

Human T_H17 cells express a functional IL-13 receptor and IL-13 attenuates IL-17A production

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Background: IL-13 is a central mediator of airway responsiveness and mucus expression in patients with allergic airway inflammation, and IL-13 is currently a therapeutic target for asthma. However, little is known about how IL-13 regulates human CD4⁺ T-cell lineages because IL-13 receptor (IL-13R) α 1, a subunit of IL-13R, has not previously been reported to exist on human T cells.

Objective: We sought to determine whether human CD4⁺ T_H17 cells express IL-13R α 1 and whether IL-13 regulates T_H17 cytokine production.

Methods: Naive human CD4⁺ cells were isolated from whole blood, activated with anti-CD3 and anti-CD28, and polarized to T_H1, T_H2, T_H17, or induced regulatory T cells in the presence of IL-13 (0–10 ng/mL). Cell supernatants, total RNA, or total protein was examined 4 days after T_H17 polarization.

Results: T_H17 cells, but not T_H0, T_H1, T_H2, or induced regulatory T cells, expressed IL-13R α 1. IL-13 attenuated IL-17A production, as well as expression of retinoic acid–related orphan receptor, runt-related transcription factor-1, and interferon regulatory factor 4 in T_H17-polarized cells. IL-13 neither inhibited IFN- γ production from T_H1 cells nor inhibited IL-4 production from T_H2 cells. Furthermore, attenuation of IL-17A production only occurred when IL-13 was present within 24 hours of T-cell activation or at the time of restimulation.

Conclusions: IL-13R α 1 is expressed on human CD4⁺ T_H17 cells, and IL-13 attenuates IL-17A production at polarization and restimulation. Although IL-13 is an attractive therapeutic target for decreasing symptoms associated with asthma, these results suggest that therapies inhibiting IL-13 production could

have adverse side effects by increasing IL-17A production. (J Allergy Clin Immunol 2011;■■■:■■■–■■■.)

Key words: IL-13, IL-13 receptor, T_H17, IL-17A, asthma

IL-13 is an important T_H2 cytokine that is upregulated in patients with allergic airway inflammation. IL-13 is deemed a central mediator of airway responsiveness and mucus expression, both hallmarks of asthma.^{1,2} Studies in mice and primates that blocked IL-13 during allergen challenge decreased inflammation, airway hyperreactivity, and mucus production,^{2–5} making IL-13 an attractive therapeutic target for asthma. IL-13 signals through the IL-13 receptor (IL-13R), also known as the type II IL-4 receptor (IL-4R), which is composed of IL-13R α 1 and IL-4R α subunits. The type I IL-4R is also composed of IL-4R α in addition to the common γ chain. Signaling through type I or type II IL-4R results in phosphorylation and activation of the downstream transcription factor signal transducer and activator of transcription (STAT) 6.^{6,7} The direct effects of IL-13 on human CD4⁺ T cells, however, have not been fully examined because IL-13R α 1 was not known to be expressed on human T cells.⁶ We have recently shown in murine CD4⁺ cells that the IL-13R α 1 subunit is expressed on T_H17 cells but not T_H0, T_H1, or T_H2 cells. IL-13R is functional on murine T_H17 cells, as indicated by the fact that IL-13 attenuated T_H17 cytokine production and increased STAT6 phosphorylation.⁸ IL-13 did not inhibit IFN- γ produced by T_H1 cells or inhibit IL-4 produced by T_H2 cells.⁸

T_H17 cells are a distinct lineage of T cells and are associated with autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, as well as protection against bacterial infections.⁹ The cytokines IL-6 and TGF- β are responsible for the differentiation of naive T cells into T_H17 cells in mice.^{10,11} Human T_H17 cell differentiation requires IL-6 and IL-1 β , and although TGF- β is not required for differentiation, it is required for expansion by inhibiting T_H1 differentiation.^{10–14} T_H17 cell differentiation requires expression of the transcription factors STAT3, retinoic acid–related orphan receptor (RORC2), runt-related transcription factor-1 (Runx1), and interferon regulatory factor (IRF) 4¹³ and the production of the cytokine IL-21.^{10,15–17} Phosphorylation of STAT3 leads to increased RORC2 and Runx1 expression, as well as IL-21 and IL-17A production.¹⁸ IL-21 is secreted from T_H17 cells and acts in an autocrine fashion, increasing IL-23 receptor (IL-23R) surface expression. This increased IL-23R expression allows for IL-23, which is produced by antigen-presenting cells, to bind to the T_H17 cell, further increasing RORC2 expression and production of T_H17 cytokines, such as IL-17A and IL-22.^{19–21}

The cytokine milieu at the time of T_H17 cell differentiation dramatically affects T_H17 cytokine production. In mice IL-4

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Abbreviations used

IL-4R: IL-4 receptor
 IL-13R: IL-13 receptor
 IL-23R: IL-23 receptor
 IRF: Interferon regulatory factor
 iTreg: Induced regulatory T
 PE: Phycoerythrin
 rh: Recombinant human
 RORC2: Retinoic acid-related orphan receptor
 Runx1: Runt-related transcription factor-1
 STAT: Signal transducer and activator of transcription

and IL-13 negatively regulate murine CD4⁺ T_H17 differentiation and cytokine production.^{8,10} Based on our findings in murine CD4⁺ T cells, we hypothesized that human subjects, like mice, express IL-13Rα1 and that IL-13 attenuates IL-17A production. We have extended our findings in human T cells to show IL-13Rα1 surface expression on human CD4⁺ T_H17 cells by means of flow cytometry. Furthermore, we found that TGF-β, IL-1β, and IL-23 were the key components required for IL-13Rα1 expression on T_H17 cells and that IL-13Rα1 was functional because it increased phosphorylation of STAT6 in T_H17 cells. IL-13 added at the time of polarization or restimulation attenuated IL-17A production in CD4⁺ T cells by decreasing RORC2, Runx1, and IRF-4 expression.

METHODS**Isolation of human T cells**

All studies were approved by the Institutional Review Board at Vanderbilt University Medical Center. Human PBMCs were isolated from the buffy coats of healthy donors by using Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ). Naive CD4⁺ T cells were isolated from the PBMCs by using a naive T-cell isolation kit (Miltenyi Biotec, Auburn, Calif). Briefly, CD4⁺ T cells were negatively selected by incubating mononuclear cells with biotin-labeled antibodies targeting non-CD4⁺ cells and CD45R0⁺ memory T cells. Biotin was conjugated to magnetic beads, and non-CD4⁺ cells and CD45R0⁺ memory T cells were bound to the column contained within a magnetic field. Naive CD4⁺ T cells were able to flow through the column, and these cells were collected. CD4⁺ naive T cells were activated by using an activation/expansion kit (Miltenyi Biotec) with anti-CD3 and anti-CD28 bound to bead particles at the ratio of 1 bead particle per 2 cells. CD4⁺ T cells were polarized to become T_H1, T_H2, T_H17, or induced regulatory T (iTreg) cells in RPMI containing 10% FBS, 1% penicillin/streptomycin, 2 mmol/L L-glutamine, 10 mmol/L HEPES, 0.1 mmol/L nonessential amino acids, and 1 mmol/L sodium pyruvate (Gibco, Carlsbad, Calif). For T_H1 polarization, naive T cells were stimulated in the presence of recombinant human (rh) IL-2 (10 ng/mL), rhIL-12 (10 ng/mL), and anti-IL-4 (10 μg/mL). For T_H2 polarization, naive T cells were stimulated in the presence of rhIL-2, rhIL-4 (10 ng/mL), and anti-IFN-γ (10 μg/mL). For T_H17 cells, naive T cells were stimulated in the presence of rhIL-2, rhIL-1β (10 ng/mL), rhTGF-β (1 ng/mL), rhIL-6 (10 ng/mL), rhIL-23 (10 ng/mL), anti-IFN-γ, and/or anti-IL-4. For iTreg cell differentiation, naive T cells were stimulated in the presence of rhIL-2 and rhTGF-β (5 ng/mL). Recombinant IL-13 (0–10 ng/mL) was added to select wells at the time of polarization. In some experiments T_H17 cells were polarized by using the above conditions, except anti-IL-13 (10 μg/mL) or IgG2a isotype control (10 μg/mL) were added at the time polarization instead of anti-IL-4. Cells were polarized for 4 days, and supernatants, total protein, or total RNA were harvested for analysis. The cells were restimulated with anti-CD3 for 24 hours in the presence of rhIL-4 or rhIL-13 (0–10 ng/mL) to stimulate effector cytokine production. All antibodies and cytokines except rhTGF-β were purchased from R&D Systems (Minneapolis, Minn). rhTGF-β was purchased from PeproTech (Rocky Hill, NJ).

Quantitative PCR

cDNA was generated from total RNA, and a real-time PCR assay was conducted with TaqMan Universal primer mix and commercially available primers and probes for IL-13Rα1 and glyceraldehyde-3-phosphate dehydrogenase (Applied Biosystems, Foster City, Calif). Real-time analysis was performed on a Step-One PCR instrument (Applied Biosystems). All real-time data were normalized to glyceraldehyde-3-phosphate dehydrogenase, and relative expression is compared with either T_H0 cells or T_H17 cells (see the Methods section in this article's Online Repository at www.jacionline.org).

Cytokine measurements

Cytokine levels were measured from cell-culture supernatants with available Duoset or Quantikine ELISA kits (R&D Systems), according to the manufacturer's instructions. Any value less than the limit of detection was assigned half the value of the lowest detectable standard.

Immunoblotting

Cells were lysed by using modified radioimmunoprecipitation assay buffer containing phenylmethylsulfonyl fluoride and 1% proteinase inhibitors, and total protein was extracted and resolved by using 10% SDS-PAGE gels, transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, Calif), and probed with antibodies against RORC2, nucleolin, or actin, all of which are from Santa Cruz Biotechnology (Santa Cruz, Calif) or phospho-STAT3, STAT3, IRF-4, or Runx1 (Cell Signaling, Beverly, Mass). Signals were amplified and visualized with infrared IRDy3-labeled secondary antibodies (LI-COR, Lincoln, Neb) and the Odyssey infrared imaging system (LI-COR). Densitometry was performed with Odyssey software (LI-COR).

Flow cytometry and intracellular staining

Four days after polarization, T_H0 and T_H17 CD4⁺ T cells were harvested, and cells were blocked with anti-FcR antibody (BD Biosciences, San Jose, Calif) and in some experiments surface stained with allophycocyanin-conjugated anti-CD4 (BD Biosciences), Pacific blue-conjugated anti-CD3, and in select experiments phycoerythrin (PE)-conjugated IL-13Rα1 (R&D Systems). For experiments examining STAT6 phosphorylation, cells were serum starved for 4 hours, and select wells were stimulated with IL-13 (10 ng/mL) for 1 hour. Cells were blocked with anti-FcR antibody and surface stained with allophycocyanin-conjugated anti-CD4 (BD Biosciences) and Pacific blue-conjugated anti-CD3. Cells were then permeabilized with cytofix/cytoperm (BD Biosciences), washed thoroughly, and stained with PE-conjugated phospho-STAT6 (BD Biosciences) or PE-conjugated IgG1κ isotype control. Cells were analyzed with an LSR II flow cytometer (BD Biosciences), and data were analyzed with Flow Jo 7.2 software (TreeStar, Inc, Ashland, Ore) (see the Methods section in this article's Online Repository).

Statistical analyses

Data are presented as means ± SEMs. Data were analyzed with ANOVA followed by the Tukey post-hoc test or a 2-tailed *t* test with GraphPad Prism version 4 software (GraphPad Software, Inc, La Jolla, Calif), with values being considered significant at a *P* value of less than .05.

RESULTS**Functional IL-13R is expressed on human T_H17 cells**

Naive human CD4⁺ T cells were activated with anti-CD3 and anti-CD28 and polarized to become T_H1, T_H2, T_H17, or iTreg cells. Total cellular RNA was collected 4 days after polarization and analyzed for IL-13Rα1 by means of real-time PCR (Fig 1, A). Flow cytometry was conducted and cells were gated on CD3⁺CD4⁺ cells to examine surface expression of IL-13Rα1. IL-13Rα1 expression was significantly increased on T_H17 cells both as percentages (Fig 1, B) and total numbers of CD3⁺CD4⁺IL-13Rα1⁺ cells (Fig 1, C) but not T_H0, T_H1, T_H2,

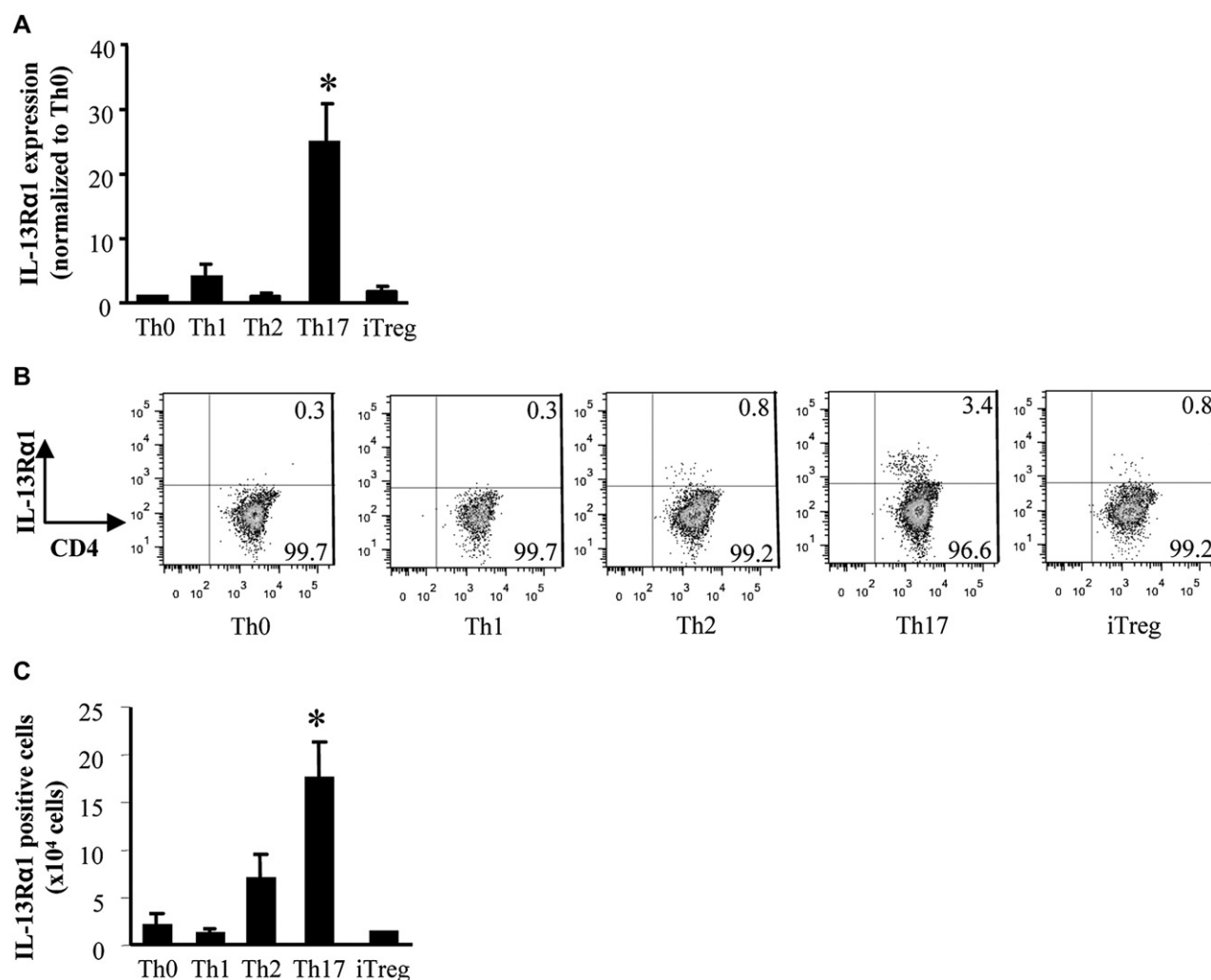


FIG 1. IL-13Rα1 expression on human T_H17 cells. CD4⁺ T cells were polarized to T_H1, T_H2, T_H17, or iTreg cells. **A**, IL-13Rα1 relative expression normalized to glyceraldehyde-3-phosphate dehydrogenase and compared with that seen in T_H0 cells. **B** and **C**, Dot plot of IL-13Rα1 surface expression on CD4⁺ CD3⁺ cells (Fig 1, **B**) and total CD4⁺ CD3⁺ IL-13Rα1⁺ cells (Fig 1, **C**). Data are composed of 5 to 6 replicates compiled from 3 independent experiments. **P* < .05 compared with T_H0 cells (ANOVA).

or iTreg cells. IL-13Rα1 message levels were slightly increased in T_H1 cells, and IL-13Rα1 surface expression was slightly increased in T_H2 cells. However, these increases were not statistically significant. Because IL-13Rα1 was thought not to be expressed in human T cells, we wanted to ensure the cells were CD3⁺CD4⁺ T cells. Therefore cells were analyzed by means of flow cytometry and sorted on live cells, and all T-cell lineages contained greater than 99% CD3⁺CD4⁺ cells (see Fig E1 in this article's Online Repository at www.jacionline.org). The other subunit of IL-13R, IL-4Rα, was also expressed on T_H17 cells and other T-cell lineages (see Fig E2 in this article's Online Repository at www.jacionline.org), showing that both components of IL-13R are expressed on human T_H17 cells. Membrane-bound IL-13Rα2 was not upregulated in T_H17 cells, as determined by means of real-time PCR (data not shown).

We further hypothesized that if IL-13Rα1 is expressed on human T_H17 cells, then the downstream transcription factor STAT6 would become phosphorylated in the presence of IL-13. To test this hypothesis, we polarized naive human T cells under T_H17-differentiating conditions for 4 days. On day 4, we serum starved the cells for

4 hours and restimulated them for 1 hour with IL-13 (0-10 ng/mL). Cells were collected, and STAT6 phosphorylation was analyzed by means of intracellular cytokine staining with a PE-conjugated phospho-STAT6 antibody or IgG1κ isotype control and flow cytometry with histograms gated for CD3⁺CD4⁺ cells. As shown in Fig 2, **A**, T_H17 cells with IL-13 stimulus showed an increase in STAT6 phosphorylation (Fig 2, **A**, right) compared with that seen in T_H0 cells with IL-13 (Fig 2, **A**, left) or T_H17 cells with no IL-13 added (Fig 2, **A**, right, gray curve). CD3⁺CD4⁺ phospho-STAT6⁺ cells were calculated and normalized to no IL-13 stimulation (Fig 2, **B**), and T_H0 cells showed no increase in STAT6 phosphorylation, although T_H17 cells had an increase in STAT6 phosphorylation in the presence of IL-13.

TGF-β, IL-1β, and IL-23 are responsible for increases in IL-13Rα1 production

Our data reveal that T_H17 cells, but not T_H1, T_H2, or iTreg cells, express functional IL-13Rα1. We therefore hypothesized that 1 or more of the cytokines involved in the T_H17 polarization process is

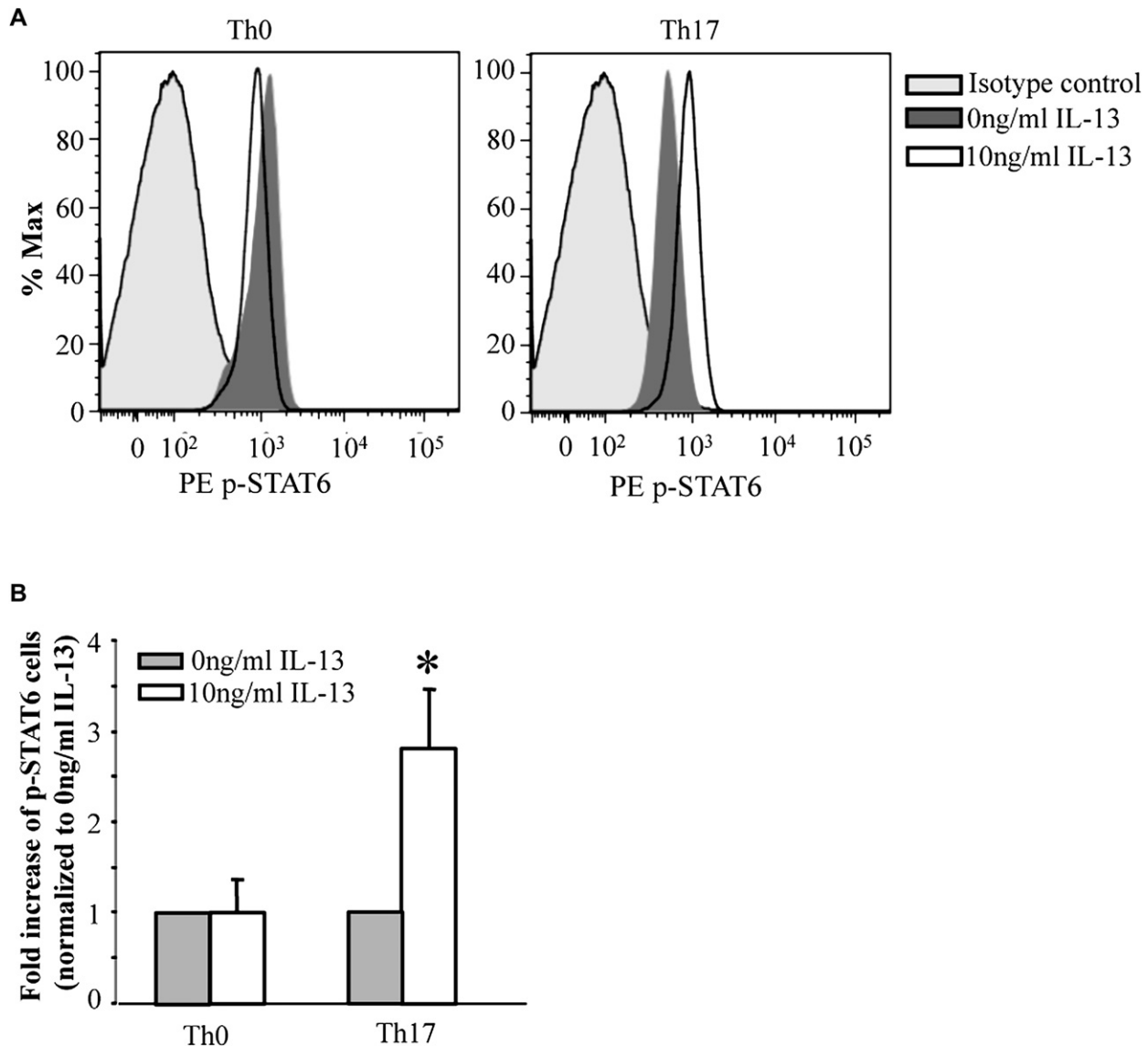


FIG 2. IL-13 increases STAT6 phosphorylation in T_H17 cells. Serum-starved T_H0 and T_H17 cells were stimulated with IL-13 (10 ng/mL) for 1 hour and examined for phospho-STAT6 (*p-STAT6*) expression by means of flow cytometry. **A**, STAT6 phosphorylation in $CD3^+CD4^+$ cells compared with the isotype control. **B**, Fold increase of phospho-STAT6 $^+$ $CD3^+CD4^+$ cells compared with 0 ng/mL IL-13 in respective cell lineages. Data are composed of 4 replicates. * $P < .05$ (ANOVA).

responsible for IL-13 α 1 expression on these cells. To test this hypothesis, we activated naive T cells in the presence of 1 or more of the cytokines necessary for T_H17 polarization (TGF- β , IL-6, IL-1 β , and/or IL-23). Although no single factor increased IL-13 α 1 expression to the level of T_H17 -polarizing conditions, the combination of TGF- β , IL-1 β , and IL-23 increased IL-13 α 1 expression significantly compared with T_H0 conditions (Fig 3). In addition, there was no difference in IL-13 α 1 expression between T_H17 -polarizing conditions and the combination of TGF- β , IL-1 β , and IL-23. These results suggest that IL-6 does not have a role in IL-13 α 1 expression on $CD4^+$ T cells.

IL-13 decreases IL-17A production from T_H17 cells

Naive $CD4^+$ T cells were polarized to T_H17 cells in the presence of IL-13 for 4 days, and supernatants were analyzed by

means of ELISA. IL-13 (10 ng/mL) significantly decreased IL-17A production (Fig 4, A), as well as IL-21 production (Fig 4, B), but IL-17A production was not attenuated when $CD4^+$ T cells were differentiated with 5 ng/mL IL-13 (data not shown). IL-13 did not decrease IL-22 production from T_H17 cells (Fig 4, C).

We also examined whether neutralizing IL-13 during polarization would increase IL-17A production from T_H17 cells. Naive T cells were polarized with T_H17 -polarizing cytokines and anti-IFN- γ and either anti-IL-4 (10 μ g/mL), anti-IL-13 (10 μ g/mL), or isotype control antibody (10 μ g/mL) was added at the time of activation. Anti-IL-13 increased IL-17A production compared with the isotype control in a similar fashion as anti-IL-4 (Fig 4, D). Lower concentrations of anti-IL-13 did not affect IL-17A production in T_H17 cells, and therefore 10 μ g/mL IL-13 antibody was used (see Fig E3 in this article's Online Repository at www.jacionline.org). Also, the baseline amount of IL-17A produced

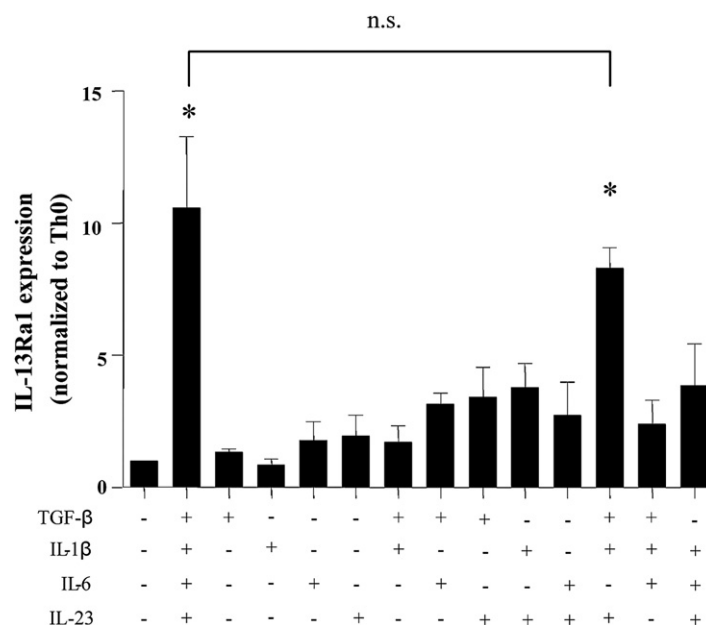


FIG 3. TGF- β , IL-1 β , and IL-23 upregulate IL-13R α 1 expression. Naive T cells were polarized by using combinations of T_H17-polarizing cytokines in the presence of anti-IL-4 and anti-IFN- γ . IL-13R α 1 expression levels determined by means of real-time PCR, normalized to glyceraldehyde-3-phosphate dehydrogenase, and compared with T_H0 cells are shown. Data are representative of 3 different experiments. *n.s.*, Not significant. **P* < .05 compared with T_H0 cells (ANOVA).

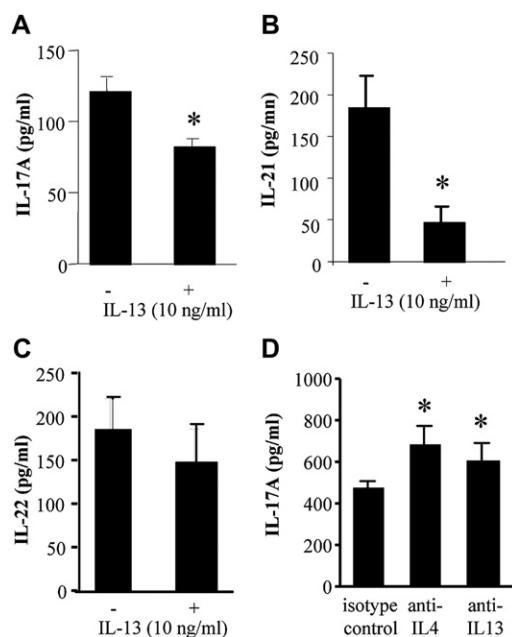


FIG 4. IL-13 attenuates T_H17 cytokine production. **A-C**, Naive T cells were polarized to become T_H17 cells in the presence of IL-13, and IL-17A, IL-21, and IL-22 levels were measured. **D**, T_H17 cells were polarized in the presence of the isotype control, anti-IL-4, or anti-IL-13 (all 10 μ g/mL), and IL-17A levels were measured. Data are from 3 experiments. **P* < .05 compared with isotype control (*t* test [Fig 4, A-C] or ANOVA [Fig 4, D]).

was greater in experiments with the IgG2a isotype control (Fig 4, D) compared with cells polarized in the absence of IgG2a antibody (Figs 4, A, and 5, A). However, the addition of either anti-IL-13 or anti-IL-4 increased IL-17A production (Fig 4,

D), showing that IL-13 negatively regulates IL-17A production in human T_H17 cells.

IL-13 specifically attenuates cytokine production from T_H17 cells but not from other T-cell subsets

Cell-culture supernatants from polarized human T cells were collected and analyzed by means of ELISA. IL-13 (10 ng/mL) attenuated IL-17A production in T_H17 cells (Fig 5, A). T_H1 production of IFN- γ and T_H2 production of IL-4 were also analyzed, and IL-13 had no effect on either IFN- γ (Fig 5, B) or IL-4 (Fig 5, C) production. iTreg cell production of IL-17A and IFN- γ was unaffected by IL-13 (Fig 5, A and B), and iTreg cells had undetectable levels of IL-4 produced (Fig 5, C). Taken together, these data show IL-13 regulates cytokine production from T_H17 cells but not from T_H1 or T_H2 cells, further supporting that only T_H17 cells express IL-13R α 1.

IL-13 attenuates STAT3 phosphorylation and RORC2, Runx1, and IRF-4 expression in T_H17 cells

Cells were activated and differentiated into T_H17 cells for 4 days in the presence or absence of IL-13, serum starved for 4 hours, and then restimulated with IL-1 β and IL-6 for 15 minutes to induce phosphorylation of STAT3. In the presence of IL-13, decreased STAT3 phosphorylation was observed compared with that seen in T_H17 cells without IL-13 added to the culture (Fig 6, A).

RORC2 is an essential transcription factor for T_H17 differentiation and cytokine production, and therefore we hypothesized that IL-13 would decrease RORC2 expression, leading to decreased IL-17A production. Western blotting was conducted, and blots are representative of 3 independent experiments. IL-13 decreased RORC2 expression in human T_H17 cells (Fig 6, B). As previously

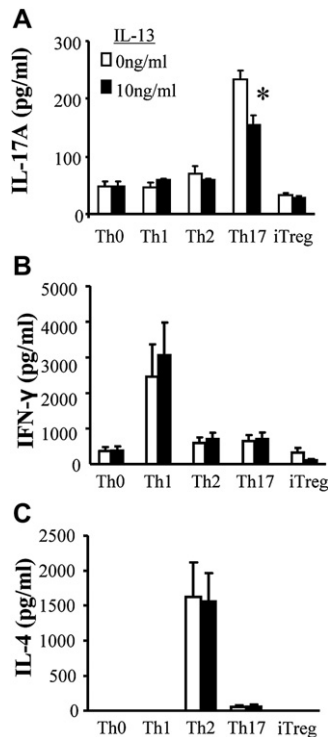


FIG 5. IL-13 attenuates IL-17A production in human T_H17 -polarized cells. $CD4^+$ T cells were polarized to become T_H0 , T_H1 , T_H2 , T_H17 , or iTreg cells after 4 days in the presence of IL-13 (10 ng/mL). Supernatants were examined by means of ELISA for cytokines. Data are composed of 5 to 6 replicates compiled from 3 independent experiments. * $P < .05$ compared with 0 ng/mL IL-13 for respective T-cell lineages (ANOVA).

mentioned, T_H17 cells have increased expression of the transcription factors IRF-4 and Runx1, both of which are upregulated during T_H17 cell differentiation.²² We determined that IL-13 decreased not only RORC2 but also Runx1 (Fig 6, C) and IRF-4 (Fig 6, D) protein expression in T_H17 -differentiated cells. These data show that IL-13 regulates IL-17A production by decreasing STAT3 phosphorylation, as well as RORC2, Runx1, and IRF-4 expression levels.

IL-13 only decreases IL-17A production within 24 hours of T_H17 cell activation and polarization

IL-13 (10 ng/mL) was added at the time of polarization, 1 day after polarization, or 2 days after polarization to determine whether IL-13 could attenuate IL-17A responses if administered after T_H17 cell polarization and activation. Cell supernatants were collected 4 days after polarization, and IL-17A levels were determined from 3 independent experiments. IL-13 attenuated IL-17A only when added at the time of polarization and had no effect on IL-17A production if administered 1 or 2 days after polarization (Fig 7, A). These data suggest that IL-13 is decreasing or inhibiting a signaling intermediate early in the differentiation of T_H17 cells.

$CD4^+$ cells were also polarized to T_H17 cells in the absence of IL-13 for 4 days and then restimulated with anti-CD3 and anti-CD28 in the presence of IL-13. When added at the time of restimulation, IL-13 inhibited IL-17A production (Fig 7, B). If T cells were replated and not restimulated with anti-CD3, then no IL-17A was detected (data not shown). Taken together, these data

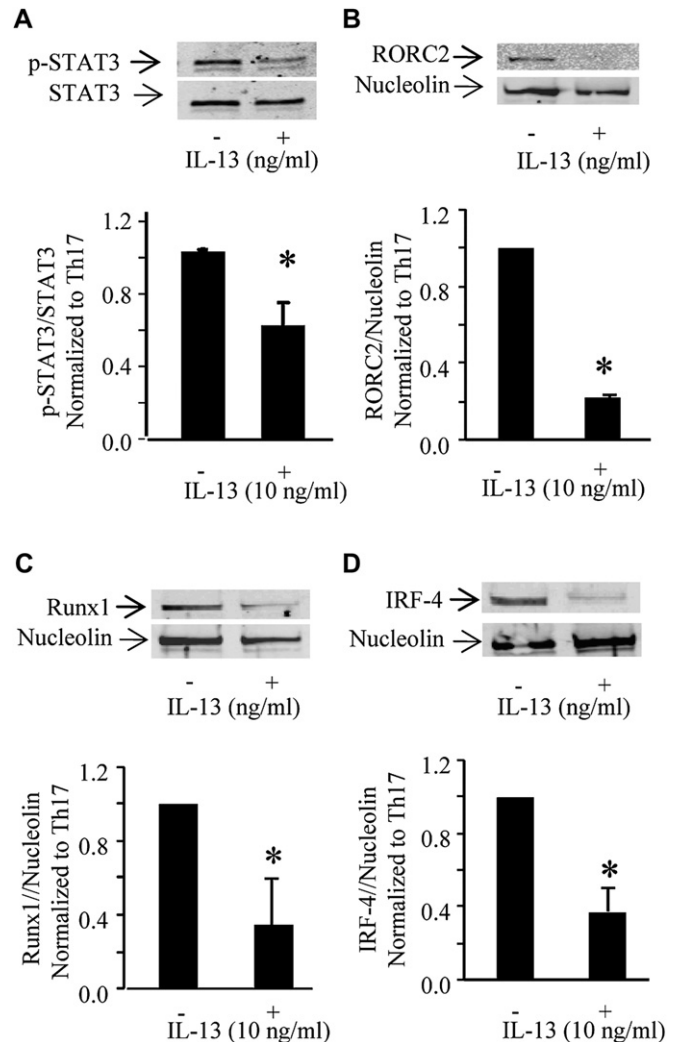


FIG 6. IL-13 attenuates expression of T_H17 transcription factors. **A**, Serum-starved T_H17 cells were stimulated with IL-1 β and IL-6 for 15 minutes, and phospho-STAT3 (p -STAT3) levels were examined. **B-D**, Nuclear protein from T_H17 cells was examined for RORC2, Runx1, and IRF-4. Densitometry was normalized to total STAT3 (Fig 6, A) or nucleolin (Fig 6, B-D) and then normalized to T_H17 cells. * $P < .05$ compared with 0 ng/mL IL-13 (t test).

suggest that IL-13 regulates IL-17A production at the time of polarization, as well as restimulation.

DISCUSSION

We have previously shown that IL-13R α 1 is expressed on murine T_H17 cells and that IL-13 attenuates IL-17A production from T_H17 cells.⁸ In this study we extend these findings to show for the first time that human T_H17 cells express IL-13R α 1 and that T_H1 , T_H2 , and iTreg cells do not. This is the first report that IL-13R α 1 is expressed in human T cells, likely because T_H17 cells were only recently discovered and IL-13R α 1 was not found in other T-cell lineages. We show IL-13R α 1 expression by means of real-time PCR and flow cytometry, revealing that it is a functional receptor because it increases phosphorylation of STAT6 in T_H17 cells but not T_H0 cells.

Very little is known about the regulation of IL-13R α 1 expression, and to our knowledge, factors regulating IL-13R α 1 have

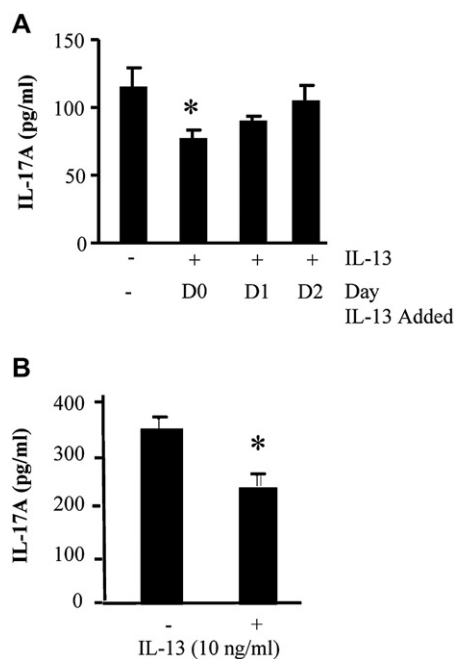


FIG 7. IL-13 decreases IL-17A levels within 24 hours of polarization or at restimulation. **A**, IL-13 was added to T_H17 cells at days 0, 1, or 2, and IL-17A levels were measured 4 days after polarization. **B**, T_H17 cells were restimulated with anti-CD3 and anti-CD28 in the presence or absence of IL-13, and IL-17A levels were measured 24 hours later. * $P < .05$ compared with T_H17 cells (ANOVA [A] or t test [B]).

only been reported for B lymphocytes, in which IL-13R α 1 levels were increased in tonsillar B cells stimulated with soluble CD40 ligand alone or in combination with IL-4 or IL-13.^{23,24} We know of no other description of the factors regulating IL-13R α 1 expression in inflammatory cells. Because only T_H17 cells and not other T-cell lineages expressed IL-13R α 1, we hypothesized that 1 or more cytokines involved in T_H17 polarization were responsible for IL-13R α 1 expression. Our data show that the combination of TGF- β , IL-1 β , and IL-23 was responsible for IL-13R α 1 expression on T cells. Although treatment with either TGF- β or IL-23 resulted in a trend toward increases in IL-13R α 1 expression, IL-1 β alone did not. Therefore the combination of all 3 cytokines being required for increased IL-13R α 1 expression was a surprising finding.

Although these experiments to determine the cytokine factors necessary for IL-13R α 1 expression were only performed in T cells, these results could possibly extend to other cell types. For instance, TGF- β levels are increased in the bronchoalveolar lavage fluid of allergic asthmatic subjects after segmental allergen challenge,²⁵ along with greater levels of IL-13. Animal models have shown that TGF- β has an important role in promoting peribronchiolar extracellular matrix deposition, airway smooth muscle cell proliferation, and mucus production in the lungs without affecting inflammation.²⁶ One mechanism by which TGF- β in combination with IL-1 β and IL-23 might affect these features of asthma could be by upregulating IL-13R α 1 expression and increasing tissue sensitivity to this cytokine. IL-1 β levels are increased in bronchoalveolar lavage fluid from subjects with stable asthma,²⁷ and IL-23 signaling was recently reported to enhance allergic airway inflammation in a murine model.²⁸ Thus the presence of these cytokines in patients with allergic airway

inflammation might positively regulate IL-13R α 1 expression. We are undertaking studies to determine whether TGF- β , IL-1 β , and IL-23 regulate IL-13R α 1 expression on airway epithelial cells, airway smooth muscle cells, and fibroblasts.

IL-13 attenuated IL-17A levels in human CD4⁺ T_H17 cells, but the amount of IL-13 necessary for inhibiting IL-17A production was greater in human subjects than in murine T_H17 cells, in which 5 ng/mL IL-13 attenuated IL-17A production.⁸ To examine the mechanism of how IL-13 decreased IL-17A, we examined the expression of the T_H17 transcription factors STAT3, RORC2, Runx1, and IRF-4. IL-13 decreased RORC2, Runx1, and IRF-4 expression, as well as decreasing STAT3 phosphorylation. This observed decrease in STAT3 phosphorylation could be accomplished by increasing suppressor of cytokine signaling 3, a known inhibitor of STAT3 in T_H17 cells,²⁹ although we have not tested this hypothesis. Further experimentation needs to be performed to determine the mechanisms of IL-13-mediated decreases in STAT3 phosphorylation. Although IL-13 decreased expression of T_H17 transcription factors and IL-21 expression, IL-13 did not attenuate IL-22 production (Fig 4, C) or IL-23R relative expression (data not shown) in human T_H17 cells. IL-23R signaling might be required for IL-22 production in mice,³⁰ and therefore one possible explanation for IL-22 expression not changing is that IL-13 does not alter IL-23R signaling, with IL-22 production therefore unchanged.

IL-13 levels are known to be increased in the sputum of patients with mild and severe asthma,³¹ and IL-13 is a mediator of mucus hyperplasia and airway hyperreactivity. Therefore IL-13 is an attractive drug target for treating allergic airway diseases, such as asthma.^{2,3} These therapeutics would block IL-13 from binding or signaling through the receptor and therefore inhibit downstream effects. Clinical trials are currently underway that block IL-13 or IL-13 signaling.³² Antibodies against IL-4R α 1 (AMG 317)³³ and IL-13 (IMA-026)³⁴ are currently in phase I and II trials. AMG 317 (75, 150, or 300 mg) was tested in patients with moderate-to-severe asthma in a randomized, double-blind, placebo-controlled study.³³ AMG 317 did not change asthma symptom scores or FEV₁, but AMG 317 (300 mg) did reduce IgE levels and reduce the number of exacerbations in patients.³³ An rIL-4 variant (pitakinra) that competitively inhibits IL-13 or IL-4 binding to the IL-4R α component of type I and type II IL-4R is also currently undergoing phase II clinical trials.³⁵ Pitakinra was tested in a randomized, double-blind, placebo-controlled study in patients with mild asthma, and it caused a smaller decrease in FEV₁ after allergen challenge.³⁵

These antibodies and small proteins that bind IL-4R α would inhibit IL-4 and IL-13 from signaling and potentially could be used for patients who do not respond well to inhaled corticosteroids or the combination of β_2 -agonists and inhaled corticosteroids. However, based on the findings of our study, we believe that blocking IL-13 in patients with T_H17 -mediated autoimmune diseases, including multiple sclerosis, could have adverse side effects by increasing IL-17A production and exacerbating a T_H17 -driven disease. Overall, this study shows that IL-13 is important in negatively regulating T_H17 cytokine production. However, further studies need to be conducted to determine the precise mechanisms for IL-13 inhibiting IL-17A production in human T_H17 cells. In contrast, IL-13 might provide a potential therapeutic for treatment of T_H17 -mediated diseases, including Crohn disease and rheumatoid arthritis, by decreasing IL-17 production. This is a very important area of research

because IL-17A levels are increased in many autoimmune diseases. Therefore the effect of determining IL-13 regulation on Th17 cell cytokine production is vast.

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Clinical implications: IL-13 is an attractive therapeutic target for decreasing symptoms associated with asthma, but blocking IL-13 in persons with Th17-mediated disease might have adverse side effects by increasing IL-17A production.

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METHODS

Flow cytometry

Human naive CD4⁺ T cells were polarized for 4 days, and 1×10^6 cells were stained for live/dead cells by using a commercially available kit (Invitrogen, Carlsbad, Calif) and washed 3 times. Cells were blocked with Fc block and stained with PE-Cy7-conjugated anti-CD4 and Pacific Blue-conjugated anti-CD3 (BD Biosciences). Cells were analyzed with an LSR II flow cytometer (BD Biosciences), and data were analyzed with Flow Jo 7.2 software (Tree Star, Inc). Histograms are gated on live CD4⁺ cells.

Quantitative PCR

cDNA was generated from total RNA, and a real-time PCR assay was conducted with TaqMan Universal primer mix and commercially available primers and probes for IL-4R α and glyceraldehyde-3-phosphate dehydrogenase (Applied Biosystems). Real-time analysis was performed on a Step-One PCR instrument (Applied Biosystems). All real-time data were normalized to glyceraldehyde-3-phosphate dehydrogenase, and relative expression is compared with that seen in either naive (T_H0) cells or T_H17 cells.

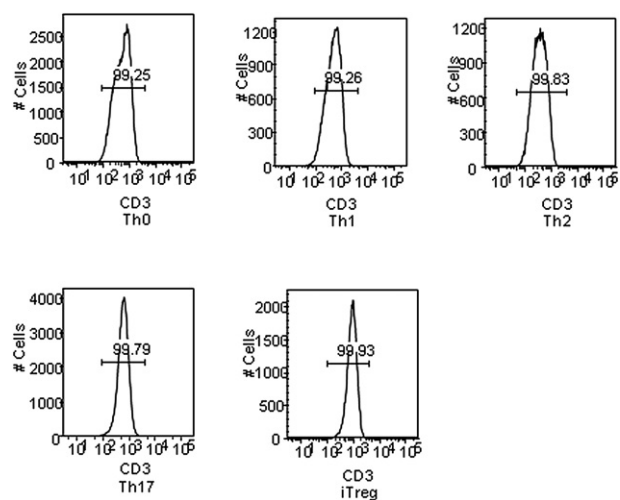


FIG E1. Isolated cells are CD3⁺CD4⁺ T cells. Histograms of cultured T cells 4 days after isolation are shown. Cells were gated on live cells that are CD4⁺ and are shown as CD3⁺ cells. Histograms are representative of 3 samples for each T-cell lineage.

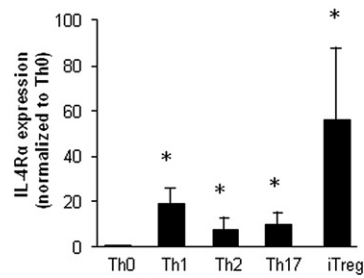


FIG E2. IL-4R α is expressed in human T_H1, T_H2, T_H17, and iTreg cells. Naive human CD4⁺ T cells were isolated from PBMCs and cultured in polarizing conditions to become naive (T_H0), T_H1, T_H2, T_H17, or iTreg cells for 4 days. Cells were lysed, and RNA was extracted. Measurement of relative expression of IL-4R α was performed by means of real-time PCR. Real-time data were normalized to glyceraldehyde-3-phosphate dehydrogenase, and relative expression is compared with that of naive (T_H0) cells. Data are composed of 5 to 6 replicates compiled from 3 independent experiments. * $P < .05$ compared with T_H0 cells (ANOVA).

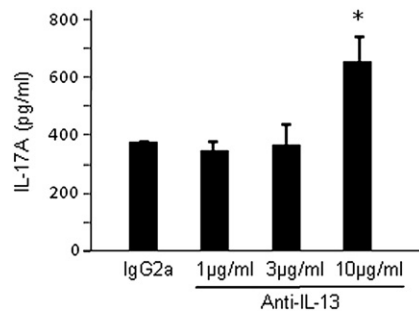


FIG E3. Naive CD4⁺ T cells were activated and polarized to T_H17 cells presence of IgG isotype control (10 µg/mL) or anti-IL-13 (1-10 µg/mL) for 4 days, and IL-17A production was measured by means of ELISA for cytokine production. Data are composed of 4 to 6 replicates. **P* < .05 compared with isotype control (ANOVA).