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Human Th17 Cells Express a Functional IL-13 Receptor and IL-13 Attenuates IL-17A Production

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Abstract

Background—IL-13 is a central mediator of airway responsiveness and mucus expression in 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 the IL-13 receptor α1 (IL-13Rα1), a subunit of the IL-13 receptor, has not previously been reported to exist on human T cells.

Objective—To determine if human CD4+ Th17 cells express IL-13Rα1 and if IL-13 regulates Th17 cytokine production.

Methods—Naïve human CD4+ cells were isolated from whole blood, activated with anti-CD3 and anti-CD28, and polarized to Th1, Th2, Th17, or induced T regulatory cells in the presence of IL-13 (0–10ng/ml). Cell supernatants, total RNA, or total protein was examined four days after Th17 polarization.

Results—Th17 cells, but not Th0, Th1, Th2 or induced T regulatory cells, expressed IL-13Rα1. IL-13 attenuated IL-17A production as well as expression of RORC2, Runx1, and IRF-4 in Th17 polarized cells. IL-13 neither inhibited IFN-γ production from Th1 cells nor inhibited IL-4 production from Th2 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-13Rα1 is expressed on human CD4+ Th17 cells, and IL-13 attenuates IL-17A production at polarization and restimulation. While 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.

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The authors have no competing interests with this study.

Author Contributions:
D.C.N. conceived the project, designed and completed experiments, analyzed data, and wrote the manuscript; M.G.B. completed experiments and helped prepare the manuscript; W.Z. was involved with experimental design and helped write the manuscript; M.M.H. completed experiments; K.G. and C.M.S. provided technical support; G.K.H, and J.K.K, provided reagents and helped revise the manuscript; R.S.P. conceived the project, designed experiments and helped write the manuscript.

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Keywords

IL-13; IL-13R; Th17; IL-17A; Asthma

Introduction

Interleukin (IL)-13 is an important T helper (Th)2 cytokine that is upregulated in 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-4R, which is composed of IL-13Rα1 and IL-4Rα subunits. The type I IL-4R is also composed of the IL-4Rα in addition to the common-gamma chain. Signaling through the type I or type II IL-4R results in phosphorylation and activation of the downstream transcription factor, STAT6.[6;7] The direct effects of IL-13 on human CD4+ T cells, however, have not been fully examined because the IL-13 receptor α1 (IL13Rα1) was not known to be expressed on human T cells.[6] We have recently shown in mouse CD4+ cells that the IL-13Rα1 subunit is expressed on Th17 cells, but not Th0, Th1, or Th2 cells. The IL-13R is functional on mouse Th17 cells, as indicated by the fact that IL-13 attenuated Th17 cytokine production, and increased STAT-6 phosphorylation.[8] IL-13 did not inhibit IFN-γ produced by Th1 cells nor inhibit IL-4 produced by Th2 cells.[8]

Th17 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 naïve T cells into Th17 cells in mice.[10;11] Human Th17 cell differentiation requires IL-6 and IL-1β and while TGF-β is not required for differentiation, it is required for expansion by inhibiting Th1 differentation.[10–14] Th17 cell differentiation requires expression of the transcription factors STAT3, RORC2, Runx1, and IRF-4[13] 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 (as reviewed in [18]). IL-21 is secreted from Th17 cells and acts in an autocrine fashion increasing IL-23R surface expression. This increased IL-23R expression allows for IL-23, produced by antigen presenting cells, to bind to the Th17 cell, further increasing RORC2 expression and production of Th17 cytokines, such as IL-17A and IL-22.[19–21]

The cytokine milieu at the time of Th17 cell differentiation dramatically affects Th17 cytokine production. In mice, IL-4 and IL-13 negatively regulate mouse CD4+ Th17 differentiation and cytokine production.[8;10] Based on our findings in mouse CD4+ T cells, we hypothesized that humans, like mice, express IL-13Rα1 and IL-13 attenuates IL-17A production. We have extended our findings in human T cells to show IL-13Rα1 surface expression on human CD4+ Th17 cells by flow cytometry. Further, we found that TGF-β, IL-1β, and IL-23 were the key components required for IL-13Rα1 expression on Th17 cells and that IL-13Rα1 was functional as it increased phosphorylation of STAT6 in Th17 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.

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Methods

Isolation of human T cells

All studies were approved by the Institutional Review Board at Vanderbilt University Medical Center. Human peripheral blood mononuclear cells were isolated from the buffy coats of healthy donors by Ficoll-paque Plus (GE Healthcare, Piscataway, NJ). Naïve CD4+ T cells were isolated from the PBMCs using a naïve T cell isolation kit (Miltenyi, Biotec, Auburn, CA). 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. Naïve CD4+ T cells were able to flow through the column, and these cells were collected. CD4+ naïve T cells were activated using an activation/expansion kit (Miltenyi) 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 Th1, Th2, Th17, or induced T regulatory (iTregs) cells in RPMI containing 10% FBS, 1% penicillin/streptomycin, 2mM L-glutamine, 10mM HEPES, 0.1mM non-essential amino acids, and 1mM sodium pyruvate (Gibco, Carlsbad, CA). For Th1 polarization, naïve T cells were stimulated in the presence of rhIL-2 (10ng/ml), rhIL-12 (10ng/ml), and anti-IL-4 (10μg/ml). For Th2 polarization naïve T cells were stimulated in the presence of rhIL-2, rhIL-4 (10ng/ml), and anti-IFN-γ (10μg/ml). For Th17 cells, naïve T cells were stimulated in the presence of rhIL-2, rhIL-1β (10ng/ml), rhTGF-β (1ng/ml), rhIL-6 (10ng/ml), rhIL-23 (10ng/ml), anti-IFN-γ, and/or anti-IL-4. For iTreg differentiation naïve T cells were stimulated in the presence of rh-IL-2 and rhTGF-β (5ng/ml). Recombinant IL-13 (0–10ng/ml) was added to select wells at the time of polarization. In some experiments Th17 cells were polarized using conditions above except anti-IL-13 (10μg/ml) or IgG2a isotype control (10μg/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. To stimulate effector cytokine production, the cells were restimulated with anti-CD3 for 24h in the presence of rhIL-4 or rhIL-13 (0 ng/ml to 10 ng/ml). All antibodies and cytokines except rhTGF-β were purchased from R & D systems (Minneapolis, MN). 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 using TaqMan Universal primer mix and commercially available primers and probes for IL-13Rα1 and GAPDH (Applied Biosystems, Foster City, CA). Real-time analysis was performed on a Step-One PCR instrument (Applied Biosystems). All real-time data was normalized to GAPDH and relative expression is compared to either Th0 cells or Th17 cells.

Cytokine measurements

Cytokine levels were measured from cell culture supernatants with available Duoset or Quantikine ELISA kits (R & D Systems) following the manufacturer's instructions. Any value below the limit of detection (B.L.D.) was assigned half the value of the lowest detectable standard.

Immunoblotting

Cells were lysed using modified RIPA (radioimmunoprecipitation assay) buffer containing PMSF and 1% proteinase inhibitors and total protein was extracted and resolved by 10% SDS-PAGE gels, transferred to a nitrocellulose membranes (BioRad, Hercules, CA), and probed with antibodies against RORC2, nucleolin, or actin all from Santa Cruz Biotechnology (Santa Cruz, CA) or phospho-STAT3, STAT3, IRF-4, or Runx1 (Cell

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Signaling, Beverly, MA). Signals were amplified and visualized with infrared IRDy3-labeled secondary antibodies (LI-COR, Lincoln, NE) and Odyssey infrared imaging system (LI-COR). Densitometry was performed using Odyssey software (LI-COR).

Flow cytometry and intracellular staining

Four days after polarization, Th0 and Th17 CD4+ T cells were harvested and cells were blocked with anti-FcR Ab (BD Biosciences) and in some experiments surface stained with APC- conjugated anti-CD4 (BD Biosciences), pacific blue conjugated anti-CD3, and in select experiments PE conjugated IL-13Rα1 (R and D Systems). For experiments examining STAT6 phosphorylation, cells were serum starved for 4 hours and select wells were stimulated with IL-13 (10ng/ml) for 1 hour. Cells were blocked with anti-FcR antibody and surface stained with APC- conjugated anti-CD4 (BD Biosciences) and pacific blue conjugated anti-CD3. Cells were then permeabolized 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 using a LSR II flow cytometer (BD Biosciences), and data were analyzed using Flow Jo 7.2 software.

Statistical analyses

Data are presented as mean ± SEM. Data were analyzed with ANOVA followed by the Tukey posthoc test or a 2-tailed T-test using GraphPad Prism version 4 with values being considered significant when $p < 0.05$

Results

A functional IL-13 receptor is expressed on human Th17 cells

Naïve human CD4+ T cells were activated with anti-CD3 and anti-CD28 and polarized to become Th1, Th2, Th17, or iTreg cells. Total cellular RNA was collected 4 days after polarization and analyzed for IL-13Rα1 by real-time PCR (Figure 1A). To examine surface expression of IL-13Rα1, flow cytometry was conducted and cells were gated on CD3+, CD4+ cells. IL-13Rα1 expression was significantly increased on Th17 cells both by percentages (Figure 1B) as well as total number of CD3+, CD4+, IL-13Rα1+ cells (Figure 1C), but not Th0, Th1, Th2, or iTreg cells. IL-13Rα1 message levels were slightly increased in Th1 cells and IL-13Rα1 surface expression was slightly increased in Th2 cells. However, these increases were not statistically significant. Since 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 flow cytometry and sorted on live cells and all T cell lineages contained greater than 99% of CD3+ CD4+ cells (Supplemental Figure 1). The other subunit of the IL-13 receptor, IL-4Rα, was also expressed on Th17 cells and other T cell lineages (Supplemental Figure 2), showing that both components of the IL-13 receptor are expressed on human Th17 cells. Membrane bound IL-13Rα2 was not upregulated in Th17 cells as determined by real-time PCR (data not shown).

We further hypothesized that if IL-13Rα1 is expressed on human Th17 cells then the downstream transcription factor, STAT6, would become phosphorylated in the presence of IL-13. To test this hypothesis, we polarized naïve human T cells under Th17 differentiating conditions for 4 days. On day 4, we serum starved the cells for 4 hours and restimulated the cells for 1 hour with IL-13 (0–10ng/ml). Cells were collected and STAT6 phosphorylation was analyzed by intracellular cytokine staining using a PE-conjugated phospho-STAT6 antibody or IgG1κ isotype control (dashed line) and flow cytometry with histograms gated for CD3+, CD4+ cells. As shown in Figure 2A, Th17 cells with IL-13 stimulus had an increase in STAT6 phosphorylation (right panel) compared to Th0 cells with IL-13 (left panel) or Th17 cells with no IL-13 added (right panel, gray shaded curve). CD3+, CD4+,

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phospho-STAT6+ cells were calculated and normalized no IL-13 stimulation (Figure 2B), and Th0 cells showed no increase in STAT6 phosphorylation, but Th17 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 Th17 cells, but not Th1, Th2 nor iTreg cells, express a functional IL-13Rα1. We therefore hypothesized that one or more of the cytokines involved in the Th17 polarization process is responsible for IL-13Rα1 expression on these cells. To test this hypothesis we activated naïve T cells in the presence of one or more of the cytokines necessary for Th17 polarization (TGF-β, IL-6, IL-1β, and/or IL-23). While no single factor increased IL-13Rα1 expression to the level of Th17 polarizing conditions, the combination of TGF-β, IL-1β, and IL-23 increased IL-13Rα1 expression significantly compared to Th0 conditions (Figure 3). In addition, there was no difference in IL-13Rα1 expression between Th17 polarizing conditions and the combination of TGF-β, IL-1β, and IL-23. These results suggest that IL-6 does not have a role in IL-13Rα1 expression on CD4+ T cells.

IL-13 decreases IL-17A production from Th17 cells

Naïve CD4+ T cells were polarized to Th17 cells in the presence of IL-13 for 4 days and supernatants were analyzed by ELISA. IL-13 (10ng/ml) significantly decreased IL-17A production (Figure 4A) as well as IL-21 production (Figure 4B), but IL-17A production was not attenuated when CD4+ T cells were differentiated with 5ng/ml of IL-13 (data not shown). IL-13 did not decrease IL-22 production from Th17 cells (Figure 4C).

We also examined if neutralizing IL-13 during polarization would increase IL-17A production from Th17 cells. Naive T cells were polarized with Th17 polarizing cytokines and anti-IFN-γ and either anti-IL4 (10μg/ml), anti-IL13 (10μg/ml), or isotype control antibody (10μg/ml) were added at the time of activation. Anti-IL-13 increased IL-17A production compared to isotype control in a similar fashion as anti-IL-4 (Figure 4D). Lower concentrations of anti-IL-13 did not affect IL-17A production in Th17 cells and therefore 10μg/ml of IL-13 antibody was used (Supplemental Figure 3). Also, the baseline amount of IL-17A produced was greater in experiments with IgG2a isotype control (Figure 4D) compared to cells polarized in the absence of IgG2a antibody (Figures 4A and 5A). However, the addition of either anti-IL-13 or anti-IL-4 increased IL-17A production (Figure 4D) showing that IL-13 negatively regulates IL-17A production in human Th17 cells.

IL-13 specifically attenuates cytokine production from Th17 cells, but not from other T cell subsets

Cell culture supernatants from polarized human T cells were collected and analyzed by ELISA. IL-13 (10ng/ml) attenuated IL-17A production in Th17 cells (Figure 5A). Th1 production of IFN-γ and Th2 production of IL-4 were also analyzed, and IL-13 had no effect on either IFN-γ (Figure 5B) or IL-4 production (Figure 5C). iTreg production of IL-17A and IFN-γ was unaffected by IL-13 (Figure 5A and 5B), and iTreg cells had undetectable levels of IL-4 produced (Figure 5C). Taken together these data show IL-13 regulates cytokine production from Th17 cells, but not from Th1 or Th2 cells, further supporting that only Th17 cells express IL-13Rα1.

IL-13 attenuates STAT3 phosphorylation and RORC2, Runx1, and IRF-4 expression in Th17 cells

Cells were activated and differentiated into Th17 cells for 4 days in the presence or absence of IL-13, serum starved for 4 hours, and then restimulated with IL-1β and IL-6 for 15 minutes to induce phosphorylation of STAT3. In the presence of IL-13, decreased STAT3

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phosphorylation was observed compared to Th17 cells without IL-13 added to the culture (Figure 6A).

RORC2 is an essential transcription factor for Th17 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 Th17 cells (Figure 6B). As previously mentioned, Th17 cells have increased expression of the transcription factors IRF-4 and Runx1, both of which are upregulated during Th17 cell differentiation.[22] We determined that IL-13 decreased not only RORC2, but also Runx1 (Figure 6C) and IRF-4 (Figure 6D) protein expression in Th17 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 Th17 cell activation and polarization

To determine if IL-13 could attenuate IL-17A responses if given after Th17 cell polarization and activation, IL-13 (10ng/ml) was added at the time of polarization, 1 day following polarization, or 2 days following polarization. Cell supernatants were collected 4 days after polarization and IL-17A levels were determined from three independent experiments. IL-13 attenuated IL-17A only when added at the time of polarization, and had no effect on IL-17A production if given 1 or 2 days after polarization (Figure 7A). These data suggest that IL-13 is decreasing or inhibiting a signaling intermediate early in the differentiation of Th17 cells.

CD4+ cells were also polarized to Th17 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 (Figure 7B). If T cells were replated and not restimulated with anti-CD3 then no IL-17A was detected (data not shown). Taken together these data suggest that IL-13 regulates IL-17A production at the time of polarization, as well as restimulation.

Discussion

We have previously shown that IL-13Rα1 is expressed on mouse Th17 cells and that IL-13 attenuates IL-17A production from Th17 cells.[8] In this study we extend these findings to show for the first time that human Th17 cells express IL-13Rα1; and that Th1, Th2, or iTreg cells do not. This is the first report that IL-13Rα1 is expressed in human T cells likely because Th17 cells were only recently discovered and IL-13Rα1 was not found in other T cell lineages. We show IL-13Rα1 expression by real-time and flow cytometry and that it is a functional receptor as it increases phosphorylation of STAT6 in Th17 cells but not Th0 cells.

Very little is known about the regulation of IL-13Rα1 expression, and to our knowledge factors regulating IL-13Rα1 have only been reported for B lymphocytes where IL-13Rα1 levels were increased in tonsillar B cells stimulated with soluble CD40L alone or in combination with IL-4 or IL-13.[23;24] We know of no other description of the factors regulating IL-13Rα1 expression in inflammatory cells. Since only Th17 cells, and not other T cell lineages, expressed IL-13Rα1, we hypothesized that one or more cytokines involved in Th17 polarization were responsible for IL-13Rα1 expression. Our data show that the combination of TGF-β, IL-1β, and IL-23 were the cytokines responsible for IL-13Rα1 expression on T cells. While treatment with either TGF-β or IL-23 trended for increases in IL-13Rα1 expression, IL-1β alone did not. Therefore, the combination of all three cytokines required for increased IL-13Rα1 expression was a surprising finding.

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While these experiments to determine the cytokine factors necessary for IL-13Rα1 expression were only performed in T cells, these results could possibly extend to other cell types. For instance, TGF-β is increased in the bronchoalveolar lavage fluid of allergic asthmatics following segmental allergen challenge,[25] along with greater levels of IL-13. Animal models have shown that TGF-β has an important role in promoting peribronchialar 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-1β and IL-23 might affect these features of asthma could be by upregulating IL-13Rα1 expression and increasing tissue sensitivity to this cytokine. IL-1β is increased in the BAL fluid from stable asthmatics[27] and IL-23 signaling was recently reported to enhance allergic airway inflammation in a mouse model.[28] Thus, the presence of these cytokines in allergic airway inflammation may positively regulate IL-13Rα1 expression. We are undertaking studies to determine if TGF-β, IL-1β, and IL-23 regulate IL-13Rα1 expression on airway epithelial cells, airway smooth muscle cells, and fibroblasts.

IL-13 attenuated IL-17A levels in human CD4+ Th17 cells, but the amount of IL-13 necessary for inhibiting IL-17A production was greater in humans than in mouse Th17 cells, in which 5ng/ml of IL-13 attenuated IL-17A production.[8] To examine the mechanism of how IL-13 decreased IL-17A we examined the expression of the Th17 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 (SOCS) 3, a known inhibitor of STAT3 in Th17 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. While IL-13 decreased expression of Th17 transcription factors and IL-21 expression, IL-13 did not attenuate IL-22 production (Figure 4C) or IL-23R relative expression (data not shown) in human Th17 cells. IL-23R signaling maybe 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 and therefore IL-22 production is unchanged.

IL-13 is 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-4Rα1 (AMG 317)[33] and IL-13 (IMA-026)[34] are currently in phase I and phase II trials. AMG 317 (75mg, 150mg, or 300mg) was tested in patients with moderate to severe asthma in a randomized, double-blinded placebo-controlled study.[33] AMG 317 did not change asthma symptom score nor FEV_1, but AMG 317 (300mg) did reduce IgE levels as well as reduce the number of exacerbations in patients.[33] A recombinant human IL-4 variant (pitrakinra) that competitively inhibits IL-13 or IL-4 binding to the IL-4Rα component of the type I and type II IL-4R is also currently undergoing Phase II clinical trials.[35] Pitrakinra was tested in a randomized, double-blinded placebo-controlled study in patients with mild asthma and pitrakinra caused a smaller decrease in FEV_1 after allergen challenge.[35]

These antibodies and small proteins that bind IL-4Rα 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 beta2- agonists and inhaled corticosteroids. However, based on the findings of our study we believe that blocking IL-13 in people with Th17-mediated autoimmune diseases, including multiple sclerosis, could have adverse side effects by increasing IL-17A production, and exacerbating a Th17 driven disease. Overall, this

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study shows that IL-13 is important in negatively regulating Th17 cytokine production. However, further studies need to be conducted to determine the precise mechanisms for IL-13 inhibiting IL-17A production in human Th17 cells. In contrast, IL-13 may provide a potential therapeutic for treatment of Th17 mediated diseases, including Crohn's disease and rheumatoid arthritis, by decreasing IL-17 production. This is a very important area of research since IL-17A is increased in many autoimmune diseases. Therefore, the impact of determining IL-13 regulation on Th17 cell cytokine production is vast.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

B.L.D	below the limit of detection
IFN	interferon
IL	interleukin
IRF	interferon regulatory factor
iTreg	induced T regulatory cells
PBMC	peripheral blood mononuclear cells
PMSF	phenylmethylsulfonyl fluoride
R	receptor
RIPA	**r**adioimmunoprecipitation assay
RORC2	retinoic acid related orphan receptor
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
STAT	signal transducers and activators of transcription
TGF	transforming growth factor
Th	T-helper

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Key Implications

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

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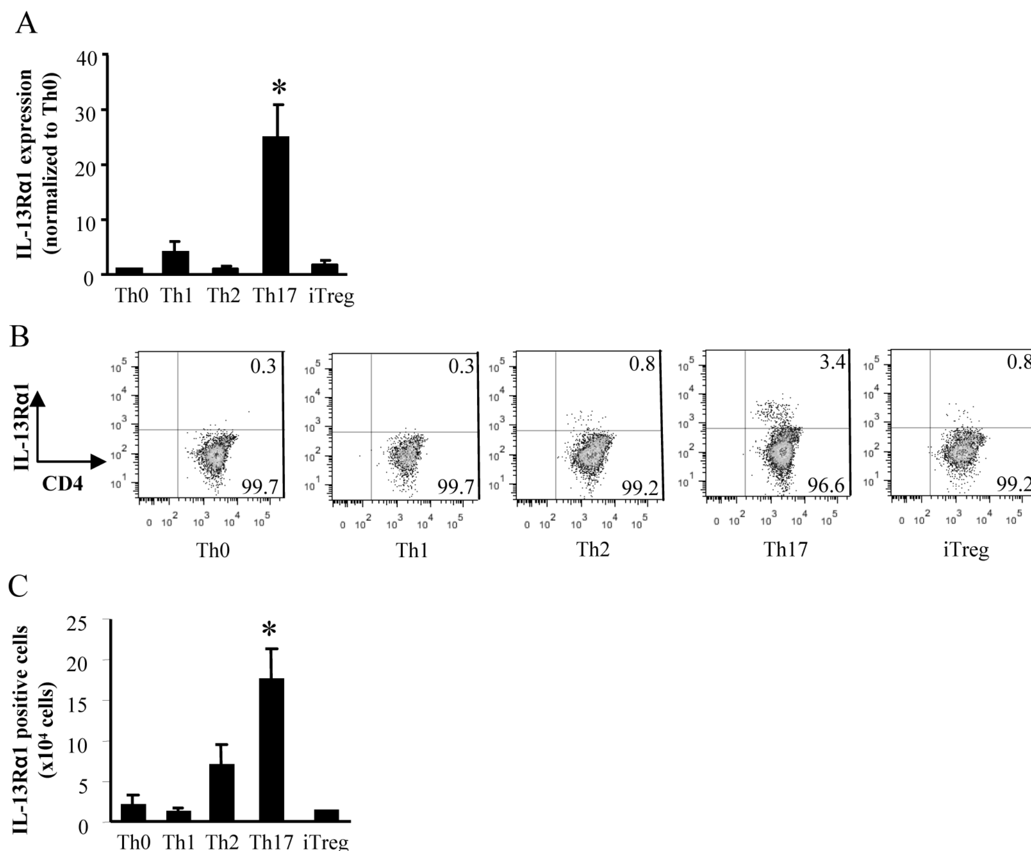

FIG. 1.

IL-13Rα1 expression on human Th17 cells. CD4+ T cells were polarized to Th1, Th2, Th17 or iTregs. **(A)**, IL-13Rα1 relative expression normalized to GAPDH and compared to Th0 cells. **(B)**, Dot plot of IL-13Rα1 surface expression on CD4+, CD3+ cells **(C),** total CD4+, CD3+ IL-13Rα1+ cells. n=5–6 from 3 experiments, *$p<0.05$ compared to Th0, ANOVA.

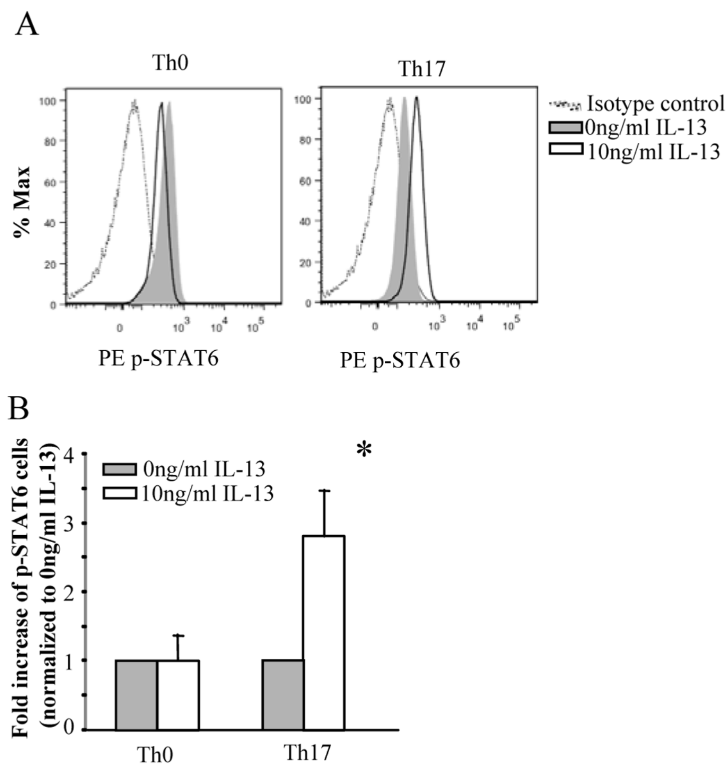

FIG. 2.

IL-13 increases STAT6 phosphorylation in Th17 cells. Serum-starved Th0 and Th17 cells were stimulated with IL-13 (10ng/ml) for 1 h and examined for phospho-STAT6 by flow cytometry. **(A).** STAT6 phosphorylation in CD3+, CD4+ cells compared to isotype control. **(B).** Fold increase of phospho-STAT6+, CD3+, CD4+ cells compared to 0ng/ml IL-13 in respective cell lineage. $n = 4$; *p<0.05, ANOVA

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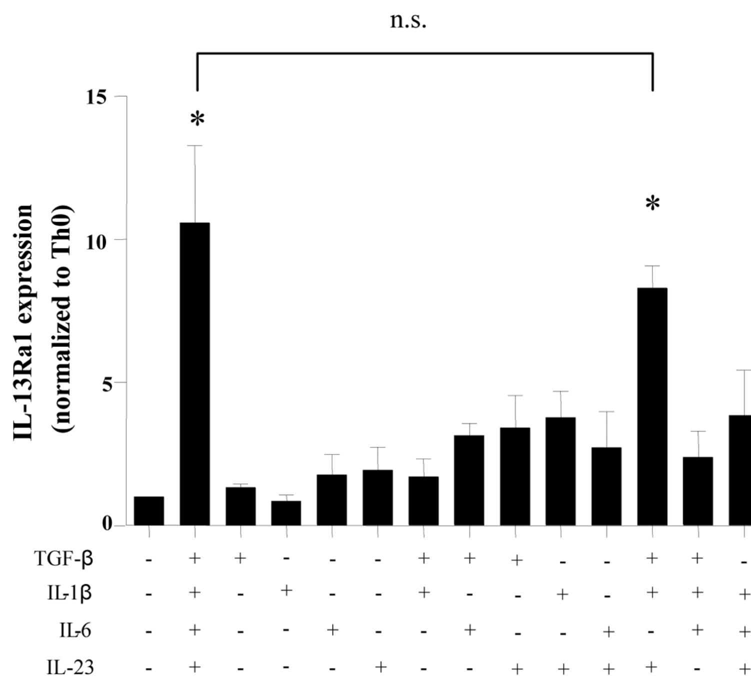

FIG. 3.

TGF-β, IL-1β, and IL-23 upregulate IL-13Rα1 expression. Naïve T cells were polarized using combinations of Th17 polarizating cytokines in the presence of anti-IL-4 and anti-IFN-γ. IL-13Rα1 expression levels determined by real-time PCR, normalized to GAPDH, and compared to Th0 cells. Data is representative from 3 different experiments, * $p<0.05$ compared to Th0, ANOVA.

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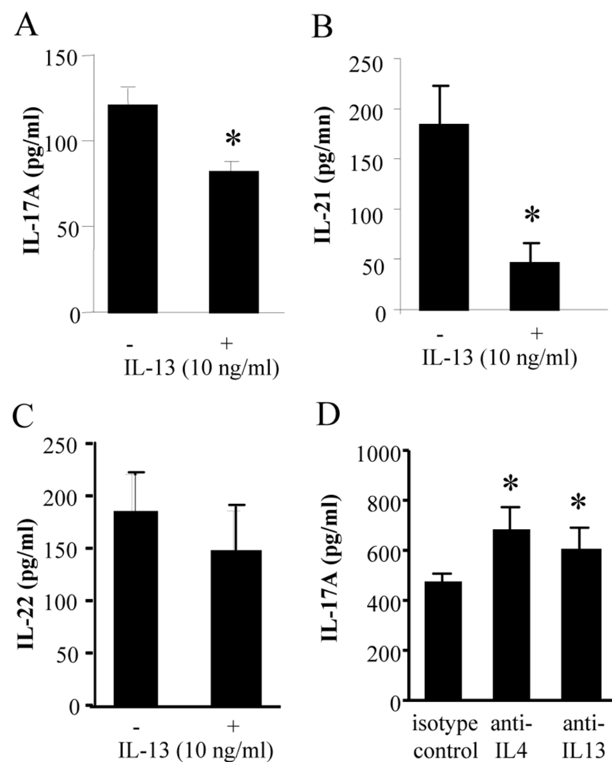

FIG. 4.

IL-13 attenuates Th17 cytokine production. **(A–C),** Naive T cells were polarized to become Th17 cells in the presence of IL-13 and IL-17A, IL-21 and IL-22 was measured. **(D),** Th17 cells were polarized in the presence of isotype control, anti-IL4 or anti-IL13 (all 10μg/ml) and IL-17A was measured. Data is from 3 experiments, * $p<0.05$ compared to isotype control, t-test (A–C) or ANOVA (D).

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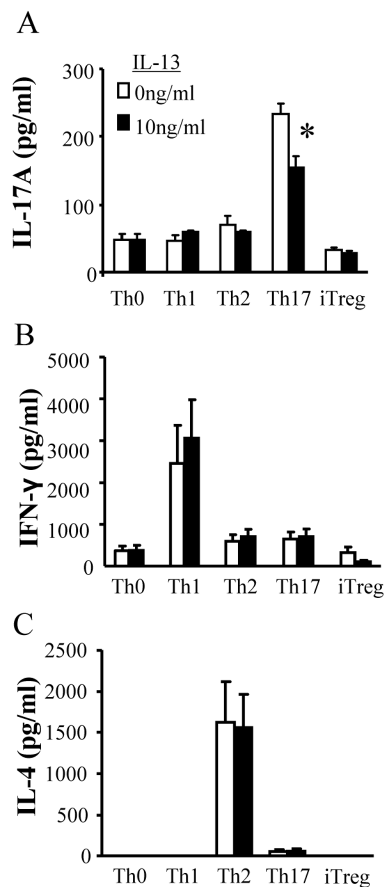

FIG. 5.

IL-13 attenuates IL-17A production in human Th17 polarized cells. CD4+ T cells were polarized to become Th0, Th1, Th2, Th17 or iTreg 4 days in the presence of IL-13 (10ng/ml). **(A–C),** Supernatants were examined by ELISA for cytokines. n= 5–6 replicates from 3 experiments, * $p<0.05$ compared to 0ng/ml of IL-13 for respective T cell lineage, ANOVA.

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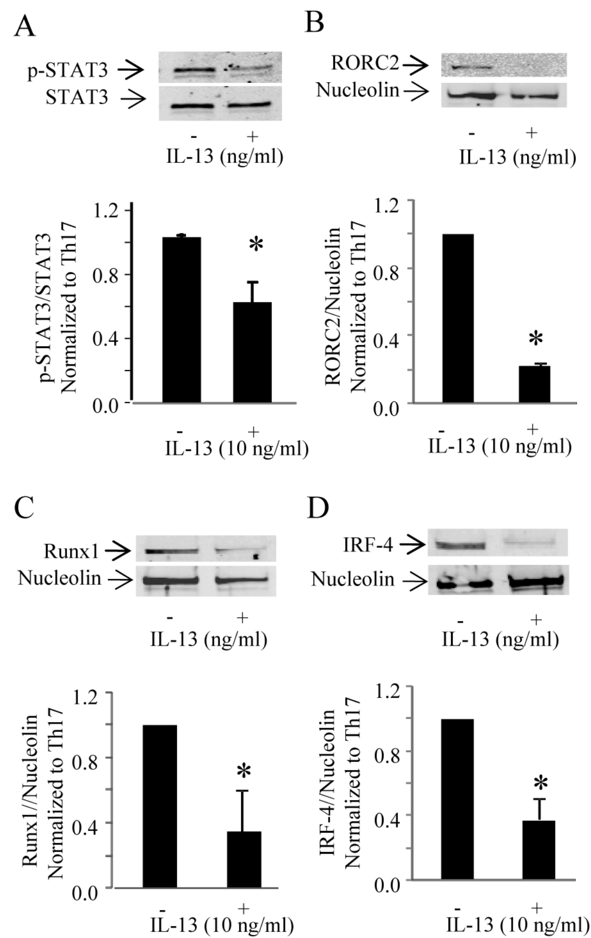

FIG. 6.

IL-13 attenuates expression of Th17 transcription factors. **(A),** Serum starved Th17 cells were stimulated with IL-1β and IL-6 for 15 min and phospho-STAT3 levels were examined. **(B–D),** Nuclear protein from Th17 cells were examined for RORC2, Runx1, and IRF-4. Densitometry was normalized to total STAT3 (A) or nucleolin (B–D) and then normalized to Th17 cells. * $p<0.05$ compared to 0ng/ml of IL-13, t-test.

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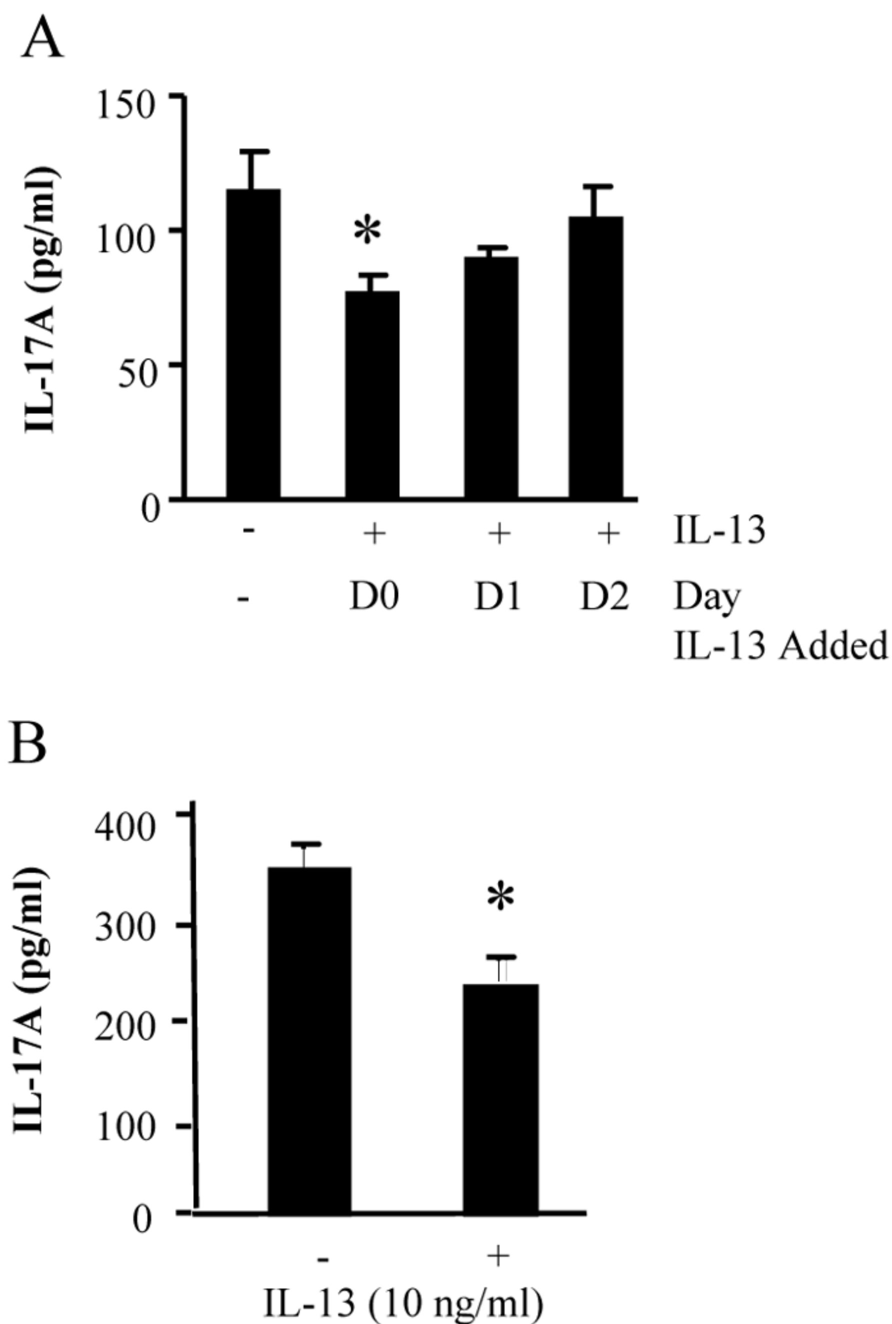

FIG. 7.

IL-13 decreases IL-17A within 24 h of polarization or at restimulation. **(A).** IL-13 was added to Th17 cells at day 0, 1, or 2 and IL-17A levels were measured 4 d after polarization. **(B).** Th17 cells were restimulated with anti-CD3 and anti-CD28 in the presence or absence of IL-13 and IL-17A was measured 24 h later. * $p<0.05$ compared to Th17, ANOVA (A) or t-test (B).