

IL-13 receptor α 2: A regulator of IL-13 and IL-4 signal transduction in primary human fibroblasts

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Background: IL-13 and IL-4 share many functional properties as a result of their use of a common receptor complex comprising IL-13 receptor α 1 (IL-13R α 1) and IL-4 receptor α (IL-4R α). The nonsignaling receptor IL-13 receptor α 2 (IL-13R α 2) binds IL-13 with high affinity and specificity and is believed to be a decoy receptor for IL-13.

Objective: We sought to examine the inhibitory effects of soluble and membrane-bound IL-13R α 2 on IL-13- and IL-4-mediated effects.

Methods: Primary human fibroblasts were grown from endobronchial biopsy specimens obtained from volunteers. Upregulation of IL-13R α 2 mRNA was measured by means of RT-PCR, and the level of surface expression was measured by means of FACS.

Results: We found that a recombinant soluble form of IL-13R α 2 blocked the effects of IL-13, but not IL-4, in fibroblasts *in vitro*. However, we found that the transmembrane form of IL-13R α 2 could attenuate both IL-13 and IL-4 responses, even though the response to TNF- α was unaffected. Furthermore, we found that IL-13R α 2 became associated with IL-4R α in the presence of IL-4. Addition of a blocking antibody to the extracellular domain of IL-13R α 2 restored responses of both IL-13 and IL-4.

Conclusion: The ability of IL-13R α 2 to regulate IL-4 was previously unrecognized in primary airway cells. These data reveal a novel role for IL-13R α 2 as a negative regulator of both IL-13 and IL-4 signaling in human bronchial fibroblasts. **Clinical implications:** It appears that IL-13R α 2 might be a powerful suppressor of T_H2-mediated responses and thus represents a potential therapeutic target for the treatment of asthma. (J Allergy Clin Immunol 2006;118:858-65.)

Key words: Allergy, cytokine receptors, signal transduction

Abbreviations used

AHR: Airway hyperresponsiveness
IL-4R α : IL-4 receptor α
IL-13R α 1: IL-13 receptor α 1
IL-13R α 2: IL-13 receptor α 2
RT-qPCR: Reverse transcription quantitative PCR
shIL-4R α : Soluble human IL-4 receptor α
STAT: Signal transducer and activator of transcription

IL-4 and IL-13 are pleiotropic cytokines with key roles in the pathogenesis of asthma and other allergic diseases.^{1,2} IL-4 is able to drive the differentiation of naive T helper cells into T_H2 lymphocytes, leading to the production of effector cytokines, such as IL-4, IL-5, IL-9, and IL-13 but not IFN- γ .^{3,4} Both IL-4 and IL-13 are associated with the induction of the ϵ isotype switch and secretion of IgE by B lymphocytes^{5,6} and enhance IgE-mediated responses by upregulating IgE receptors on B lymphocytes, mast cells, and basophils.^{7,8} They also induce vascular cell adhesion molecule 1 on vascular endothelium and thus direct the migration of T lymphocytes, monocytes, basophils, and eosinophils to the sites of inflammation.⁹ In asthma, IL-4 and IL-13 not only contribute to airway inflammation but also to airflow obstruction through remodeling effects, including induction of mucin gene expression in epithelial cells, leading to the hypersecretion of mucus.¹⁰ Both cytokines inhibit eosinophil apoptosis and promote eosinophilic inflammation by inducing chemotaxis and activation through the increased expression of eotaxin.¹¹

Although they only exhibit 25% homology, IL-13 shares many functional properties with IL-4 as a result of the common IL-4 receptor α (IL-4R α) component in their receptors.^{12,13} The IL-13 receptor α 1 (IL-13R α 1)/IL-4R α complex can act as an alternative IL-4 receptor, especially in cells that lack the common γ chain that usually forms a heterodimer with IL-4R α to bind IL-4.¹⁴ A second IL-13-binding protein, IL-13 receptor α 2 (IL-13R α 2), has also been identified.^{15,16} IL-13R α 2 binds IL-13 with high affinity but does not bind to IL-4.¹⁷ Despite this increased binding affinity, IL-13R α 2 is believed to be nonsignaling, thereby serving as a decoy receptor. Thus the presence of IL-13R α 1 and IL-4R α on

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the cell surface renders cells responsive to IL-13, and IL-13R α 2 is not required for functional ligand/receptor interactions. Similarly, the expression of IL-13R α 2 *in vitro* does not make cells responsive to IL-13. The discovery of a soluble receptor, homologous to IL-13R α 2, in the serum and urine of mice has lent support to the concept that IL-13R α 2 has a negative regulatory role as a decoy receptor.¹⁸

Despite their shared receptor components, IL-4 and IL-13 have some distinct nonoverlapping functions. For example, IL-4 is essential for the differentiation of uncommitted T cells to the T_H2 phenotype, whereas IL-13 appears to have a separate and distinct role in allergic inflammation. Specific blockade of IL-13 by soluble IL-13R α 2 to allergen-challenged mice can reverse airway hyperresponsiveness (AHR) and mucus production.¹⁹ Furthermore, acute administration of IL-13 induces many features of the allergic phenotype, such as mucus cell hyperplasia, eosinophilic infiltration, and AHR, in non-immunized mice.²⁰ The fact that IL-13 appears to play a significant role in allergic responses can be partially explained by the fact that the production of IL-13 continues for a prolonged period of time when compared with the kinetics of IL-4.²¹

There has been considerable interest in IL-13R α 2 because of its ability to bind IL-13 with high affinity and its short cytoplasmic tail, which lacks obvious signaling motifs. Although neither IL-13R α 1 nor IL-4R α appears to be regulated during T_H2-mediated inflammatory responses, several studies have shown that IL-13R α 2 is upregulated by both IL-4 and IL-13 in bronchial epithelial cells and fibroblasts.²²⁻²⁴ It has also been shown that treatment of epithelial cells with IFN- γ increases surface expression of IL-13R α 2 through mobilization from intracellular stores,²⁵ suggesting that IL-13R α 2 plays a key role in responding to and regulating the T_H1/T_H2 balance.

The exact mechanism by which IL-13R α 2 regulates IL-13 is still under investigation. It has been suggested that IL-13R α 2 present on the cell surface competes with the IL-4R α /IL-13R α 1 complex for IL-13. Alternatively, a soluble form of the receptor might be present in interstitial spaces, where it can sequester IL-13 to limit its bioavailability and suppress activation of receptor complexes on the cell surface. However, to date, no soluble form of IL-13R α 2 has been found in human subjects.

We have previously observed that primary human bronchial epithelial cells derived from some donors, but not others, failed to respond to IL-13 and IL-4 despite normal responses to other cytokines, such as TNF- α .²⁶ Further investigation suggested that surface levels of IL-13R α 2 were inversely correlated with the cellular responses to IL-13 and IL-4.²⁶ In the present study we have compared the ability of the soluble and transmembrane forms of IL-13R α 2 to regulate response to IL-4 and IL-13 in primary bronchial fibroblasts. We report that although soluble IL-13R α 2 does not suppress responses to IL-4, the membrane-bound form of IL-13R α 2 can attenuate IL-4 signaling through a physical association with IL-4R α . This appears to be a novel mechanism that

is distinct from the ability of IL-13R α 2 to regulate IL-13 signaling by means of sequestration of the ligand.

METHODS

Reagents

Eotaxin ELISA kit, recombinant sIL-4R α and sIL-13R α 2.Fc, and anti-IL-13R α 2 antibody were obtained from R&D Systems (Abingdon, United Kingdom). Recombinant IL-13 and IL-4 were purchased from Peprotech (London, United Kingdom). A monoclonal anti-IL-13R α 2 from Diaclone (IDS, Boldon, Tyne and Wear, United Kingdom) was used for neutralization experiments and FACs assays, and an anti-IL-13R α 2 from R&D Systems was used for immunoprecipitation experiments.

Primary human cell culture

Bronchial fibroblasts were grown from endobronchial biopsy specimens obtained from volunteers older than 18 years, as previously described.²⁷ Written informed consent was obtained from all volunteers, and ethical approval was obtained from the Southampton and South West Hampshire Local Ethics Research Committee. Biopsy specimens were placed in chilled Dulbecco's modified Eagle's medium (Invitrogen, Paisley, United Kingdom) with 10% FBS and finely cut with a scalpel. The pieces were then placed into 25-cm³ flasks and incubated for 5 to 7 days until fibroblasts migrated out from the tissue and proliferated. The tissue was then removed, and fibroblasts were harvested and passaged; all studies were performed on cells between the third and sixth passages. For assay, fibroblasts were plated into 24-well trays (2×10^4 cells) and allowed to grow to confluence before being rendered quiescent for 24 hours in Ultraculture (Cambrex Bio, Nottingham, UK) serum-free medium.

IL-13R α 2 gene expression

Primer and probe detection kits were obtained from PrimerDesign (Southampton, United Kingdom). Reverse transcription quantitative PCR (RT-qPCR) analysis of IL-13R α 2 gene expression was performed on DNase-treated RNA extracted from fibroblasts with TRIzol (Life Technologies, Strathclyde, UK). Total RNA (1 μ g) was reverse transcribed with avian myeloblastosis virus transcriptase (Promega, Southampton, UK) and random hexamers for IL-13R α 2 mRNA and 18S rRNA analysis. Real-time detection used an iCyclerIQ detection system with a PCR protocol as follows: 42 cycles at 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 15 seconds. IL-13R α 2 signals were normalized to 18S rRNA, and relative quantification was performed with the cycle threshold (CT) method.

Flow cytometric analysis

Fibroblasts were detached from their culture plates with trypsin. A single cell suspension was then washed in PBS containing 2% FCS. Aliquots of this cell suspension were then incubated for 1 hour at 4°C with a primary antibody to IL-13R α 2 before washing. The cells were then resuspended in PBS/1% BSA containing fluorescein isothiocyanate-conjugated anti-mouse secondary antibody. After 30 minutes in the dark, the cells were washed in cold PBS for analysis with a FACScan flow cytometer (BD Biosciences, Oxford, UK).

Enzyme immunoassay for eotaxin

Fibroblasts were exposed to stimuli and inhibitors at the concentrations indicated in the Results section, and supernatants were harvested by means of centrifugation 24 or 48 hours later. Supernatants were stored at -80°C until analysis of eotaxin by means of ELISA (R&D Systems, Abingdon, UK), according to the manufacturer's protocol. Cell-culture supernatants were assayed without

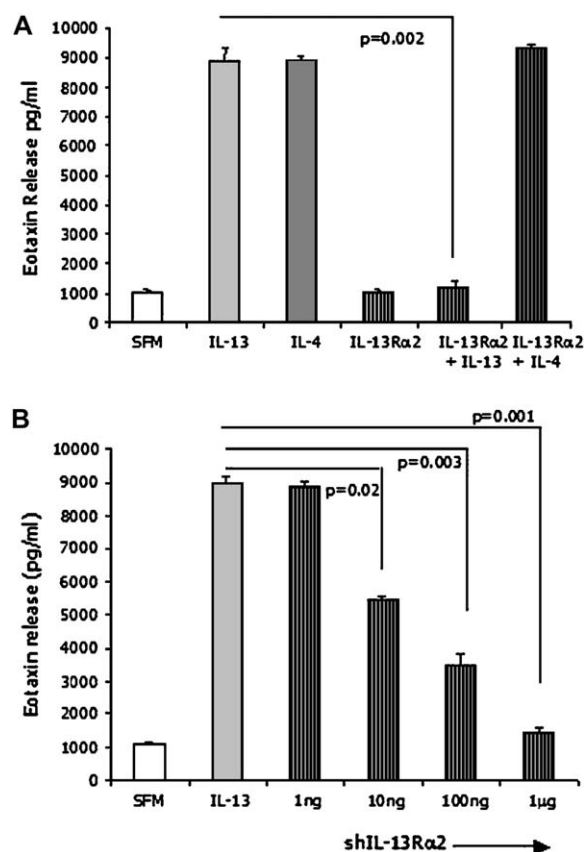


FIG 1. Attenuation of IL-13-mediated effects by soluble receptors. **A**, Primary human fibroblasts were treated with IL-13 or IL-4 (10 ng/mL) in the presence or absence of soluble receptor (1 μ g/mL) for 24 hours. The supernatants were assayed for eotaxin by means of ELISA. **B**, Fibroblasts were treated with IL-13 (10 ng/mL) in the presence of increasing concentrations of IL-13R α 2 (0–1 μ g/mL) for 24 hours ($n = 4$). *SFM*, Serum free media.

any purification or concentration, and cytokine release was expressed as picograms per milliliter.

Signal transducer and activator of transcription 6 phosphorylation

Primary human fibroblasts were grown to confluence before being serum starved for 24 hours. Cells were pretreated in serum-free medium for 24 hours in the absence or presence of IL-13 (10 ng/mL). The cells were then stimulated with IL-13 or IL-4 (10 ng/mL) for 60 minutes. Supernatants were removed, and cells were solubilized in boiling SDS sample buffer before being subjected to SDS-PAGE and Western Blotting with a phospho-signal transducer and activator of transcription (STAT) 6 antibody (New England Biolabs, Herts, UK). A pan-STAT6 antibody was used as a loading control.

Immunoprecipitation of IL-13R α 2/IL-4R α complexes

Cell extracts were prepared by lysing the cells in ice-cold buffer containing PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 2 μ g/mL leupeptin, and 5 μ g/mL aprotinin on ice for 30 minutes. The cleared supernatant was incubated with 5 μ g of anti-IL-13R α 2 antibody overnight at 4°C. The antibody complex was then captured by using Protein A/G Agarose beads (Santa Cruz Biotechnology, Heidelberg, Germany) and then boiled in denaturing sample buffer. The released

proteins were analyzed by means of Western blotting with antibodies against IL-4R α , using anti-IL-13R α 2 as a loading control.

Statistical analysis

Because the data were not normally distributed, results were analyzed with nonparametric tests and summarized as the median and interquartile range. Statistical comparisons were made with the Wilcoxon rank sum test. A *P* value of less than .05 was considered significant.

RESULTS

Neutralization of IL-13 by soluble IL-13R α 2

We first investigated the ability of a recombinant soluble form of IL-13R α 2 that lacked the transmembrane and cytoplasmic domain of the receptor (shIL-13R α 2) to attenuate responses to IL-13 or IL-4 in fibroblasts. Increasing amounts of shIL-13R α 2 were preincubated with IL-13 or IL-4 for 6 hours before being added to fibroblasts for 24 hours. The supernatants were then collected and assayed for eotaxin release by means of ELISA. Although soluble human IL-4 receptor α (shIL13R α 2) inhibited IL-13-mediated responses in a dose-dependent manner, it had no effect on IL-4-mediated effects in fibroblasts (Fig 1). These results are consistent with our previous findings that IL-13R α 2 binds IL-13 with high affinity but is unable to bind and sequester IL-4.¹⁷ Because the affinity of IL-13R α 2 for IL-13 is significantly greater than that of IL-13R α 1, these data suggest that shIL-13R α 2 is able to successfully compete with the membrane IL-13R α 1/IL-4R α receptor complex for IL-13 binding, thus preventing activation of the STAT6 signaling pathway.

Attenuation of both IL-13 and IL-4 signaling by IL-13R α 2

We previously observed that surface levels of IL-13R α 2 in primary bronchial epithelial cells were variable and inversely correlated with the magnitude of IL-13- or IL-4-stimulated IL-8 release.²⁶ Thus cells with high surface levels of IL-13R α 2 failed to respond to IL-13 or IL-4, even though they responded to TNF- α . To test the hypothesis that membrane-anchored IL-13R α 2 can inhibit both IL-13 and IL-4 signaling, we first exploited the known ability of IL-13 to upregulate IL-13R α 2 expression.^{22,24} Thus primary cultures of fibroblasts were pretreated with IL-13 (10 ng/mL) for 24 hours by using serum free media (SFM) as a control. Upregulation of IL-13R α 2 was confirmed at both the mRNA and protein levels. Thus by using RT-qPCR, significant upregulation of IL-13R α 2 mRNA expression was observed in fibroblasts exposed to IL-13, but not to IFN- γ , for 24 hours (Fig 2, A). Surface expression of IL-13R α 2 followed mRNA expression and was maximal at 24 hours after IL-13 treatment and persisted at 48 hours (Fig 2, B).

Having upregulated IL-13R α 2, the cells were then washed and restimulated with IL-13, IL-4, or TNF- α for a further 24 hours. As expected, IL-13-mediated eotaxin release was significantly reduced in fibroblasts that had high surface levels of IL-13R α 2 as a result of pretreatment

with IL-13. Furthermore, IL-4-mediated responses were also attenuated in fibroblasts that had high surface levels of IL-13R α 2 when compared with control cells that had not been pretreated with IL-13 (Fig 3). In contrast, the responses of the fibroblasts to TNF- α was unaffected by pretreatment with IL-13 or the presence of a neutralizing antibody (data not shown).

We then examined the levels of STAT6 phosphorylation. We were unable to detect any phosphorylated STAT6 in fibroblasts stimulated with either IL-13 or IL-4 if they had been pretreated with IL-13 to upregulate IL-13R α 2. This contrasted with the significant amount of STAT6 phosphorylation observed in the control samples, which had been pretreated in the absence of IL-13 (Fig 3) and then stimulated with IL-4 or IL-13. The results indicate that pretreatment with IL-13 and the associated upregulation of IL-13R α 2 are able to inhibit both IL-13 and IL-4 signal transduction.

Blockade of IL-13R α 2 on cell membrane

Although IL-4 and IL-13 can induce suppressors of cytokine signaling (SOCS) proteins^{28,29} as a feedback mechanism to suppress their own signaling,³⁰ our previous findings that high basal levels of IL-13R α 2 in epithelial cells affected their ability to respond to IL-4 led us to investigate the possibility that IL-13R α 2 directly inhibited IL-4 signaling. IL-13R α 2 was upregulated by pretreating fibroblasts with IL-13 before they were stimulated with either IL-4 or IL-13 in the absence or presence of a blocking antibody to the extracellular domain of IL-13R α 2 to test this hypothesis. In the presence of this antibody, the inhibitory effect of IL-13R α 2 on IL-13-mediated responses was abolished, and there was no significant difference in eotaxin release when compared with control values. Furthermore, IL-4-mediated eotaxin release was also restored in the presence of this blocking antibody (Fig 3). As a control, we showed that TNF- α responses were unaffected by the presence or absence of the blocking antibody. These data suggest that although IL-13R α 2 does not bind to IL-4, it has the potential to block IL-4-mediated responses, probably through a physical interaction, which can be sterically blocked by the IL-13R α 2 antibody.

Association of IL-4R α and IL-13R α 2

To investigate the possibility that IL-13R α 2 inhibited IL-4 signaling by physically associating with IL-4R α , we performed immunoprecipitation experiments with antibodies to either IL-13R α 2 or IL-4R α . This revealed that when fibroblasts were pretreated with IL-13 for 24 hours before stimulation in the absence or presence of IL-4, IL-13R α 2 was coprecipitated with IL-4R α in an IL-4-dependent manner (Fig 4). In cells that had not been pretreated with IL-13, no detectable coprecipitation was observed in the presence of IL-4. Similarly, no coprecipitation of IL-13R α 2 and IL-4R α was observed when IL-13-pretreated cells were stimulated with IL-4 in the presence of the IL-13R α 2 antibody, suggesting that the antibody caused steric hindrance to inhibit the physical

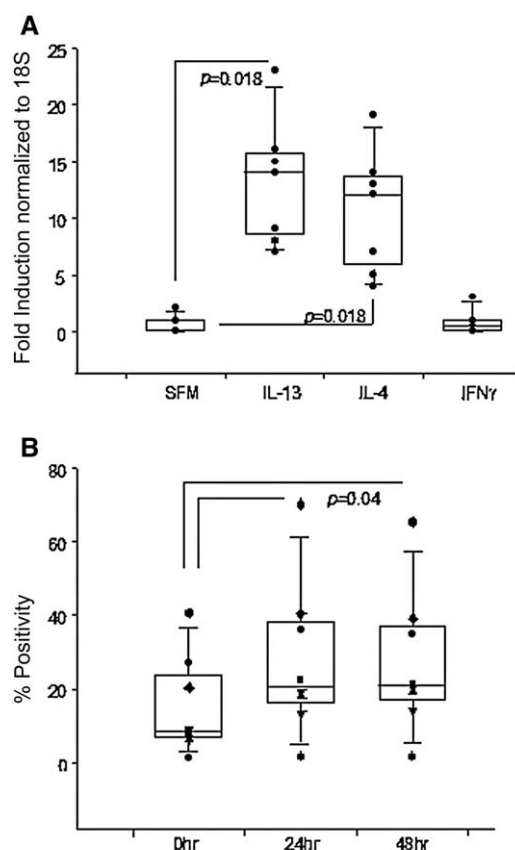


FIG 2. Pretreatment of fibroblasts leads to an increase in IL-13R α 2 levels. **A**, Induction of IL-13R α 2 gene expression was measured by means of RT-qPCR and normalized to 18S rRNA. Both IL-13 and IL-4 significantly upregulated IL-13R α 2 gene expression (24 hours), whereas IFN- γ had no effect. *SFM*, Serum free media. **B**, Primary human fibroblasts were treated with IL-13 (10 ng/mL) for the times indicated. Cells were stained with an IL-13R α 2 antibody (Diacclone) and analyzed by means of flow cytometry. Pretreating fibroblasts with IL-13 for 24 hours doubled the expression of IL-13R α 2 on the cell surface, which persisted at 48 hours. Similar results were obtained for IL-4 (data not shown). *Symbols* represent individual volunteers ($n = 8$). Statistical analysis was performed with the Wilcoxon rank sum test.

interaction between the 2 receptors. This would explain the increase in IL-4-stimulated eotaxin production observed in the presence of the blocking antibody. Taken together, our data lead us to propose that an IL-4-dependent physical interaction between IL-4R α and IL-13R α 2 prevents IL-4-stimulated signal transduction.

Natural variation in IL-13R α 2 affects IL-4-mediated responses

In the study described above, we used IL-13 to upregulate IL-13R α 2. However, analysis of basal IL-13R α 2 levels in fibroblasts from different donors revealed that, like epithelial cells, there was a natural variation in expression of the receptor, with some subjects expressing higher levels than others. Therefore we

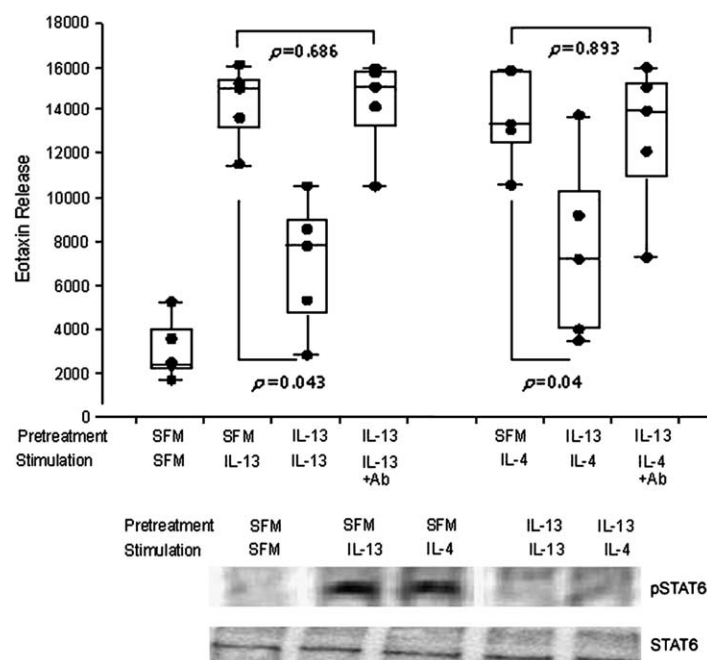


FIG 3. Increased IL-13R α 2 expression leads to attenuation of IL-13 and IL-4 responses. Primary human fibroblasts were pretreated with IL-13 (10 ng/mL) for 24 hours. The cells were then stimulated with IL-13 or IL-4 (10 ng/mL) for a further 24 hours in the presence or absence of an IL-13R α 2 neutralizing antibody (Diacclone). The supernatants were assayed for eotaxin by means of ELISA. Symbols represent individual volunteers ($n = 8$). Statistical analysis was performed with the Wilcoxon rank sum test. In the lower panel STAT6 phosphorylation was determined by means of Western blotting with a phospho-STAT6 antibody, with a pan-STAT6 antibody as a loading control. SFM, Serum free media; Ab, antibody.

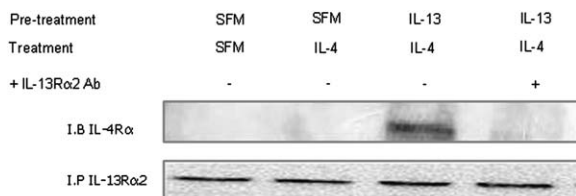


FIG 4. Immunoprecipitation of IL-13R α 2/IL-4R α complex. Primary human fibroblasts were pretreated without (SFM) or with IL-13 (10 ng/mL) for 24 hours before being treated with IL-4 in the absence or presence of a neutralizing IL-13R α 2 antibody (Ab; Diacclone) for 24 hours. IL-13R α 2 was immunoprecipitated from cell lysates, and then Western blotting was carried out with an IL-4R α antibody (R&D Systems). IL-13R α 2 antibody was used as a control for loading. The blot is representative of 3 independent experiments.

exploited this natural variation to analyze responses to IL-4 without any pretreatment to upregulate IL-13R α 2 levels. Thus when fibroblasts were graded as high (mean fluorescence intensity $> 40\%$) or low (mean fluorescence intensity $< 5\%$) expressers of IL-13R α 2, this revealed a clear difference in the magnitude of their responses to IL-4 (Fig 5). Thus the ability of membrane-anchored IL-13R α 2 to directly regulate both IL-13- and IL-4-mediated responses would appear to be of functional significance in controlling fibroblast responses to these key T_H2 cytokines.

DISCUSSION

Many cytokines, such as IL-5, IL-6, and IL-9, have soluble receptors³¹ that modulate their functions. Production of these isoforms can occur either through alternative splicing or through proteolytic cleavage of the membrane-anchored protein from the cell surface. Although some soluble receptors, such as the soluble IL-6 receptor, are agonists, most soluble receptors are antagonists because they compete with their membrane counterparts for their ligands. In the present study we show that soluble IL-13R α 2 attenuated the effects of IL-13 in primary fibroblasts without affecting responses to IL-4, suggesting that it acts as a decoy receptor that sequesters ligand. Consistent with this proposal, we have previously characterized the binding of IL-13 to its receptor components and shown that IL-13R α 2 binds IL-13 with a significantly higher affinity than IL-13R α 1, a property that is derived primarily from its extremely slow dissociation rate.¹⁷ Thus once IL-13 has been captured by IL-13R α 2, it is effectively sequestered from the lower-affinity IL-13R α 1 signaling receptor. A soluble form of IL-13R α 2 has been found in the serum and urine of mice, and it has been found that administration of soluble IL-13R α 2 attenuated IL-13-induced AHR in murine models of allergic inflammation,¹⁹ suggesting a potential therapeutic role for soluble IL-13R α 2. To our knowledge, there are no reports of a soluble form of IL-13R α 2 in human subjects. Although a recent

report has shown that trypsin treatment can promote release of the ectodomain of IL-13R α 2 from cells transfected to overexpress IL-13R α 2,³² we been unable to detect constitutive release of shIL-13R α 2 in fibroblast culture supernatants.

IL-13R α 2 is overexpressed in a vast majority of high-grade astrocytomas, and it has been proposed as a tumor-specific antigen.³³ Studies of the expression of the membrane-anchored form of IL-13R α 2 in tumor cells suggest that it also functions as a negative regulator of IL-13 in these cells. However, it has been shown that it not only competes with IL-13R α 1 for binding of IL-13, but it also promotes IL-13 internalization, resulting in marked suppression of IL-13 activity.³⁴ This property has been exploited for tumor targeting by using an IL-13 *Pseudomonas* exotoxin fusion protein. This is a highly specific cytotoxin to medulloblastoma cell lines expressing IL-13R α 2 and has led to the proposal that IL-13R α 2 might serve as a tumor-specific antigen for active immunotherapy of brain tumors.³⁵ Similarly, lung fibroblasts isolated from the most severe form of idiopathic interstitial pneumonia (usual interstitial pneumonia) exhibit increased expression of IL-4 and IL-13 receptor subunits, and these can be targeted with an IL-13 *Pseudomonas* exotoxin fusion protein.³⁶ Significantly, this effect was dependent on expression of not only IL-13R α 2 but also IL-4R α .

In contrast with tumor cells or usual interstitial pneumonia fibroblasts, studies of normal nasal and bronchial epithelial cells suggest that IL-13R α 2 occurs mainly as an intracellular molecule, which is rapidly mobilized from intracellular stores after treatment with IFN- γ .²⁵ In these studies upregulation of IL-13R α 2 surface expression in response to IFN- γ was found to be rapid, did not require protein synthesis, and resulted in diminished IL-13 signaling. Other studies have shown that IL-13R α 2 gene expression is regulated by a variety of factors, such as IL-10, IL-12, and IL-13, as well as IFN- γ , and that this regulation might well be cell type specific. We used RT-qPCR and FACS to show that IL-13R α 2 mRNA and protein expression can be increased in the presence of either IL-13 or IL-4 in fibroblasts, as we reported previously.³⁷ These results are also consistent with a report by Yoshikawa et al,³⁸ who found that IL-4 and TNF- α synergistically upregulated the cell-surface expression of IL-13R α 2 on fibroblasts. In similar experiments they pretreated fibroblasts with IL-4 to upregulate IL-13R α 2. This led to an inhibition of STAT6 phosphorylation when the cells were subjected to a further stimulation of IL-13.

We have previously observed that the level of IL-13R α 2 on the surface of primary epithelial cells is variable and inversely correlated to their responsiveness not only to IL-13 but also to IL-4, despite responding normally to TNF- α . In contrast with previous studies, which suggested that the majority of IL-13R α 2 is intracellular,²⁵ we found that epithelial cells or fibroblasts treated with IL-13 for 24 hours had increased levels of IL-13R α 2 on their surface. When the responsiveness of these cells to IL-13 and IL-4 was compared with that of

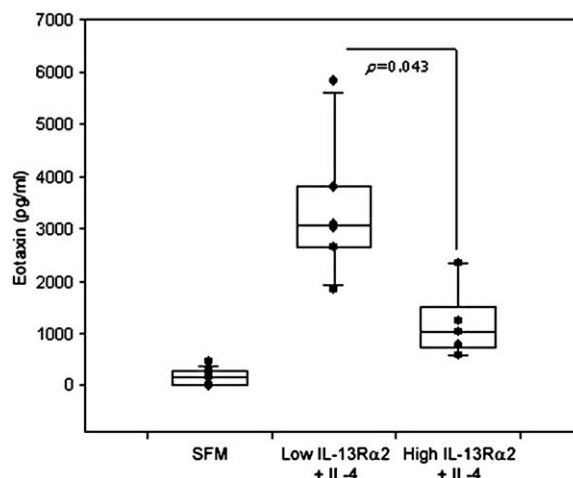


FIG 5. IL-4-mediated eotaxin release from primary human fibroblasts. Primary human fibroblasts ($n = 10$) were assessed for surface IL-13R α 2 levels by means of FACS analysis and graded according to whether they were high (mean fluorescence intensity $>40\%$) or low (mean fluorescence intensity $<5\%$) expressors of IL-13R α 2. In parallel cultures were grown to confluence before being treated with IL-4 (10 ng/mL) for 24 hours. Supernatants were assayed for eotaxin by using ELISA. Statistical analysis was performed with the Mann-Whitney U test.

cells that had been left untreated, there was a significant inhibition of both IL-13- and IL-4-mediated effects. It is known that T_H2 cytokines regulate their own activity through a feedback mechanism linked to increased expression of SOCS proteins that suppress IL-13 or IL-4 signaling.³⁰ Thus although it could be argued that the upregulation of IL-13R α 2 was only coincidentally related to the inhibition of IL-4 responses, the fact that neutralizing antibodies to IL-13R α 2 prevented the inhibition of the IL-4-stimulated responses strongly supports a causal relationship.

The involvement of IL-13R α 2 is also supported by evidence that IL-13R α 2 forms a complex with IL-4R α when cells are exposed to IL-4. The fact that IL-13R α 2 associates with IL-13R α 1 when cells are exposed to IL-13, but not IL-4, rules out the possibility that IL-13R α 2 inhibits IL-4 signaling by sequestering IL-13R α 1.³⁹ Similarly, IL-13R α 2 is unable to bind IL-4, and therefore it would be unable to inhibit IL-4 signaling by sequestering the ligand.

A more likely explanation is that the short cytoplasmic tail of IL-13R α 2 might interfere with the association or activation of signaling molecules, which, in turn, prevents downstream signal cascade. A similar mechanism has been linked to the abnormal proliferation of glioblastoma cells,³⁹ in which IL-4-mediated antitumor activity in rodent experimental gliomas is abrogated by aberrant expression of IL-13R α 2 by the glioblastoma cells. This involves the cytoplasmic tail of IL-13R α 2, which partly blocks IL-4-mediated activation of STAT6 and upregulates the activation of STAT3, although the latter does not require a direct physical interaction between STAT3 and IL-13R α 2.⁴⁰ Furthermore, although glioblastoma

cells fail to express the alternative IL-4R α /common γ chain receptor complex, this form of the IL-4 receptor is present on both epithelial cells and fibroblasts, suggesting that IL-13R α 2 has the ability to inhibit IL-4R α in either signaling configuration. This possibility is consistent with the observation that IL-13R α 2 inhibits IL-4R α by an intracellular mechanism involving an interaction with its short cytoplasmic tail.³⁹

Our study represents the first demonstration that the interaction between IL-4R α and IL-13R α 2 is a normal regulatory mechanism for IL-4 signaling in fibroblasts. The potential physiologic relevance of this finding is evident in our studies in fibroblasts that exhibit a natural variation in the expression of IL-13R α 2 (Fig 5). Although it would be anticipated that high IL-13R α 2 levels would attenuate IL-13 responses, it would not have been expected that it would also have an effect on IL-4-mediated effects. This natural variation in the levels of IL-13R α 2 appears to have a marked effect on the cell responsiveness to both IL-13 and IL-4 when there is a high level of IL-13R α 2 on the cell surface. Thus the functional consequence of this natural variation *in vitro* might play a significant role in the progression of inflammatory and remodeling processes, particularly in subjects with low levels of IL-13R α 2 because they are lacking in a key mechanism to regulate these 2 important cytokines.

It is easy to speculate that the ability of IL-13 and IL-4 to augment IL-13R α 2 expression would serve as a negative feedback loop to ensure that the effects of IL-13 are continually regulated. Previous studies have suggested that this regulation of IL-13 might actually lead to an increase in IL-4 responses by rendering more free IL-13R α 1/IL-4R α receptor complexes available for IL-4 binding. However, our data suggest that this is not the case. We propose that IL-13R α 2 inhibits IL-4 signal transduction in a way that is probably distinct from its ability to regulate IL-13 signaling. It will now be of interest to determine whether there is an hierarchic relationship between IL-13 and IL-4R α for binding of IL-13R α 2 or whether IL-13R α 2 can bind to and coregulate both proteins at once (ie, whether IL-13R α 2 can bind IL-13 when it is involved in a physical interaction with IL-4R α). Such a multifunctional interaction would identify the transmembrane form of IL-13R α 2 as a powerful suppressor of T_H2-mediated responses.

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