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

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Capsule Summary

IL-17 and TNF α synergistically induce surface expression of IL-13R α 2 on primary lung fibroblasts, rendering them unresponsive to IL-13. Neutralizing antibodies to IL-13R α 2 restored IL-13-mediated signaling and transcriptome studies confirmed IL-13R α 2 is an IL-13 decoy receptor.

Keywords

Severe asthma; IL-13; IL-13R α 2; IL-17; inflammation

To the Editor:

IL-13 is a pleiotropic cytokine that provokes diverse pathophysiological outcomes. While its effect during gastrointestinal (GI) helminth infection is prototypic of a protective Th2 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 Th2 inflammation including *CCL26*, *CCL11*, *POSTN*, and *MUC5AC* (2).

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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 *IL13RA2* transcript and therefore IL-13R α 2 is only expressed 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 (NLF) were grown to confluence and incubated with TNF α , IL-4 and IL-17 for 72 hours, at which time cells lysates were analyzed by qPCR with primers specific for *IL13RA2* (Methods in Online Repository). While TNF α and IL-4 synergistically induced *IL13RA2* as described (6), a combination of TNF α and IL-17 induced higher expression (Fig 1A). In contrast, *IL13RA1* decreased in the presence of TNF α (Fig 1B).

IL-13R α 2 mRNA progressively increased over time, peaking at 72 hours (Fig 1C) and longer incubations did not consistently result in any further increase (data not shown); therefore, 72 hrs was chosen as the ideal endpoint for subsequent experiments. To determine whether IL-13R α 2 expression was sustainable, NLF 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. Upon removal of TNF α and IL-17, IL-13R α 2 expression declined between 24 hours and 72 hours post-wash, though remained elevated (Fig 1D). We confirmed that primary lung fibroblasts derived from healthy adult donors (ALF) also manifested similar synergy to TNF α and IL-17 (Fig E1). Using flow cytometry, we verified that the transcriptional induction of IL-13R α 2 was associated with augmented surface expression of protein (Fig 1E-F). In order to ascertain if 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 1G).

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 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 real time quantitative PCR for *CCL26* (eotaxin-3) expression, as a marker for the canonical IL-13 signaling through IL-13R α 1. Whereas the expression of *CCL26* increased in a dose-dependent fashion upon IL-13 stimulation in control wells where IL-13R α 2 was at baseline levels, *CCL26* expression was abrogated in conditions where IL-13R α 2 expression was augmented (Fig 2A-B). Similar data were obtained with ALF (Fig E2).

While 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 down-regulation of IL-13R α 1 or dampening of intracellular signaling networks could have also led to the attenuation of IL-13 bioactivity following TNF/IL-17

treatment. To investigate these possibilities, NLF were first incubated with TNF/IL-17 to induce high IL-13R α 2 expression and prior to the addition of IL-13, some wells were treated with a highly selective neutralizing anti-human IL-13R α 2 monoclonal antibody. Subsequently, wells were treated with IL-13 in increasing concentrations for 24 hours and *CCL26* expression was assayed by qPCR. As before, in wells expressing basal levels of IL-13R α 2, *CCL26* was induced in a dose-dependent fashion following IL-13 stimulation. In wells where 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 restoration of *CCL26* expression in a dose-dependent fashion, confirming that the diminution of IL-13 activity by TNF/IL-17 was due to increased expression of IL-13R α 2 (Fig 2C).

While 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 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 NLF with or without TNF/IL17 pretreatment, in the presence or absence of the anti-IL-13R α 2 mAb were 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 (i.e. $2^{3.6}$, $q\text{-value}=6\times 10^{-8}$) over baseline (Fig 2D and E3). Upon TNF/IL17 pretreatment, this diminished to 3.2-fold ($2^{1.7}$), in accord with the above-described IL-13R α 2 mediated inhibition. Surprisingly however, while 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 2D and E3) that were distinguishable from the pretreatment controls. Blocking IL-13R α 2 with the Ab restored IL-13-induced *CCL26* expression (Fig 2D and E3). These data indicate that in NLF, 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 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 activity (9) and therefore may not benefit from blockade of IL-13, and perhaps the mechanisms we describe above already operate in these patients to quench endogenous IL-13 activity via IL-13R α 2. Indeed, due caution must be exercised, as blocking IL-13/IL-13R α 1 interactions in these patients might actually lead to disease aggravation, as IL-13 has been shown to down modulate inflammation induced by TNF α and IL-17 (10).

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Fig 1.

TNF α and IL-17 synergistically induce IL-13R α 2. Primary NLF (A, B) were incubated with specified cytokines for 72 hr. Kinetics of *IL13RA2* expression in NLF, assayed at 24, 48 or 72 hr post stimulation (C) and decay post-cytokine withdrawal (D). Flow cytometric measurement of IL-13R α 1 and IL-13R α 2 on ALF surface post cytokine stimulation (E, F). Expression of *Il13ra1* and *Il13ra2* transcripts in mouse lungs following TNF α and IL-17 administration (G).

Fig 2.

NLF were treated with TNF α and IL-17 for 72 hr, washed and then treated with IL-13 in increasing concentrations (0.1 to 2.5 ng/ml) for 24 hours. Increased *IL13RA2* induction is associated with loss of IL-13 induced *CCL26* production (A-B). Recovery of IL-13 induced *CCL26* expression by blocking IL-13Ra2 with mAb (C). Pairwise analyses of transcriptome signature of NLF treated with IL-13 $\pm$ TNF/IL-17 pretreatment (PreTx), $\pm$ anti-IL-13Ra2 mAb (D). Also refer to E Fig 3.