson. L.A.B. is funded by a European Union FP7 Marie Curie fellowship (grant no. 275781).

Disclosure of potential conflict of interest: T. Ort is employed by Janssen R&D. A. Fisher's institution has received or has grants pending from MedImmune. L. A. Borthwick is employed by Newcastle University, which has received funding from the European Union Research Fellowship Programme. The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2014.05.019>

METHODS

Cell culture

Human lung fibroblasts were purchased from the American Type Culture Collection (ATCC CCL-75, Rockville, Md). Adult primary lung fibroblasts were isolated from healthy adult donors at Newcastle University, Newcastle upon Tyne, United Kingdom, as previously described.^{E1} All participants provided informed consent before participation in this study. All aspects of the study were approved by Newcastle and North Tyneside Local Research Ethics Committee (Ref. 2001/179). Fibroblasts were incubated in Dulbecco's modified Eagle medium (Life Technologies, Carlsbad, Calif) supplemented with 10% FBS, 4 mM L-glutamine, 100 units/mL of penicillin G, and 100 µg/mL of streptomycin. Cells were passaged every 4 to 7 days, and passages 4 to 9 were used in all experiments. Confluent fibroblasts were removed from flasks and wells by treatment with 0.05% trypsin in 0.53 mM EDTA and resuspended in serum-supplemented media to quench trypsin.

Animals

Female BALB/c mice were purchased from Taconic Biotechnology (Germantown, NY). Experiments commenced when mice were 15 weeks old. All mice experiments were performed under protocols approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee and the National Institutes of Health Interagency Animal Model Committee. On days 1, 2, 8, and 9, mice were anesthetized with ketamine/xylazine and challenged intratracheally with saline or 1 µg of recombinant murine TNF-α and IL-17, singly, or in combination, diluted in 30 µL of saline. Mice were sacrificed on day 10, and their lungs were harvested and processed for RNA extraction.

Reagents

Recombinant human TNF-α, IL-4, IL-13, IL-17, and recombinant murine TNF-α were purchased from Peprotech (Rocky Hill, NJ). Recombinant mouse IL-17 was purchased from Biolegend (San Diego, Calif). Purified mouse anti-human IL-13Rα2 mAb was provided by Janssen Biotech (formerly Centocor Biotech, Springhouse, Pa).

Cytokine stimulation of fibroblasts

Fibroblasts were plated in Corning Costar 24-well cell culture plates (Corning Life Sciences, Tewksbury, Mass), with cell numbers ranging from 30,000 to 50,000 per well per experiment. Cells were stimulated with the human recombinant TNF-α, IL-4, and IL-17 A at concentrations of 20 ng/mL and IL-13 at concentrations of 0.1, 0.5, 2.5, and 20 ng/mL. Fibroblasts were incubated with the above cytokines for periods of 24 to 96 hours.

Real-time quantitative PCR

Total RNA was extracted with an RNeasy kit (Qiagen, Valencia, Calif) or MagMAX-96 Total RNA Isolation Kit (Life Technologies) according to manufacturer instructions. Complementary DNA was synthesized using the Superscript II (Life Technologies). Real-time quantitative PCR was performed with an ABI 7900HT using SYBR green chemistry. Data were analyzed using the comparative Ct method using SAGE software (<http://sage.niaid.nih.gov>). The table of primers used for quantitative PCR is given in Table E1. To assay for IL-13 bioactivity, *CCL26* (eotaxin-3) was chosen on the basis of previous reports.^{E2,E3}

Microarray design and hybridization

Two different passages of NLFs were first incubated with TNF/IL-17 for 72 hours to induce high IL-13Ra2 expression and some wells were treated with a

highly selective neutralizing anti-human IL-13Ra2 mAb during the last 1 hour. Subsequently, the wells were washed with media, and treated with IL-13 for 20 hours. RNA quality was verified by using Bioanalyzer with RNA Integrity Number greater than 9.0 for all samples. Amplification and labeling of the RNA samples were performed using the Illumina TotalPrep RNA Amplification (Applied Biosystems) and an input of 500 ng of total RNA per sample. Biotinylated aRNA was hybridized to Illumina HumanHT-12 v4.0 Expression BeadChip (GEO Accession GPL10558) having 47,323 unique probes, using reagents provided, and imaged using the Illumina HiScan-SQ.

Microarray expression analysis

Signal data were extracted from the image files with the Gene Expression module (v. 1.9.0) of the GenomeStudio software (v. 2011.1) from Illumina, Inc (San Diego, Calif). Signal intensities were converted to log₂ scale. Calculation of detection *P* values is described in the GenomeStudio Gene Expression Module User Guide. Data for array probes with insufficient signal (detection *P* value < .1 in at least 2 arrays) were removed from the data set. After dropping nonperforming probes, Quantile normalization was applied across all arrays. ANOVA was performed on the normalized log₂ expression estimates to test for mRNA expression differences. A *P* value of .05 was used for the statistical significance cutoff, after adjusting for the familywise error rate using the Benjamini-Hochberg method to account for multiple testing. Statistical analysis was performed using JMP/Genomics software (version 6.0; SAS Institute, Inc, Cary, NC). Full protocol and microarray data are accessible through GEO Series accession number GSE56338 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56338>).

Flow cytometry

Fluorescence-activated cell sorting (FACS) analysis of cell surface receptors for IL-13 was performed with allophycocyanin-conjugated mouse anti-human IL-13Ra1 mAb (RnD Systems, Minneapolis, Minn) and phycoerythrin-conjugated mouse anti-human IL-13Ra2 mAb (Cell Sciences, Canton, Mass). Fibroblasts were briefly detached using 0.05% trypsin solution (Life Technologies) and incubated with manufacturer-recommended concentrations of anti-human IL-13Rα1 or IL-13Rα2 for 30 minutes at 4°C in FACS buffer (PBS supplemented with 1% FBS). Cells were washed twice with FACS buffer and acquired with BD FACS Canto II. Data were analyzed with FlowJo software (version 9.7.4; www.flowjo.com).

Statistics

Individual replicate data are presented for each representative experiment, with group means as bars ± SEM. Unless otherwise specified, statistical significance was determined by using the unpaired 2-tailed *t* test and differences were considered significant at *P* < .05. Analysis was performed using GraphPad Prism (version 6.00; www.graphpad.com).

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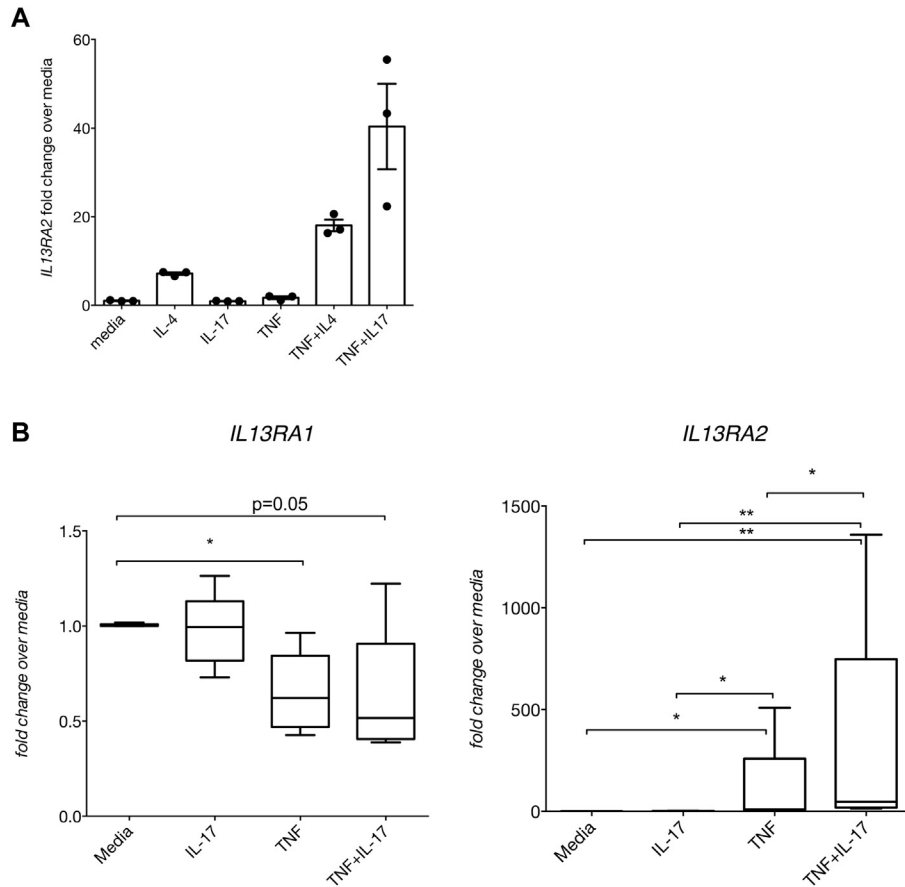


FIG E1. TNF- α and IL-17 synergistically induce IL-13R α 2 in human adult lung fibroblasts (ALFs). **A**, Primary lung fibroblasts from a healthy adult donor were treated with indicated cytokines for 72 hours. Transcript abundance of *IL13RA2* was measured by using quantitative PCR. **B**, Primary ALFs from 5 healthy donors were cultured with indicated cytokines for 72 hours. Transcript abundance of *IL13RA1* and *IL13RA2* was quantified by using quantitative PCR. Box-and-whisker plots depict 25th-75th percentile range with median drawn inside, and error bars depict Tukey's interquartile range. Ratio paired *t* test used for calculating statistical significance between relevant treatment groups. **P* < .05; ***P* < .01.

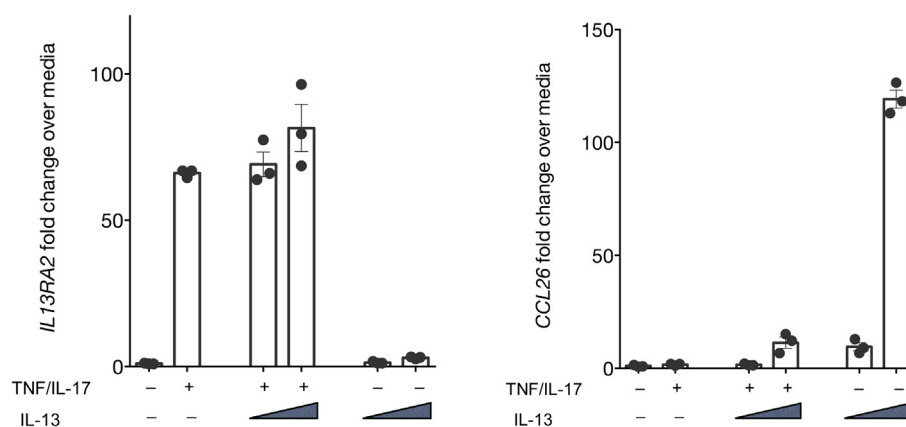
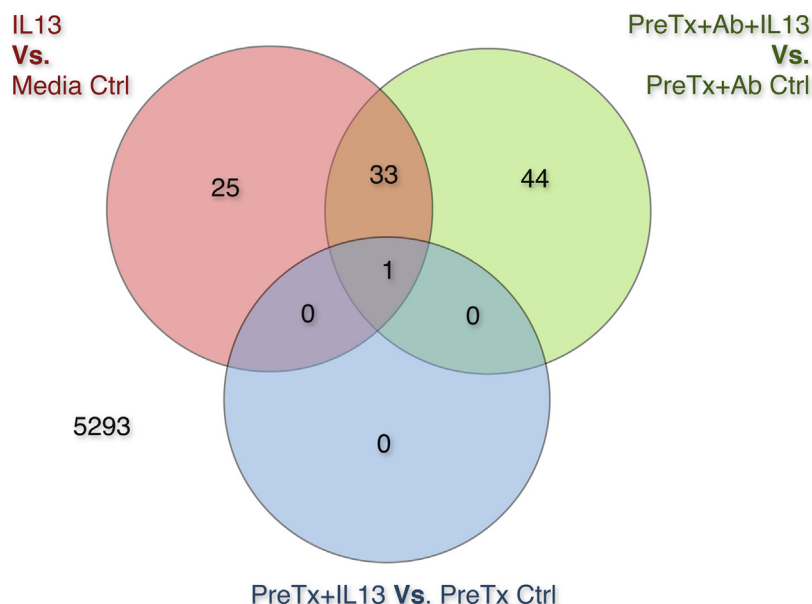


FIG E2. Induction of IL-13R α 2 by TNF- α and IL-17 in ALFs is associated with diminution of *CCL26* expression by IL-13. Representative ALFs from a healthy donor were cultured with or without TNF- α and IL-17 for 72 hours. Media were replaced without or with low (0.5 ng/mL) or high (5 ng/mL) concentrations of IL-13 for 24 hours, at which time cells were harvested for RNA and quantitative PCR was performed for *IL13RA2* and *CCL26*. ALFs, Adult lung fibroblasts.

A

	Pre Tx (72 hr)	Wash	mAb	Tx (20 hr)	Group Designation
1	None	Yes	None	None	Media
2	None	Yes	None	IL-13	IL-13
3	TNF/IL-17	Yes	None	None	PreTx Ctrl
4	TNF/IL-17	Yes	None	IL-13	PreTx+IL13
5	TNF/IL-17	Yes	Anti-IL13Rα2	None	PreTx+Ab Ctrl
6	TNF/IL-17	Yes	Anti-IL13Rα2	IL-13	PreTx+Ab+IL13

C



B

Gene	Media Control	IL13	PreTx+IL13	PreTx+Ab+IL13	PreTx+Ab Ctrl	PreTx Ctrl	Legend
CCL26	0	3.63	2.13	4.18	0.35	0.36	3.00
CCL11	0	1.78	0.39	1.31	0.13	0.11	2.00
ANPEP	0	1.55	0.80	1.57	-0.08	0.12	1.00
C1QTNF1	0	1.45	2.58	2.93	2.22	2.40	0.00
C11orf70	0	1.12	0.64	1.46	0.34	0.26	-1.00
COL17A1	0	1.11	0.16	0.66	-0.06	-0.05	-2.00
CDH6	0	1.08	0.15	0.74	-0.62	-0.57	-3.00
PTGIS	0	1.08	-0.48	0.07	-0.51	-0.64	
CCL2	0	1.01	1.41	1.59	1.18	1.31	
INHBE	0	0.99	0.21	0.62	0.01	-0.06	
SPINT2	0	0.99	-0.33	0.42	-0.59	-0.61	
PPFIB2	0	0.95	0.03	0.76	-0.10	-0.04	
LRRCSB	0	0.95	-0.04	0.31	-0.13	-0.16	
TNC	0	0.95	0.78	1.40	0.54	0.39	
SFR1	0	0.94	-0.07	0.46	-0.34	-0.22	
TMEM171	0	0.85	0.07	0.72	-0.26	-0.17	
C11orf70	0	0.81	0.60	1.34	0.30	0.19	
IL13RA2	0	0.78	3.64	3.89	3.21	3.36	
SLIT2	0	0.77	1.19	1.89	0.54	0.71	
ST8SIA1	0	0.76	0.19	0.95	0.04	0.07	
BATF3	0	0.75	0.32	1.02	-0.01	0.03	
RGS2	0	0.75	0.45	0.86	0.75	0.63	
EFNB2	0	0.74	-0.71	0.06	-0.84	-1.00	
TNC	0	0.72	0.70	1.22	0.38	0.26	
NFIL3	0	0.71	-0.29	0.56	-0.18	-0.09	
LMCD1	0	0.69	-0.24	0.05	-0.11	-0.37	
BHLHE40	0	0.69	-0.68	-0.32	-0.75	-0.67	
VCAM1	0	0.68	1.23	1.39	1.02	1.19	
LIMA1	0	0.66	0.05	0.77	-0.16	-0.14	
CPA4	0	0.66	-0.62	-0.12	-0.72	-0.85	
SOC51	0	0.66	0.82	1.31	0.72	0.71	
SGK223	0	0.65	-0.21	-0.24	-0.26	-0.35	
HK1	0	0.65	-0.13	0.24	-0.40	-0.35	
COL6A2	0	0.61	0.23	0.60	-0.07	-0.03	
LYPD1	0	0.60	-0.21	0.15	-0.20	-0.19	
EGFLAM	0	0.60	-0.26	0.17	-0.37	-0.38	
LOX	0	0.58	0.14	0.34	-0.10	0.04	
ANGPTL4	0	0.57	-0.50	-0.01	-0.73	-0.66	
KLF2	0	0.57	-1.14	-0.38	-1.22	-1.21	
IL17RA	0	0.56	0.01	0.24	-0.14	-0.05	
NNMT	0	0.56	-0.62	-0.02	-0.58	-0.86	
LYPD1	0	0.54	-0.04	0.24	-0.12	-0.17	
TMTC1	0	0.54	0.14	0.26	-0.03	-0.09	
PRRX1	0	0.54	-0.19	0.21	-0.23	-0.01	
SH2D4A	0	0.53	1.04	1.43	0.89	0.96	
FRMD4A	0	0.53	-0.53	-0.22	-0.60	-0.63	
PLD6	0	0.52	-0.03	0.01	-0.19	-0.15	
DCUN1D3	0	0.51	-0.18	0.09	-0.25	-0.20	
PCDH18	0	0.51	0.71	1.00	0.70	0.41	
TNFRSF21	0	0.50	1.35	1.82	1.31	1.36	
AHI1	0	0.49	0.41	0.79	0.17	0.17	
ASB1	0	0.48	-0.09	0.26	-0.33	-0.24	
MXRA5	0	0.45	1.33	1.67	1.10	1.15	
TGM2	0	0.45	-1.26	-0.70	-1.67	-1.48	
SORD	0	0.44	0.05	0.62	0.05	0.13	
COL6A1	0	0.42	0.33	0.78	0.08	-0.12	
PTGER4	0	0.42	0.03	0.45	-0.09	-0.22	
LDLR	0	0.40	-0.45	-0.14	-0.78	-0.70	
LBH	0	0.38	-0.96	-0.25	-0.81	-1.22	
COL6A2	0	0.37	0.05	0.30	-0.21	-0.12	
SLC26A6	0	0.35	0.56	1.24	0.57	0.81	
RHOV	0	0.32	0.18	0.94	0.12	0.13	
MAP3K8	0	0.31	0.52	1.03	0.51	0.60	
NABP1	0	0.29	0.07	0.56	0.00	-0.01	
INSIG1	0	0.28	-0.34	0.00	-0.54	-0.45	
OXTR	0	0.28	-2.12	-1.69	-2.53	-2.58	
COL7A1	0	0.23	1.31	1.66	1.12	1.10	
SFRP1	0	0.22	-0.89	-0.41	-1.00	-1.15	
RASSF2	0	0.15	-0.19	0.32	-0.26	-0.45	
HMHAI1	0	0.13	0.34	0.75	0.08	0.12	
LXN	0	0.12	1.57	2.63	1.53	1.55	
STEAP2	0	0.08	0.49	1.12	0.46	0.49	
SIPA1L2	0	0.06	0.16	0.69	0.14	0.14	
STEAP2	0	0.05	0.51	1.03	0.37	0.47	
METRN1	0	0.01	0.13	0.43	-0.11	-0.09	
STEAP1B	0	-0.01	0.98	1.82	0.82	1.10	
MFSD6	0	-0.02	0.37	0.18	0.70	0.79	
FMO3	0	-0.04	0.12	0.01	0.57	0.23	
NRA2	0	-0.09	1.04	0.54	1.09	1.28	
ILMN_1705	0	-0.10	0.69	1.53	0.55	0.84	
DIO2	0	-0.13	1.70	1.12	1.88	1.98	
GRB14	0	-0.13	0.76	0.20	0.90	0.87	
NUPR1	0	-0.14	-0.39	-0.79	-0.20	-0.31	
FMO3	0	-0.20	0.20	-0.04	0.53	0.36	
STEAP1	0	-0.23	1.03	1.94	0.75	1.09	
FAM43A	0	-0.26	0.26	-0.30	0.25	0.26	
UBE2C	0	-0.30	0.49	0.09	0.73	0.56	
MT1JP	0	-0.33	0.10	-0.50	0.14	-0.08	
NDRG1	0	-0.40	-0.22	-0.55	-0.04	0.11	
MT1X	0	-0.41	0.40	0.01	0.64	0.57	
CDC20	0	-0.42	0.66	0.21	0.83	0.88	
BTG1	0	-0.43	0.37	-0.09	0.57	0.85	
MT1M	0	-0.43	0.20	-0.23	0.42	0.13	
CLDN1	0	-0.52	1.75	0.99	1.47	1.82	
FST	0	-0.52	0.02	-0.04	-0.06	0.05	
MTSS1	0	-0.56	-0.50	-0.90	-0.37	-0.27	
MALL	0	-0.58	-2.13	-2.30	-2.03	-1.99	
STC1	0	-0.59	0.66	0.40	0.78	1.09	
HSPB7	0	-0.60	-2.00	-2.04	-1.98	-2.07	
KISS1	0	-0.63	-1.37	-1.35	-1.35	-1.36	
DRAM1	0	-0.76	1.02	0.47	0.93	1.14	
ADAMTS1	0	-0.78	0.79	0.36	0.79	0.72	
IGFBP3	0	-1.07	-0.12	-0.67	0.38	0.19	

FIG E3. Expression profile of differentially expressed transcripts in response to IL-13, $\pm$ IL-13R α 2 induction, $\pm$ anti-IL-13R α 2 mAb. **A**, List of experimental groups and their treatment conditions that were studied in quadruplicates by microarray. **B**, Heat-map of log2 expression level, relative to media-only control. After pretreatment to induce IL-13R α 2, IL-13 was added in the presence or absence of

TABLE E1. Primer sequences used for quantitative PCR

Species	Gene	Forward	Reverse
<i>H sapiens</i>	<i>IL13RA1</i>	TGAGTCTGCTGTGACTGAGC	TCAGGTTCACACGGGAAGTT
<i>H sapiens</i>	<i>IL13RA2</i>	GGTCTTCCGGATGAAGGCTA	GATATTGCCTCTCTCCCCGC
<i>H sapiens</i>	<i>CCL26</i>	CTGGGTGCGAAGCTATGAAT	TCTTGCCTCTTTTGGTAGTGAA
<i>H sapiens</i>	<i>GAPDH</i>	AGCCACATCGCTAGACAC	GCCCAATACGACCAAATCC
<i>M musculus</i>	<i>Hprt</i>	GCCCTTGACTATAATGAGTACTTCAGG	TTCAACTTGCCTCATCTTAGG
<i>M musculus</i>	<i>Il13ra1</i>	CCTGAAGGAGCCAGTCCAAA	GCCCACCTGCAGACAGATTT
<i>M musculus</i>	<i>Il13ra2</i>	GGAAAGGAGGACAAAGAGGTC	GATTAGTGTGCTGAAAGCTCTACTC

anti-IL-13R α 2-blocking mAb. Entries for IL13 with *q* value > 0.05 (comparing media control) are not color coded. **C**, Venn diagram of statistically significant expression changes, showing the number of probes having log2 fold change > 0.5 (up or down) and false discovery rate-adjusted *P* value < .05. *Ab*, Antibody.