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TNFα/IL-17 synergy inhibits IL-13 bioactivity via IL-13Rα2 induction

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Capsule Summary

IL-17 and TNFα synergistically induce surface expression of IL-13Rα2 on primary lung fibroblasts, rendering them unresponsive to IL-13. Neutralizing antibodies to IL-13Rα2 restored IL-13-mediated signaling and transcriptome studies confirmed IL-13Rα2 is an IL-13 decoy receptor.

Keywords

Severe asthma; IL-13; IL-13Rα2; 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-13Rα1/IL-4Rα heterodimer to induce several genes specific to Th2 inflammation including CCL26, CCL11, POSTN, and MUC5AC (2).
IL-13Rα2 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-13Rα2 is only expressed as a cell surface protein (3). The factors that regulate the expression of IL-13Rα2 are unclear and the biological function of the endogenously expressed IL-13Rα2 remains controversial (4, 5). In this study, we show that the inflammatory cytokines TNFα and IL-17, often associated with severe asthma, synergize to induce IL-13Rα2 in primary human lung fibroblasts and mouse lungs, and evaluate its biological role using a novel IL-13Rα2 blocking antibody.

Primary human neonatal lung fibroblasts (NLF) were grown to confluence and incubated with TNFα, 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 TNFα and IL-4 synergistically induced IL13RA2 as described (6), a combination of TNFα and IL-17 induced higher expression (Fig 1A). In contrast, IL13RA1 decreased in the presence of TNFα (Fig 1B). IL-13Rα2 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-13Rα2 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 TNFα and IL-17, IL-13Rα2 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 TNFα and IL-17 (Fig E1). Using flow cytometry, we verified that the transcriptional induction of IL-13Rα2 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-13Rα2 transcripts were strikingly higher in the lungs of mice that received both TNF and IL-17, while IL-13Rα1 message changed negligibly (Fig 1G).

Next, we examined whether TNF/IL-17-induced expression of IL-13Rα2 was capable of altering the biological effects of IL-13. NLFs were incubated with TNF/IL-17 for 72 hours to upregulate IL-13Rα2. 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-13Rα1. Whereas the expression of CCL26 increased in a dose-dependent fashion upon IL-13 stimulation in control wells where IL-13Rα2 was at baseline levels, CCL26 expression was abrogated in conditions where IL-13Rα2 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-13Rα2 was likely due to the IL-13Rα2 receptor functioning as a decoy, a number of other alternative explanations, such as concomitant down-regulation of IL-13Rα1 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-13Ra2 expression and prior to the addition of IL-13, some wells were treated with a highly selective neutralizing anti-human IL-13Ra2 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-13Ra2, CCL26 was induced in a dose-dependent fashion following IL-13 stimulation. In wells were IL-13Ra2 was induced with TNF/IL-17, CCL26 expression was suppressed. However, in wells treated with anti-IL-13Ra2 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-13Ra2 (Fig 2C).

While the above results confirm potent decoy activity for IL-13Ra2 (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-13Ra2 mAb were compared. With this approach, the effects of IL-13 signaling through IL-13Ra1 and IL-13Ra2 could be distinguished. CCL26 was the most abundant IL-13 induced transcript in this genome-wide screen, upregulated 12.3-fold (i.e. 2^{1.6}, q-value=6×10^{-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-13Ra2 mediated inhibition. Surprisingly however, while IL-13 binding to IL-13Ra2 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-13Ra2 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-13Ra2 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-13Ra1 interactions (8). Steroid-resistant and severe asthma subtypes are often associated with neutrophilia, TNFα 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-13Ra2. Indeed, due caution must be exercised, as blocking IL-13/IL-13Ra1 interactions in these patients might actually lead to disease aggravation, as IL-13 has been shown to down modulate inflammation induced by TNFα and IL-17 (10).

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References

Fig 1.
TNFα and IL-17 synergistically induce IL-13Rα2. 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-13Rα1 and IL-13Rα2 on ALF surface post cytokine stimulation (E, F). Expression of Il13ra1 and Il13ra2 transcripts in mouse lungs following TNFα and IL-17 administration (G).
Figure 2.
NLF were treated with TNFα 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-13Rα2 with mAb (C). Pairwise analyses of transcriptome signature of NLF treated with IL-13 ± TNF/IL-17 pretreatment (PreTx), ± anti-IL-13Ra2 mAb (D). Also refer to E Fig 3.