The Modulation of Pulmonary Fibrosis by IL-13Ra2

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This work was funded by: Science Foundation Ireland
Abstract

Pulmonary Fibrosis is a progressive and fatal disease that involves the remodelling of the distal airspace and the lung parenchyma which results in compromised gas exchange. The median survival time once diagnosed is less than 3 yrs. IL-13 has been shown to play a role in a number of inflammatory and fibrotic diseases. IL-13 modulates its effector functions via a complex receptor system that includes the IL-4Rα, IL-13Rα1 and the IL-13Rα2. IL-13Rα1 binds IL-13 with low affinity yet when it forms a complex with IL-4α, it binds with much higher affinity inducing the effector functions of IL-13. IL-13Rα2 binds IL-13 with high affinity but has a short cytoplasmic tail and has been shown to act as a non-signalling decoy receptor. Transfection of fibroblasts and epithelial cells with IL-13Rα2 inhibited the IL-13 induction of soluble collagen, TGF-β and CCL17. Adenoviral overexpression of IL-13Rα2 in the lung reduced bleomycin induced fibrosis. Our work shows that overexpression of IL-13Rα2 inhibits the IL-13 induction of fibrotic markers in vitro and inhibits bleomycin induced pulmonary fibrosis. In summary our study highlights the antifibrotic nature of IL-13Rα2.

Key Words:

Pulmonary Fibrosis, IL-13Rα2, Bleomycin,
Introduction

Pulmonary Fibrosis is a progressive and fatal disease that involves the remodelling of the distal airspace and the lung parenchyma which results in compromised gas exchange (17). The median survival time once diagnosed is less than 3 yrs (1). IL-13 is a 12 kDa profibrotic cytokine produced by T_{H}2 cells, mast cells, eosinophils, and basophils (9, 30). IL-13 has been shown to play a role in a number of inflammatory and fibrotic diseases e.g. asthma, idiopathic pulmonary fibrosis, systemic sclerosis and granulomatous lung disease (7, 12, 27). It shares 30% homology with the Th2 cytokine IL-4 and they share many effector functions. They also share signalling pathways and transcription factors namely STAT6 (33). When IL-13 and IL-4 were neutralised independently it emerged that IL-13 was the dominant cytokine in driving the pathogenesis of fibrosis (8, 15, 16). The phenotype of transgenic mice expressing IL-13 on a lung specific promoter demonstrates sub-epithelial airway fibrosis and alveolar remodelling (32). The neutralisation of IL-13 using antibodies specific for the cytokine or gene deletion has been shown to inhibit bleomycin and fluorescein isothiocyanate (FITC) induced collagen production (2, 16). IL-13 modulates its effector functions via a complex receptor system that includes the IL-4Rα, IL-13Rα1 and the IL-13Rα2. IL-13Rα1 binds IL-13 with low affinity yet when it forms a complex with IL-4α, it binds with much higher affinity inducing the effector functions of IL-13 (34). This receptor complex can be expressed on lymphoid and non lymphoid cells. IL-13Rα2 binds IL-13 with high affinity but has a short cytoplasmic tail and has been shown by several investigators to act as a non-signalling decoy receptor (34). In contrast some studies have found that IL-13 signals through IL-13Rα2 to induce TGF-β and subsequently fibrosis (10, 11). The expression of all three receptors of the
IL-13 receptor system has been shown to increase in fibroblasts from patients with pulmonary fibrosis compared to normal fibroblasts (13). IL-13 and its receptors have also been shown to be increased in the lungs and blood of patients with IPF (20, 22). The fibrotic milieu that is caused by IL-13, including cytokine and chemokine production and extracellular matrix deposition could potentially be reduced by the administration IL-13Rα2.

In mice, IL-13Ra2 exists in two forms; discrete transcripts, generated by alternative splicing, encode soluble and membrane bound forms of the receptor (5, 25). In contrast to mice, the human soluble form of IL-13Ra2 is generated by cleavage of the membrane bound receptor by MMP8 (6). In this study we show that overexpression of IL-13Ra2, either as the membrane bound form or the soluble receptor, IL-13Ra2Δ10, inhibits the IL-13 induction of fibrotic markers in vitro and inhibits bleomycin induced pulmonary fibrosis. This highlights the antifibrotic nature of IL-13Ra2.
Materials and Methods

Cloning of IL-13Rα2 into pcDNA3.1/V5-His TOPO

PCR was carried out using Phusion High fidelity enzyme (New England Biolabs, Ipswich, MA). Typically 1µl of cDNA, generated from mouse lung mRNA, was added to a master mix and amplified with primers specific for IL-13Rα2 (5’- TGGAGCACACCTGGAGGACCC -3’, 3’- ACAGAGGGTGATCTTCATAAGC -5’). The PCR product was then cloned into pcDNA™ 3.1/V5-His TOPO® TA expression vector (Invitrogen, Carlsbad, CA).

Transfection of IL-13 receptor alpha 2-V5

NIH 3T3 fibroblasts and MLE epithelial cells were seeded at 1x10⁵, and transfected overnight with a complex formed using TRANSIT 2020 reagent (Mirus, Madison, WI) in Optimem (Gibco, Life Technologies, Carlsbad, CA) and 1µg of pcDNA3.1.IL-13Rα2-V5. Cells were serum starved for 6hrs and stimulated with 50ng/ml of IL-13 (Biolegend, San Diego, CA) and incubated for 48hr before being analysed for fibrotic endpoints.

RNA extraction, cDNA synthesis and PCR

Total RNA was isolated from NIH3T3 cells and mouse tissue using an RNEasy Kit (Qiagen, Manchester, UK), and reverse-transcribed into cDNA using Superscript III reverse transcriptase (Invitrogen, Paisley, UK). Quantitative real-time RT-PCR was performed with Taqman® Gene Assay probes (Applied Biosystems, Paisley, UK). To determine gene expression at a basal level, ΔCt values were normalised to the endogenous control of 18S ribosomal RNA. The ΔΔCt method was used to relatively quantify the levels in gene expression, with 18S rRNA as an endogenous control.
Western Blotting

Protein samples were quantified using Bradford reagent and run on a 10% SDS polyacrylamide gel by electrophoresis. Samples were transferred to PVDF membrane and blocked in 5% marvel. Membranes were probed with primary V5 antibody (Invitrogen), Stat 6, phospho-Stat6 or Gapdh (Cell signalling) overnight at 4°C and appropriate secondary for 1hr at RT. The membrane was then incubated with 1:1 Luminol enhancer solution: stable peroxidise buffer (Pierce, Rockford, IL) and rotated for 10mins and then transferred to a autoradiography cassette and exposed to Fuji RX film.

Animal Model of Pulmonary Fibrosis

Pulmonary fibrosis was induced as previously described (3). Briefly, 6-8week C57BL/6 mice were anaesthetised with ketamine/xylazine and instilled intratracheally with 1U/kg bleomycin. Three days post bleomycin administration mice were anaesthetised as above and 50µl of PBS containing 2 x 10^8 PFU adenovirus-IL-13Rα2, IL-13Rα2Δ10 or LacZ was administered intranasally using a micropipette. Mice were sacrificed on days 10 and 14 to assess the effect of IL-13Rα2 overexpression on fibrosis. The adenovirus was generated and rescued in collaboration with Prof. Jack Gauldie at McMaster University into a pDC3.16 microbix vector.

Sircol soluble collagen assay

Sircol collagen assay protocol was followed as manufacturer’s instructions. Briefly, 1ml of collagen dye was added to samples and mixed for 30mins. Samples were centrifuged at 12,000 RPM for 10mins. Collage pellets were washed with acid salt wash and centrifuged at
12,000 RPM for 10mins. Supernatant was discarded and pellet resuspended. Plates were read at 555nm and collagen was expressed as µg/ml.

**ELISA**

ELISAs were performed for IFNγ, active TGF-β, and CCL17 (R and D systems, Wiesbaden, Germany). Briefly, 96-well plates (Nunc, Roskilde, Denmark) were coated with 50 µl/well of the appropriate antibody for 24 h and then washed with PBS, and 0.05% Tween 20 (wash buffer). Nonspecific binding sites were blocked with 5% milk. Samples were incubated for 1 h at 37°C. 50 µl/well of the appropriate biotinylated antibody added for 45 min at 37°C. Streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) was added, and the plates incubated for 30 min at 37°C. Chromogen substrate (DAKO, Carpinteria, CA) was then added, and plates incubated at room temperature. Plates were read at 490 nm in an automated microplate reader (Bio-Tek Instruments, Winooski, VT). Standards were log(1/2) dilutions of recombinant cytokine/chemokine (50 µl/well).

**Statistics**

Data are presented as means ± SE. Data were analysed using the Mann Whitney U test. P values ≤ 0.05 were considered significant.
**Results**

**Time course of expression of transfected IL-13Rα2**

NIH3T3 fibroblasts were seeded into a 12 well plate and left for 24hrs. Cells were then transfected with pcDNA3.1/IL-13Rα2.V5 for 24 and 48hrs. Whole cell lysates (fig.1.A) and supernatants (fig.1.B) were isolated and assayed for IL-13Rα2 using western blotting. Membranes were probed with a primary anti V5 antibody and an anti mouse HRP-linked secondary. Cellular overexpression can be seen at 24hr and 48hr whereas secreted levels are only detectable at 48hrs. Therefore the 48hr time point was optimal to assess the inhibitory effects of IL-13Rα2.

**IL-13Rα2 Inhibits IL-13 induction of collagen, TGF-β and CCL17 in epithelial cells and fibroblasts.**

To determine the role of IL-13Rα2 in inhibiting IL-13 biology, NIH3T3 fibroblasts and MLE-12 alveolar epithelial cells were cultured in 12 well plates and transfected with pcDNA3.1/V5.IL-13Rα2 and stimulated with 50ng/ml IL-13 for 48hours. Supernatants were taken and assayed for soluble collagen using the Sircol soluble collagen assay. IL-13 significantly induced collagen production versus controls in both the fibroblasts (fig.2A) and epithelial cells (fig.2B), with more being produced by the fibroblast. IL-13Rα2 overexpression resulted in a reduction of soluble collagen production significantly in the fibroblasts. This suggests a protective role for the receptor in the production of extracellular matrix in response to IL-13.

Supernatants were also assayed for active TGF-β by ELISA. IL-13 induced TGF-β1 in both NIH3T3 fibroblasts (fig.2C) and MLE-12 epithelial cells (fig.2D). Overexpression of IL-13Rα2 in NIH3T3 and MLE-12 cells significantly decreased TGF-β1 in response to IL-13.
stimulation. This shows an inhibitory role of IL-13Rα2 against the IL-13 induction of a cytokine which plays a major role in fibrosis progression.

Supernatants were assayed for CCL17 by ELISA. CCL17 is a chemokine involved in chemotaxis of cells expressing CCR4. IL-13 significantly induced CCL17 in the epithelial cell and this was significantly reduced when transfected with IL-13Rα2 (fig. 2F). Although the fibroblasts followed a similar pattern to the epithelial cell, the decrease was not statistically significant (fig. 2E).

Control cells were treated with transfection reagent TransIT 2020 (Mirrus) alone to ensure it had no effect on collagen, TGF-β or CCL17 production. The cells were also transfected with an empty expression vector pCDNA3.1/V5-His-TOPO to ensure that it had no effect on these secretory proteins.

**Overexpression of IL-13Ra2 or IL-13Ra2D10 in NIH3T3 fibroblasts alters the signalling capability of IL-13.**

NIH3T3 fibroblasts were transiently transfected as described above. Prior to stimulation with IL-13 cells were serum starved for 24h. IL-13 stimulation was carried out for 0, 5, 15, 30, 60 and 120 minutes. Figure 3 shows that in untransfected cells Stat6 phosphotylation occurs at 15 minutes after IL-13 stimulation. However in transfected cells this phosphorylation is reduced at 30 and 60mins illustrating the inhibitory nature of IL-13Rα2.

**Basal levels of the IL-13 receptor complex in NIH3T3 fibroblasts, MLE cells and C57BL/6 mouse lung.**

Real time PCR was used to quantify the basal level of the IL-13 receptor complex, IL-13Rα1, IL-13Rα2 and IL4Rα, in NIH3T3 fibroblasts, MLE cells, and the lungs of C57BL/6 mice.
All three receptors are present in NIH3T3 fibroblasts and C57BL/6 mouse lung, with mouse lung having relatively higher copy numbers of each receptor compared to NIH3T3 fibroblasts.

**Adenoviral overexpression of IL-13Rα2 in cells and the lung.**

HEK293 cells were infected with our AV.IL-13Rα2 to assess IL-13Rα2 protein production as shown in figure 5 AV.IL-13Rα2 overexpressed IL-13Rα2 as observed by western blot showing efficacy of the virus. A.IL-13Rα2Δ10 soluble splice variant also showed increased protein in the supernatant (Figure 5B). Mice instilled with bleomycin were treated 3 days later with adenoviral IL-13Rα2, IL-13Rα2Δ10 or LacZ control and assessed at day 10 and 14 for IL-13Rα2 gene expression as measured by the ∆∆CT method using 18s as an endogenous control. (Figure 6) At day 10 IL-13Rα2 and IL-13Rα2Δ10 were significantly higher in AV.IL-13Rα2 and AV.IL-13Rα2Δ10 treated animals compared to bleomycin alone. On day 14 (11 days post infection) as seen in Figure 6B, there was still elevated IL-13Rα2 and IL-13Rα2Δ10 although these figures were not significant.

To assess the effect of adenoviral transduction on IFN-γ production in the mouse lung we carried out an ELISA. There was no significant difference between groups (Figure 7) so we can confidently say that anti-fibrotic effects observed are due to the overexpression of IL-13Rα2 in these experiments and not due to non-specific adenoviral induced anti-inflammatory anti-fibrotic properties of IFN-γ.

**Adenoviral overexpression of IL-13Rα2 and IL-13Rα2Δ10 inhibit soluble collagen production**

Bleomycin or PBS treated mice were anaesthetised and administered PBS, AV.IL-13Rα2, AV.IL-13Rα2Δ10 or AV.LacZ intranasally 3 days post bleomycin instillation. On day 10 and
14 lungs were harvested to assess the effects of IL-13Rα2 overexpression on collagen deposition in the lung. (Fig.8.) Bleomycin significantly induced soluble collagen compared to PBS treated controls at day 10 (Fig.8A). AV.IL-13Rα2 and IL-13Rα2Δ10 significantly reduced soluble collagen in comparison to bleomycin alone treated mice. Bleomycin + Adv.LacZ treated mice had significantly more collagen than PBS treated controls and there was no difference between this group and bleomycin alone treated group showing it had no effect on collagen deposition. Day 14 (Fig.8B) saw a significant increase in soluble collagen in bleomycin treated mice as compared to PBS treated controls. Overexpression of IL-13Rα2 in the mouse lung had no effect on collagen deposition by day 14. This is most likely due to the dissipated IL-13Rα2 gene expression at this time as shown in Fig.6.

**Adenoviral overexpression of IL-13Rα2 and IL-13Rα2Δ10 does not affect TGFβ production.**

Day 3 post bleomycin administration mice were treated with PBS, AV.IL-13Rα2, AV.IL-13Rα2Δ10 and AV.LacZ. Lungs were harvested on day 10 and 14 to assess the effects of IL-13Rα2 on TGFβ production (Figure 9). There was no significant change between treatment groups.

**Adenoviral overexpression of IL-13Rα2 and IL-13Rα2Δ10 inhibits CCL17 chemokine production**

Day 3 post bleomycin administration mice were treated with PBS, AV.IL-13Rα2, AV.IL-13Rα2Δ10 and AV.LacZ. Lungs were harvested on day 10 and 14 to assess the effects of IL-13Rα2 on CCL17 chemokine production. (Fig.10) Lungs were homogenised as described and protein isolated. CCL17 production was measured by ELISA Figure 6 shows that on both day
10 and 14 bleomycin significantly induced CCL17 production verses control mice. CCL17 also increases over time in the bleomycin group showing disease progression. Adenoviral overexpression of IL-13Rα2 and its soluble splice variant IL-13Rα2Δ10 significantly reduced CCL17 production on both day 10 and 14. AV.LacZ had significantly higher CCL17 on day 14 compared to control mice showing it did not affect CCL17 production. We found similar results assaying for CCL22 but these were not as significant (Data not shown).
The current study has demonstrated that IL-13Rα2 overexpression attenuates the fibrotic response in bleomycin induced lung injury. When overexpressed in the lung using an adenovirus, IL-13Rα2 and its soluble splice variant IL-13Rα2Δ10 significantly reduced soluble collagen and the Th2 chemokine CCL17. These endpoints are crucial to the development of idiopathic pulmonary fibrosis. We have previously shown that CCL17 has a pivotal role in bleomycin induced pulmonary fibrosis. When CCL17 was neutralised in a bleomycin model of pulmonary fibrosis there was a significant reduction in lung collagen content and infiltrating leukocyte population in BAL fluid (3). CCL17 has been detected in the serum of IPF patients (23) and appears to be associated with an adverse outcome. CCL17 has recently been shown to be chemotactic for fibroblasts (14). Using an in vivo wound model in CCL17 transgenic mouse, they showed that the overexpression of CCL17 accelerated wound healing (14). This was associated with an increase of CCR4 positive fibroblasts in the wound tissue (14). We and others have found CCL17 to be localised to epithelial cells, and to be a major source of CCL17 in pulmonary fibrosis (3, 31). Our findings that epithelial cells show a significant upregulation of CCL17 with IL-13 stimulation, supports the role of the epithelial cell as a source of CCL17. The inhibition of CCL17 by IL-13Ra2 in epithelial cells and the bleomycin model is further support for the role of Th2 chemokines in the fibrotic response. We also observed that CCL17 was reduced at both day 10 and 14 following bleomycin induction of lung injury when IL-13Ra2 was overexpressed, while collagen was only reduced at day 10. This may suggest that CCL17 is a critical biomarker of epithelial injury rather than causative in the process.

The overexpression of IL-13Ra2 reduced the production of active TGF-β by epithelial cells and fibroblasts. TGF-β plays a pivotal role in the progression of fibrosis (18, 24) by...
collagen production, fibroproliferation, differentiation of fibroblasts to myofibroblasts and
epithelial to mesenchymal transition (4). Most studies have indicated that IL-13Ra2 is a
decoy receptor however it has also been suggested that IL-13 signals through IL-13Ra2 to
induce TGF-β and fibrosis progression (10). Silencing of IL-13Ra2 reduced TGF-β
production and collagen deposition in a murine model of bleomycin induced pulmonary
fibrosis (10). In contrast we have shown that IL-13Ra2 inhibits TGF-β expression in
epithelial cells and fibroblasts in response to IL-13. These apparently opposing findings may
be due to the fact that we overexpressed both the transmembrane and soluble receptor as
opposed to the silencing IL-13Ra2. Furthermore Fichtner Feigl et al. overexpressed IL-
13Ra2 in the MM6 macrophage cell line which does not constitutively express IL-13Ra2
whereas both the NIH-3T3 and MLE-12 cells do constitutively express the receptor. Here we
are suggesting that in our hands both the soluble and membrane bound IL-13Ra2 are
inhibitory receptors for IL-13.

Similarly, Wilson et al. found that IL-13Ra2 deficient mice did not have increased fibrosis in
the bleomycin model. They suggest that bleomycin induced pulmonary fibrosis is IL-13 and
IL-13Ra2 independent (29). Consistent with our findings, IL-13Ra2 deficiency has
previously been shown to lead to increases in collagen deposition and treatment with IL-
13Ra2 fusion protein has been shown to reduce collagen production(28). We show here that
the adenoviral overexpression of the IL-13Ra2 in the lung inhibits collagen deposition in the
bleomycin model. This was associated with attenuation of the IL-13 induced fibrotic
phenotype in key effector cells, namely the fibroblast and epithelial cell. This shows the
therapeutic potential that IL-13Ra2 and its soluble splice variant may have in the modulation
of a disease that currently has very limited treatment options. The IL-13Ra2-fc fusion protein
is another method of exploiting the inhibitory properties of IL-13Ra2. It has been used to
treat IL-13 induced IgE produced by peripheral blood mononuclear cells from flea allergen-
sensitized canines (26). Mentik-Kane et al. showed in a model of schistosomiasis that IL-
13Rα2 was elevated in serum post *S. mansoni* infection (19). They found that IL-13Rα2
deficiency resulted in decreased survival, increased granuloma size and increased liver
fibrosis (19). Recently Murray et al have elegantly demonstrated that targeting IL-13 directly
with tralokinumab also attenuates fibrosis and epithelial damage, highlighting the importance
of targeting this ligand/receptor complex (21).

Although the use of IL-13Rα2 in treating fibrosis has been previously shown (19), the genetic
overexpression of the receptor locally in the lung is a novel approach. The soluble variant of
IL-13Rα2 is not present in the human gene but the transmembrane receptor is cleaved and
solubilised, allowing it to act to inhibit IL-13. Previous studies have shown that fibroblasts
isolated from IPF patients have a hyperresponsiveness to IL-13, potentially due to the lack of
IL-13Rα2 regulation (13, 20). This shows the potential important role IL-13Rα2 plays in the
pathogenesis of fibrosis by limiting chronic IL-13 exposure.

In conclusion we have shown that overexpression of the transmembrane and soluble version
of IL-13Rα2 acts to modulate pulmonary fibrosis both at an *in vitro* and *in vivo* level.
Modulation of IL-13Rα2 expression may therefore represent a viable therapeutic option in
pulmonary fibrosis.
References:


Figure legends

Figure.1 Time course of cytosolic and secreted IL-13Rα2.V5 expression in NIH3T3 fibroblasts

NIH3T3 fibroblasts were seeded into a 12 well plate and left for 24hrs. Cells were then transfected with pcDNA3.1/IL-13Rα2.V5 for 24 and 48hrs. Whole cell lysates (A) and supernatants (B) were isolated and assayed for IL-13Rα2 using western blotting. Lysates were run on a 10% SDS-PAGE gel and transferred to an activated PVDF membrane. Membranes were probed with a primary anti V5 antibody and an anti mouse HRP-linked secondary. IL-13Rα2 was overexpressed in NIH3T3 fibroblasts possessing a V5 tag. 1.Reagent alone 2. Untransfected 3.Transfected 4. Transfected 5.Reagent alone 6. Untransfected 7.Transfected 8. Transfected. This is a representative blot.

Figure.2 IL-13Rα2 inhibits IL-13 induced collagen, TGF-β and CCL17 production in fibroblasts and epithelial cells.

NIH3T3 fibroblasts (A) and MLE-12 lung epithelial cells (B) were transfected with IL13Rα2.V5. Cells were serum starved for 6 hours and stimulated with IL-13. The cells were then assessed for soluble collagen using the Sircol soluble collagen assay 48 hours posts stimulation. IL-13 significantly induced collagen in fibroblasts and epithelial cells and was inhibited with IL-13Rα2 overexpression. Data measured in micrograms/ml.

NIH3T3 fibroblasts (C) and MLE-12 lung epithelial cells (D) were transfected with IL13Rα2.V5 and stimulated with IL-13. The cells were then assessed for active TGF-β using
ELISA (R and D systems) 48 hours post stimulation. IL-13 induced TGF-β in fibroblasts and epithelial cells and was inhibited with IL-13Rα2 overexpression. Data measured in pg/ml.

NIH3T3 fibroblasts (E) and MLE-12 lung epithelial cells (F) were transfected with IL13Rα2.V5 and stimulated with IL-13. The cells were then assessed for CCL17 using ELISA (R and D systems) 48 hours post stimulation. CCL17 was induced in epithelial cells upon IL-13 stimulation and inhibited with IL-13Rα2 overexpression. Data measured in pg/ml.

Statistical analysis used is Mann Whitney U test * P<0.05 ** P<0.01 ***P<0.001. n= minimum of 6

**Figure 3** IL-13Rα2 overexpression affects IL-13 signaling via Stat6

NIH3T3 fibroblasts were transiently transfected with IL-13Rα2.V5. or IL-13Rα2Δ10.V5. Cells were serum starved for 24h and stimulated with IL-13 for 0, 5, 15, 30, 60 and 120 minutes. Whole cell lysates were isolated and run on a 10% SDS-PAGE gel and transferred to an activated PVDF membrane. Membranes were probed with a primary antibody for Stat6 and phospho-Stat6 and appropriate secondary antibody. A=untransfected B=IL-13Rα2.V5 transfected C=IL-13Rα2Δ10.V5 transfected.

**Figure 4. Basal expression of the IL-13 Receptor complex in NIH3T3 cells and C57BL/6 mouse lung**

RNA was isolated from NIH3T3 cells and from the lungs of C57Bl/6 mice. RNA was reverse transcribed into cDNA and real time PCR was carried out for the IL-13 receptor complex components; IL-13Rα1, IL-13Rα2 and IL4Rα. Endogenous gene expression in NIH3T3 Fibroblasts (n=5) and C57BL/6 mice (n=6) is graphed as ΔCt compared to endogenous control of 18SrRNA.
Figure 5. Adenoviral overexpression of IL-13Ra2 in HEK cells

HEK 293 cells were cultured in a 12 well plate and infected with Adv.IL-13Ra2 and splice variant Adv.IL-13Ra2∆10. Whole cell lysates (A) and supernatants (B) were isolated and run on a 10% SDS-PAGE gel and transferred to an activated PVDF membrane. Membranes were probed with a primary antibody for IL-13Ra2 and a HRP-linked anti goat secondary.


Figure 6. Adenoviral overexpression of IL-13Ra2 in the lung.

3 days post Bleomycin administration Adenoviral IL-13Ra2 was administered intranasally to mice. Lungs were taken at Day10 (A) and 14 (B) to assess IL-13Ra2 overexpression. Whole lung RNA was isolated using the TRI reagent method. Complementary DNA was made and real time quantitative PCR performed using Taqman gene expression assays for IL-13Ra2. The ΔΔCT method was used along with 18s endogenous control. Adenoviral overexpression of IL-13Ra2 in the lungs was detected at day 10 but had dissipated by day 14.

*P=<0.05  **P=<0.01 Mann Whitney U Test.

Figure 7. Adenoviral over-expression of IL-13Ra2 in the mouse lung has no effect on IFN-γ production

Adenoviral IL-13Ra2 was administered intranasally to mice. Lungs were harvested to assess total lung IFN-γ protein levels. Lungs were homogenised in complete protease
inhibitor cocktail in PBS and protein isolated. IL-13Rα2 overexpression had no effect on IFN-γ production. IFN-γ was measured by ELISA in pg/ml.

Figure 8. Adenoviral overexpression of IL-13Rα2 reduces soluble collagen as measured on Day 10 and but not Day 14

3 days post Bleomycin administration Adenoviral IL-13Ra2 was administered intranasally to mice. Lungs were taken at Day 10 (A) and 14 (B) to assess soluble collagen content. Lungs were homogenised in complete protease inhibitor cocktail in PBS and assayed for soluble collagen using the Sircol soluble collagen assay. Overexpression of IL-13Rα2 inhibited soluble collagen production.

*P=<0.05 **p=<0.01 ***P=<0.001 Mann Whitney U Test

Figure 9. Adenoviral overexpression of IL-13Rα2 has no effect on TGFβ production

3 days post Bleomycin administration Adenoviral IL-13Ra2 was administered intranasally to mice. Lungs were taken at Day 10 and 14 to assess total lung TGFβ levels. Lungs were homogenised in complete protease inhibitor cocktail in PBS and protein isolated. IL-13Rα2 overexpression has no significant effect on TGFβ production. TGFβ was measured by ELISA in pg/ml.

Figure 10. Adenoviral overexpression of IL-13Rα2 inhibits CCL17 on Day 10 and 14 in bleomycin induced fibrosis

3 days post Bleomycin administration Adenoviral IL-13Ra2 was administered intranasally to mice. Lungs were taken at Day 10 and 14 to assess total lung CCL17 protein levels. Lungs
were homogenised in complete protease inhibitor cocktail in PBS and protein isolated. IL-13Rα2 overexpression inhibits CCL17 production. CCL17 was measured by ELISA in pg/ml.

*P=<0.05 **p=<0.01 Mann Whitney U Test.
Fig. 1

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50KDa
V5
Fig. 2

A

B

C

D

E

F

Fibroblasts

Epithelial cell
Fig. 3

A

B

C

GAPDH

Total STAT6

Phospho STAT6

0           5          15         30          60        120 mins IL-13

110kDa

37kDa

110kDa

37kDa

110kDa

0           5          15          30          60        120 mins IL-13
Fig. 4

IL-13Rα1

Target Ct value normalised to 18S

NIHST3, MLE-12, C57BL/6 Lung

IL13Rα2

Target Ct value normalised to 18S

NIHST3, MLE-12, C57BL/6 Lung

IL4Rα

Target Ct value normalised to 18S

NIHST3, MLE-12, C57BL/6 Lung

Fig. 4
Fig. 7
Fig. 9

(A) Day 10

(B) Day 14

Control, Control + Ra2, Control + D10, Control + LacZ, Bleo, Bleo + Ra2, Bleo + D10, Bleo + LacZ

pg/ml
Fig. 10

Day 10

Day 14

A

B