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The association of the cytoplasmic domains of interleukin 4 receptor alpha and interleukin 13 receptor alpha 2 regulates interleukin 4 signaling

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Interleukin-4 (IL-4) and Interleukin-13 (IL-13), key cytokines in the pathogenesis of allergic inflammatory disease, mediate their effects *via* a receptor composed of IL-13Rα1 and IL-4Rα. A third (decoy) receptor called IL-13Rα2 regulates interleukin signaling through this receptor complex. We employed a variety of biophysical and cell-based techniques to decipher the role of this decoy receptor in mediating IL-4 signaling though the IL-4Rα–IL-13Rα1 receptor complex. Surface plasmon resonance (SPR) analysis showed that IL-13Rα2 does not bind IL-4, and does not affect binding of IL-4 to IL-4Rα. These results indicate that the extracellular domains of IL-4Rα and IL-13Rα2 are not involved in the regulation of IL-4 signaling by IL-13Rα2. We next used a two-hybrid system to show that the cytoplasmic domains of IL-4Rα and IL-13Rα2 interact, and that the secondary structure of the IL-13Rα2 intracellular domain is critical for this interaction. The cellular relevance of this interaction was next investigated. BEAS-2B bronchial epithelial cells that stably express full length IL-13Rα2, or IL-13Rα2 lacking its cytoplasmic domain, were established. Over expression of IL-13Rα2 attenuated IL-4 and IL-13 mediated STAT6 phosphorylation. IL-13Rα2 lacking its cytoplasmic domain continued to attenuate IL-13-mediated signaling, but had no effect on IL-4-mediated STAT6 signaling. Our results suggest that the physical interaction between the cytoplasmic domains of IL-13Rα2 and IL-4Rα regulates IL-4 signaling through the IL-4Rα–IL-13Rα1 receptor complex.

Introduction

Interleukin 4 (IL-4) and Interleukin 13 (IL-13) play important roles in the pathogenesis of asthma and other atopic diseases by inducing the expression of adhesion molecules, cytokines and chemokines, such as vascular cell adhesion molecule (VCAM)-1 and monocyte chemoattractant protein-1 (MCP-1), which direct the migration of T lymphocytes, monocytes, basophils and eosinophils to inflammation sites.[1–3] In asthma, IL-4 and IL-13 act on bronchial epithelial cells and fibroblasts to induce eotaxin, which is an eosinophil chemoattractant and inhibitor of eosinophil apoptosis, to promote allergic airway inflammation.[4,5] IL-4 and IL-13 also induce epithelial mucin gene expression and promote goblet cell hyperplasia, leading to mucus plugging and airflow obstruction.[6,7]

IL-13 mediates its effects *via* its cognate receptor, a heterodimer composed of the IL-13 binding protein, IL-13Rα1 and the Interleukin-4 receptor α chain (IL-4Rα).[8–10] The IL-13Rα1–IL-4Rα complex also acts as a receptor for IL-4, especially in cells lacking the common gamma chain (γc) that usually forms a complex with IL-4Rα to bind IL-4.[11,12] Binding of either IL-13 or IL-4 to the IL-13Rα1–IL-4Rα receptor complex initiates a phosphorylation cascade that results in STAT6 dimerization; STAT6 then translocates to the nucleus where it induces inflammatory gene expression.

A second IL-13 receptor, IL-13Rα2, has been identified that shares 37% homology with IL-13Rα1 and binds IL-13 with high affinity but it does not bind IL-4.[13,14] Traditionally IL-13Rα2 has been thought of as a decoy receptor that sequesters IL-13 from its signaling receptor complex, IL-13Rα1–IL-4Rα.[13–15] Consistent with this, IL-13Rα2 has been shown to have the characteristics of a negative regulator, with a fast association rate but an exceptionally slow dissociation rate.[16] IL-13Rα2 has a short cytoplasmic tail that lacks any obvious signaling motif and is unable to initiate a signal through the STAT6 pathway. However it has been reported that glioblastoma cells express abnormally high levels of IL-13Rα2 that serve to inhibit IL-4 signaling and overcome IL-4 mediated growth arrest.[17] While this initial work suggested a potentially aberrant role for IL-13Rα2 in malignancy, the levels of IL-13Rα2 on the surface of primary bronchial fibroblasts have been found to be inversely

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correlated to their responsiveness not only to IL-13, but also to IL-4.[18,19] These observations highlighted a previously unrecognized role for IL-13Rα2 in normal cell function. As in the glioblastoma cells, IL-13Rα2 was found to be associated with IL-4Rα when the fibroblasts were treated with IL-4, suggesting that a physical interaction between IL-13Rα2 and IL-4Rα was also required for this regulatory mechanism. Although the original studies[17] suggested that the cytoplasmic tail of IL-13Rα2 is required for inhibition of IL-4 signaling, extracellular binding of neutralizing antibodies to IL-13Rα2 can also prevent the inhibition of IL-4-stimulated responses by IL-13Rα2 in bronchial fibroblasts.[20,21] This may be a result of the antibody causing steric hindrance around IL-13Rα2, blocking its interaction with IL-4Rα, but it remains unclear whether IL-13Rα2 exerts its regulatory effect on the binding of IL-4 to IL-4Rα *via* interaction of the extracellular domains of the two receptors, or *via* the interaction of the cytoplasmic domains of IL-4Rα and IL-13α2. Here we employ a variety of *in vitro* and cell-based techniques to examine the physical interaction of both the extracellular and cytoplasmic domains of IL-13Rα2 and IL-4Rα, to gain a better understanding of the molecular mechanism of regulation of IL-4 signaling by IL-13Rα2.

Material and methods

DNA synthesis and sequencing was carried out by Eurofins MWG Operon. All restriction endonucleases were purchased from New England Biolabs; T4 DNA ligase was purchased from Promega. All other molecular biology reagents were purchased from New England Biolabs, Fisher Scientific or Promega and were used as directed by the manufacturer. Chemical reagents were purchased from Sigma Aldrich, Fisher Scientific or Merck and were used as received. DNA purification was carried out using QIAquick PCR Purification Kits and plasmid purification was carried out using QIAGEN Plasmid Mini Kit (QIAGEN). The fidelity of all generated plasmids was verified by sequencing. The CRIM plasmids pAH68 and pAH69 were obtained from the *E. coli* Genetic Stock Centre at Yale University, USA. Surface plasmon resonance (SPR) measurements were carried out with a BIAcore 2000. CM5 sensor chips, HBS buffer (10 mM Hepes with 150 mM NaCl, 3.4 mM EDTA and 0.005% surfactant P20), amine coupling kit and regeneration agents were supplied by GE Healthcare, unless otherwise stated. Recombinant soluble human IL-13Rα2/IgG_1 chimera (shIL-13Rα2.Fc) and shIL-4Rα.Fc were obtained from R&D Systems. Recombinant IL-13 and IL-4 were purchased from Peprotech. SDS-PAGE reagents were from Amersham Life Sciences. Transfection regents were obtained from Qiagen.

Surface plasmon resonance (SPR) measurements

The molecular interactions between shIL-13Rα2.Fc, shIL-4Rα.Fc and IL-4 or IL-13 were determined by SPR measurements[22–24] using a BIAcore 2000™ biosensor as previously detailed.[16] shIL-4Rα.Fc and or shIL-13Rα2.Fc was diluted in HBS running buffer (10 mM Hepes, pH 7.4, 150 mM NaCl, 3.4 mM EDTA and 0.005% surfactant P20) and coupled to a CM5 sensor chip surface using the manufacturer's protocol. IL-4 and IL-13 were diluted from stock to the desired concentration (200, 400, 600, 800 or 1000 nM) in HBS buffer. To determine kinetic constants, sensograms were collected at 25 °C, flow rate 50 μl min^{-1} and data collection rate of 1 Hz. Sensograms were recorded and normalised to a base line of 0 resonance units (RU). Equivalent concentrations of each protein were injected over an untreated surface to serve as blank sensograms for subtraction of bulk refractive index background. The sensor chip surface was regenerated between runs with a 1 minute injection of 10 mM HCl, at 10 μl min^{-1}. The resultant sensograms were evaluated using the BIA evaluation 2.0 software to provide kinetic data.

Construction of IL-4Rα–IL13-αR2 bacterial reverse two-hybrid systems (RTHS)

The cytoplasmic domain of IL-4Rα was amplified by PCR and cloned into pTHCP14[25] using KpnI and XhoI restriction endonucleases to give pTHCP14-IL-4Rα. The cytoplasmic tail of IL-13Rα2 was amplified by PCR and cloned into pTHCP14-IL-4Rα with SalI and SacI restriction endonucleases to give plasmid pTHCP14-IL-4Rα–IL-13Rα2. The cassettes encoding IL-4Rα.P22 and IL-13Rα2.434 fusion proteins were integrated onto the chromosome of *E. coli* strain SNS126[25] using plasmids pAH68 and pAH69[26] as previously described[27,28] to give the IL-4Rα–IL-13Rα2 RTHS. The IL-4Rα–IL-13$Rα2^{P366A}$ RTHS was generated by site-directed mutagenesis (SDM), cloned into pTHCP14-IL-4Rα–IL-13Rα2 with AgeI and SacI. The IL-4Rα–IL-13$Rα2^{P374A}$ RTHS was generated by SDM, and cloned into pTHCP14-IL-4Rα–IL-13Rα2 with SalI and HindIII. The IL-4Rα–IL-13$Rα2^{P366A,P374A}$ RTHS was generated as above, except IL-13Rα2 was amplified by PCR using the forward and reverse mutagenic primers used for the P366A and P374A mutants, and cloned into pTHCP14-IL-4Rα–IL-13Rα2 with AgeI and HindIII. Protein–protein interactions in the RTHS were assessed as below.

ortho-Nitrophenyl-β-galactosidase (ONPG) assays and drop spotting

Protein–protein interactions were assessed by ONPG assay and drop spotting as previously detailed.[29,30] Briefly, for ONPG assays, overnight culture of each RTHS were subcultured and incubated at 37 °C for 2 h with shaking to OD600 of 0.5–0.7. IPTG was then added to a final concentration of 0, 2.5, 5, 10, 15, 25, 50, 100, and 250 μM and the cultures incubated for a further 1 h at 37 °C. The OD600 was recorded for all the samples before ONPG assays were conducted using a standard protocol. For drop-spotting, 2 μl of overnight cultures of each RTHS were diluted in 10% glycerol solution and ten-fold serial dilutions prepared in a sterile 96-well plate for each strain. 2.5 μl of each dilution was drop-spotted onto selective plates (200 ml Agar, 25 ml minimal media A, 10 ml 50% glycerol, 250 ml 1 M $MgSO_4$, 50 μg ml^{-1} Ampicillin and 25 μg ml^{-1} Spectinomycin), containing 25 μg ml^{-1} Kanamycin, 2.5 mM 3-amino-1,2,4-triazole, with or without 25 μM IPTG. Each strain was also spotted on LB-agar, supplemented with 100 μM IPTG, to demonstrate that the proteins expressed were not toxic to the host organism.

STAT6 phosphorylation

BEAS-2B cells over expressing IL-13Rα2 or IL-13Rα2 cytoplasmic mutations were established. Briefly, IL-13Rα2 was amplified by PCR from cDNA using the following primers: Forward GTTGTTCTCGATGGCTTTCGTTTGGC and Reverse GTTGGTAG CTTTCATGTATCACACAGAAAAATTCTGG. The PCR product was digested and cloned in pcDNA3.1(–) vector (Invitrogen Paisley, UK). Receptor expression was confirmed by Flow Cytometry. Cells were grown to confluence before treatment with IL-4 or IL-13 (10 ng ml^{-1}) for 60 minutes in the absence or presence of an IL-13Rα2 neutralising Ab (10 μg ml^{-1}). The cells were then solubilised in boiling SDS sample buffer before being subjected to SDS-PAGE and western Blotting with a phospho-STAT6 antibody (Cell Signaling Technology), using a pan STAT6 antibody as loading control, as described previously.[20]

Results

Kinetic analysis of IL-4 binding to the extracellular domain of IL-4Rα

The role of the interaction of the extracellular domains of IL-4Rα with IL-13Rα2 on interleukin signaling mediated through the IL-4Rα–IL-13Rα1 receptor was assessed by SPR. The binding of IL-4 and IL-13 to IL-4Rα was evaluated with soluble human IL-4Rα/IgG_1 chimera (shIL-4Rα.Fc) immobilised onto a CM5 sensor chip surface. The dissociation constant ($K_D = k_{off}/k_{on}$) for IL-4 binding to shIL-4Rα.Fc was calculated to be 412 ± 81 pM (Fig. 1A, data represents mean ± S.D, n = 10 for all values quoted). As expected, IL-13 did not bind shIL-4Rα.Fc (Fig. 1B), but bound soluble human IL-13Rα2/IgG_1 chimera (shIL-13Rα2.Fc) immobilised onto a sensor chip surface with a K_D of 1.96 ± 1.7 nM. To assess the effect of the interaction of the extracellular domains of IL-4Rα and IL-13Rα2 on IL-4 signaling, 1200 RU hIL-4Rα and 600 RU shIL-13Rα2 were immobilised to the sensor chip surface. The presence of shIL-13α2.Fc had no significant effect on the binding of IL-4 to IL-4Rα (K_D = 402 ± 87 pM, Fig. 1C). Increasing the concentrations of shIL-13Rα2.Fc to 1800 RU did not affect the binding of IL-4 to shIL-4Rα.Fc (K_D = 400 ± 130 pM). IL-4 did not bind to shIL-13Rα2.Fc immobilised onto a CM5 sensor chip. IL-13 bound the mixture of 1200 RU hIL-4Rα and 600 RU shIL-13Rα2 with a K_D of 2.10 ± 1.6 nM (Fig. 1D), and the mixture of 1200 RU hIL-4Rα and 1800 RU shIL-13Rα2 with a K_D of 1.82 ± 2.2 nM. These data extend our previous studies,[11,18] demonstrating that IL-13Rα2 binds IL-13, but is neither able to sequester IL-4 from its cognate receptor (IL-4Rα), nor to antagonize this interaction. The K_D values obtained by SPR for the IL-4:IL-4Rα interaction are comparable to those previously observed in cell-based assays.[31,32]

Two-hybrid analysis of the association of IL-4Rα and IL-13Rα2 cytoplasmic domains

The above SPR data demonstrates that while the extracellular domain IL-13Rα2 directly competes with IL-13Rα1 for IL-13, it does not affect the binding of IL-4 to IL-4Rα. We therefore turned our attention to assessing the role of the cytoplasmic

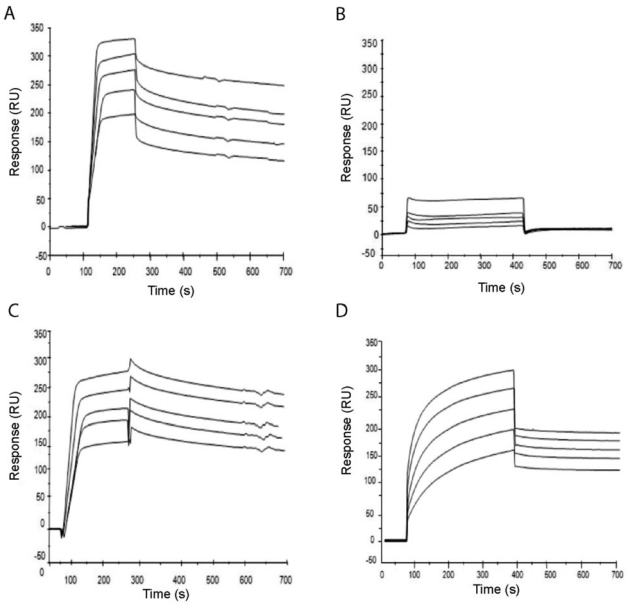

Fig. 1 SPR analysis of IL-4 and IL-13 binding to IL-4Rα and IL-13Rα2. Representative blank-corrected sensograms; concentration used lower to upper curve is 200, 400, 600, 800 and 1000 nM (A) IL-4 binds IL-4Rα with a K_D of 412 ± 81 pM. (B) IL-13 does not bind IL-4Rα. (C) Binding of IL-4 to IL-4Rα is not affected by the presence of IL-13Rα2; K_D = 402 ± 87 pM. (D) IL-13 binds the immobilised mixture of IL-4Rα and IL-13Rα2 with a K_D of 2.10 ± 1.6 nM.

domain of IL-13Rα2 in regulating the IL-4 signaling through IL-4Rα. As the cytoplasmic domain of IL-13Rα2 is relatively small and not ideal for binding studies using SPR, we used a bacterial reverse two-hybrid system (RTHS) to evaluate whether there was a direct interaction between the cytoplasmic domains of IL-4Rα and IL-13Rα2. The bacterial RTHS is based on the bacteriophage regulatory system, using chimeric repressor fusions and promoter sequences to link the interaction of targeted fusion protein heterodimers to the expression of three reporter genes; HIS3 (imidazole glycerol phosphate dehydratase) and KanR (aminoglycoside 3′-phosphotransferase for kanamycin resistance) are two chemically-tunable, conditionally selective reporter genes.[25,27] The third reporter gene, LacZ (β-galactosidase) is used to quantify the protein–protein interaction through β-galactosidase assays. An *E. coli* RTHS strain was constructed that chromosomally expressed the cytoplasmic domain of IL-13Rα2 as an N-terminal fusion with the 434 phage repressor domain, and the cytoplasmic domain of IL-4Rα as an N-terminal fusion with the chimeric P22 phage repressor domain (both regulated by IPTG *via* a Ptac promoter). Interaction of the targeted proteins (IL-13Rα2 with IL-4Rα) brings together the 434 and P22 repressor domains, forming a functional repressor that binds onto the corresponding operator regions upstream of the three reporter genes (on the chromosome of the RTHS strain), and prevents transcription of the reporter genes (Fig. 2A). This results in the host strain being unable to survive or grow on selective media lacking histidine and containing kanamycin, in an IPTG-dependent manner.

We initially used *o*-nitro-β-galactosidase (ONPG) assays to determine if the cytoplasmic tail of IL-13Rα2 interacted with

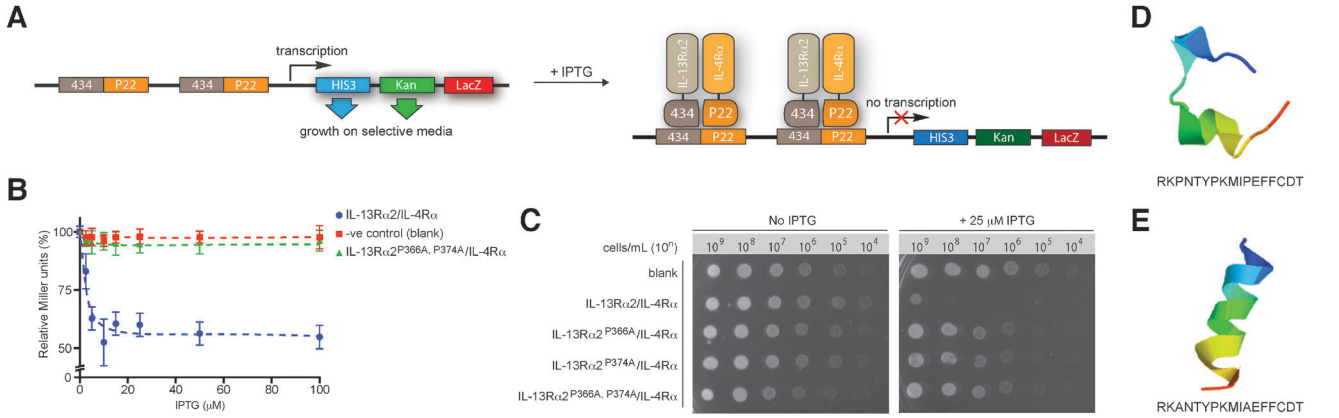

Fig. 2 Assessing the interaction of IL-4Rα and IL-13Rα2 cytoplasmic domains. (A) In the bacterial RTHS, the expression of the P22-IL-4Rα and 434-IL-13Rα2 fusion proteins is induced by IPTG; if IL-4Rα and IL-13Rα2 interact, they will bring together P22 and 434 to form a functional repressor that prevents expression of the downstream reporter genes, thus preventing survival and growth on minimal media. (C) Drop-spotting serial dilutions (2.5 μL of $\sim 10^n$ cells per ml) of the blank and IL-4Rα–IL-13Rα2, RTHS onto selective media plates with and without IPTG. A significant reduction in growth is observed for the IL-4Rα–IL-13Rα2 RTHS upon induction of protein expression with IPTG, indicating formation of a functional repressor, but not for the blank. This interaction is not observed for mutant IL-13Rα2^{P366A}, IL-13Rα2^{P374A}, or IL-13Rα2^{P366A,P374A} receptors. (D) Prediction of the structure of the cytoplasmic domain of IL-13Rα2 with the *de novo* peptide structure prediction software PepFold. (E) Prediction of the structure of the cytoplasmic domain of IL-13Rα2^{P366A,P374A} with PepFold.

the intracellular domain of IL-4Rα. A RTHS strain containing only the repressor domains was used as a negative control. A dose dependent decrease of β-galactosidase activity was observed in the ONPG assays with increasing IPTG concentration with the IL-13Rα2–IL-4Rα RTHS; increasing IPTG levels had no effect on the β-galactosidase activity of the negative control RTHS strain (Fig. 2B). The formation of a functional repressor in the IL-13Rα2–IL-4Rα RTHS strain was further demonstrated by drop-spotting ten-fold serial dilutions of this, and the control RTHS strain onto selective plates without or with 25 μM IPTG. The negative control strain showed full growth on selective media regardless of IPTG levels, whereas the growth of the IL-13Rα2–IL-4Rα RTHS was significantly affected by the presence of IPTG (Fig. 2C, 6 spots of growth in the absence of IPTG *versus* 1 spot of growth in the presence of 25 μM IPTG). The possibility that the IPTG-dependent loss of growth was due to toxicity of the fusion proteins to the host strain was probed by repeating this experiment on LB-agar plates. No IPTG-dependent repression of growth was observed indicating that the loss of β-galactosidase activity and the inability to survive on selective media is due to dimerization of the cytosolic domains of IL-13Rα2 with IL-4Rα.

As an additional control, we sought to assess the importance of the secondary structure of the cytoplasmic tail of IL-13Rα2 for the observed interaction. We sought key residues to mutate, but analysis did not reveal structural motifs typical of a regulatory domain, however the sequence (amino acids 364–380, RKPNTYPKMIPEFFCDT) contains three proline residues. Prolines are known to confer structural rigidity to peptide chains, and proline-rich motifs have been extensively demonstrated to be critical to protein–protein interactions.[33] We therefore hypothesized that the loss of these proline residues would affect the interaction of IL-13Rα2 with IL-4Rα. In order to probe this hypothesis, we used the *de novo* peptide structure prediction software PepFold,[34–36] which has been specifically designed for short amino acid sequences. The structures predicted by PepFold suggested that the secondary structure of the cytoplasmic tail of IL-13Rα2 would be significantly altered by mutation of proline residues 366 and 374 to alanine (Fig. 2D and E). To determine whether the predicted structure change would affect the interaction between IL-13Rα2 and IL-4Rα cytoplasmic tails, a double point mutant IL-13Rα2^{P366A,P374A}–IL-4Rα RTHS strain was generated. This strain did not show IPTG-dependent loss of β-galactosidase activity in ONPG assays (Fig. 2B), and the growth of this strain on minimal media was also not inhibited by IPTG (Fig. 2B), suggesting that the cytoplasmic domains of IL-13Rα2^{P366A,P374A} and IL-4Rα do not interact. In addition, single point mutants RTHS were generated; IL-13Rα2^{P366A}–IL-4Rα RTHS and IL-13Rα2^{P374A}–IL-4Rα did not show any IPTG-dependent inhibition of growth (Fig. 2C) demonstrating that mutation of either proline 366 or 374 to alanine is sufficient to disrupt this interaction. This may be due to elimination of a recognised proline-rich binding motif, or a result of changes in secondary structure that result from these point mutations.

The role of the cytoplasmic domain of IL-13Rα2 in regulating IL-4 signaling in cells

To assess the biological significance of the identified interaction of the cytoplasmic domains of IL-13Rα2 and IL-4Rα, and its affect on IL-4 signaling, two BEAS-2B cell lines (human bronchial epithelium) were established; one stably expressing full length IL-13Rα2 receptor (BEAS-2B-FL) and the other expressing the IL-13Rα2 receptor without its cytoplasmic domain (BEAS-2B-NT). These cells were treated with IL-13 or IL-4 for 30 min before being solubilized and prepared for Western blotting. Over expression of full length or truncated IL13Rα2 led to the inhibition of IL-13 stimulated STAT6 phosphorylation (Fig. 3). The addition of an IL-13Rα2 neutralising antibody restored IL-13 signaling *via* STAT6 in both transfected cells lines (Fig. 3) suggesting that IL13Rα2 directly competes with IL13Rα1 for IL-13 binding,

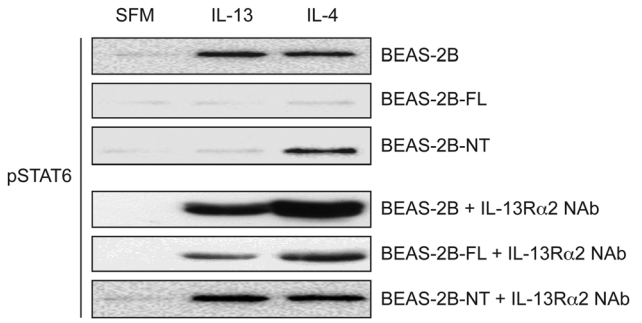

Fig. 3 Stable transfection of BEAS-2B cells with plasmids expressing full length IL-13Rα2 receptor (FL) or IL-13Rα2 receptor without its cytoplasmic domain (NT). Native BEAS-2B cells and stably transfected cell lines (FL and NT) were treated with IL-4, IL-13 or serum free media (SFM) in the presence (top 3 blots) or absence (bottom 3 blots) of an IL-13Rα2 neutralising antibody (NAb), before being assessed by Western blotting to monitor STAT6 phosphorylation. The presented data are representative of 3 independent experiments.

with IL-13Rα2 simply acting as a decoy receptor by sequestering IL-13 from IL-13Rα1 with no requirement for the cytoplasmic domain.

In cells treated with IL-4 however, inhibition of STAT6 phosphorylation was only observed in cells expressing full length IL13Rα2; STAT-6 phosphorylation was not affected in cells expressing the truncated IL13Rα2 (Fig. 3). This points to a key role for the cytoplasmic tail of IL-13Rα2 in modulating IL-4 response. Furthermore, as previously described,[20,21] an IL-13Rα2 neutralising antibody restored STAT6 signaling in BEAS2B-FL cells treated with IL-4. As our SPR data demonstrates, this is not due to the antibody preventing IL-13Rα2 binding IL-4, but likely a result of additional extracellular steric hindrance preventing assembly of the IL-4Rα–IL-13Rα2 receptor complex, and the resulting association of their cytoplasmic domains.

We attempted to explore the cellular effect of mutating the proline residues in the cytoplasmic domain of IL-13Rα2 (identified as important for the protein–protein interaction by two-hybrid analysis) by establishing cell lines that stably expressed IL-13Rα2^{P366A}, IL-13Rα2^{P374A} IL-13Rα2^{P366A,P374A}. However, we were unable to obtain significant levels of receptor expression in the resulting cell lines to draw meaningful conclusions. It is possible that the introduced mutations in the cytoplasmic domain of IL-13Rα2 compromise its insertion into the cell membrane, and/or leave the mutant receptor vulnerable to protease attack.[33]

Discussion

The cytoplasmic domain of IL-13Rα2 is composed of only 17 amino acids,[37] and therefore may have been viewed as having little effect on the modulation of interleukin signalling by this receptor. This is true in the case of IL-13 signaling, however, our data suggests that the cytoplasmic tail of IL-13Rα2 plays a key role in modulating IL-4 signaling through its interaction with the cytoplasmic tail of IL-4Rα. One possible mechanism is that the IL-13Rα2 cytosolic domain interferes with the association or activation of signaling molecules, which in turn prevent downstream signal cascades. The intracellular domain of IL-4Rα contains a membrane proximal Box-1 sequence that serves as a docking site for JAK1. It would be reasonable to speculate that the regulation of IL-4 signaling by IL-13Rα2 occurs *via* this site, as it is situated close to the membrane and therefore is in close proximity to the cytoplasmic domain of IL-13Rα2 when the two receptors associate.[38] As the phosphorylation cascade that leads to activation of STAT6 is initiated by JAK1, blocking the interaction of this signaling molecule with its receptor complex would completely inhibit the entire STAT6 signaling pathway. A similar mechanism has been linked to the abnormal proliferation of glioblastoma cells, in which IL-4-mediated antitumor activity in rodent experimental gliomas is abrogated by aberrant expression of IL-13Rα2 by the glioblastoma cells.[17] Furthermore, although glioblastoma cells fail to express the alternative IL-4Rα/common γ chain receptor complex, this form of the IL-4 receptor is present on both epithelial cells and fibroblasts, suggesting that IL-13Rα2 may have the ability to inhibit IL-4Rα in either signaling receptor complex. The data from our RTHS experiments suggest that mutation or deletion of one or more of the proline residues lead to a loss of association between IL-4Rα and IL-13Rα2. These results imply that conformational features may be important in the regulatory mechanism of IL-4, with this domain providing a rigid structure that blocks docking of JAK1 to IL-4Rα. In glioblastoma cells, the upregulation of IL-13Rα2 leads to the activation of STAT3, although this effect does not require a direct physical interaction between STAT3 and IL-13Rα2. A hypothesis consistent with these observations is that the role of IL-13Rα2 in IL-4 signaling is to regulate the STAT6 pathway, thus reducing inflammation, whilst initiating the STAT3 pathway which in the case of fibroblasts would lead to survival and an enhancement of fibrotic responses.

The work here has utilized a variety of biochemical and cell-based techniques to demonstrate the significant role played by the cytoplasmic tail of IL-13Rα2 in regulating IL-4 signaling. The IL-4/IL-13 pathway is an extremely important mediator of inflammatory responses. Thus a complete understanding of how IL-13Rα2 regulates these two important cytokines may lead to the development of a novel therapy for asthma or other inflammatory conditions that selectively targets these cytokines.

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