

Cytokine regulation of IL-13R α 2 and IL-13R α 1 in vivo and in vitro

Tao Zheng, MD,^a Zhou Zhu, MD, PhD,^a Wei Liu, MD,^a Chun Geun Lee, MD, PhD,^a
Qingsheng Chen, MD,^a Robert J. Homer, MD, PhD,^{b,c} and Jack A. Elias, MD^a

New Haven and West Haven, Conn

Background: IL-13 signals via a high-affinity receptor that includes IL-4R α and IL-13R α 1 and binds to the decoy receptor IL-13R α 2. The processes that regulate the expression of these receptor subunits, however, are poorly defined.

Objective: These studies were designed to define the regulation of IL-13R components by T_H2 and T_H1 cytokines in vivo and in vitro.

Methods: Northern analysis, in situ hybridization, RT-PCR analysis, and immunoprecipitation were used to define the expression of IL-13R α 1 and IL-13R α 2 in lungs from lung targeted overexpression mice and lung fragments and cells in culture.

Results: IL-13R α 2 and IL-13R α 1 mRNA were detected at modest levels in lungs from control mice. In contrast, transgenic IL-13 caused a marked increase in IL-13R α 2 and IL-13R α 1 mRNA; this was most prominent in airway epithelial cells and macrophages. The effects of IL-13 on IL-13R α 2 were associated with comparable increases in protein production and were mediated by a blood leukocyte-independent and IL-4R α -dependent mechanism. IL-13 stimulation of IL-13R α 1 was mediated via a blood leukocyte-dependent and partially IL-4R α -dependent pathway. These effects were not specific for IL-13, because transgenic IL-4, IL-10, and IFN- γ also stimulated IL-13R α 2 mRNA accumulation while stimulating—not altering and inhibiting—IL-13R α 1 mRNA accumulation, respectively. These regulatory events were mediated, at least in part, by direct effects of these cytokines, because IL-13, IL-4, and IFN- γ had similar effects on IL-13R α 2 and/or IL-13R α 1 in epithelial cells and macrophages in in vitro culture.

Conclusion: IL-13R α 2 and IL-13R α 1 are highly regulated in vivo and in vitro. These regulatory events might control IL-13 responses at sites of inflammation. (J Allergy Clin Immunol 2003;111:720-8.)

Key words: Interleukin 13, asthma, T_H2 cytokine, IL-13 receptor, IL-4, IL-10, IL-13 transgenic mouse

IL-13 is a pleiotropic product of a gene on chromosome 5 at q 31 in human beings and on chromosome 11 in mice that is produced in large quantities by T_H2 cells and lesser

Abbreviations used

CC10:	Clara cell 10-kd protein
Dox:	Doxycycline
HBSS:	Hanks' balanced salt solution
IL-13R:	IL-13 receptor
IL-4R:	IL-4 receptor
ISH:	In situ hybridization
NHBE:	Normal human bronchial epithelial cell
rh:	Recombinant human
rm:	Recombinant murine
rtTA:	Reverse tetracycline transactivator
tTS:	Transcriptional suppressor

quantities by T_H1 cells.¹ Early studies highlighted the similarities of IL-4 and IL-13, including their ability to upregulate MHC II and CD23 on macrophages,^{1,2} IgE synthesis by B cells³ and vascular cell adhesion molecule 1 on endothelial cells.⁴ It was subsequently demonstrated that IL-13 plays distinct roles in a variety of tissue responses, including the T_H2-dependent expulsion of parasites,⁵ hepatic fibrosis in schistosomiasis,⁶ and allergen-induced pulmonary inflammation and physiologic dysregulation.^{7,8} The importance of the latter is reflected in studies from our laboratory and others that demonstrated that IL-13 is a potent inducer of eosinophilic inflammation, mucus metaplasia, airway fibrosis, and airways hyperresponsiveness in the murine airway and that these effects are mediated, in part, by complex alterations in chemokines, matrix metalloproteinases, cathepsins, and transforming growth factor β 1.⁹⁻¹¹

The effects of IL-13 are mediated by a complex receptor (R) system that includes IL-4 receptor α (IL-4R α), IL-13 receptor α 1 (IL-13R α 1), and IL-13 receptor α 2 (IL-13R α 2).¹²⁻¹⁷ IL-13R α 1 binds IL-13 with low affinity. It also combines with IL-4R α to form a high-affinity IL-13 binding complex that renders a variety of cells, including B cells, basophils, eosinophils, mast cells, endothelial cells, fibroblasts, monocytes, epithelial cells, and smooth muscle cells, responsive to IL-13.^{18,19} IL-13R α 2 binds IL-13 with greater avidity than IL-13R α 1.¹⁹ IL-13R α 2 also exists in a soluble form,¹⁷ has a short cytoplasmic tail without intracellular signaling motifs,^{19,20} and does not confer tissue responsiveness to IL-13 in the presence of IL-4R α .^{19,21} IL-13R α 2 also exists as a preformed molecule that is largely intracellular and is rapidly mobilized to the cell surface by IFN- γ .²² As a result, it has been assumed that IL-13R α 2 functions as a decoy receptor that feeds back to control IL-13

From ^athe Section of Pulmonary and Critical Care Medicine, Department of Internal Medicine, and ^bthe Department of Pathology, Yale University School of Medicine, New Haven, and ^cPathology and Laboratory Medicine Service, VA-CT Health Care System, West Haven.

Supported by National Institutes of Health Grants RO1-HL-61904, RO1-HL-64242, RO1-HL-66571, and P50-HL-56389 (J. A. Elias).

Received for publication September 16, 2002; revised December 29, 2002; accepted for publication January 9, 2003.

Reprint requests: Jack A. Elias, MD, Department of Internal Medicine, Yale University School of Medicine, 333 Cedar Street—105 LCI, PO Box 208057, New Haven, CT 06520-8057.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1383

effects in tissues. Surprisingly, little else is known about the processes that regulate the expression of IL-13R α 2 or IL-13R α 1 in vivo or in vitro.

We hypothesized that the biologic effects of IL-13 are regulated by the ability of a number of inflammatory mediators to regulate IL-13 expression at sites of inflammation. To test this hypothesis, we used overexpression transgenic mice and tissue culture techniques to characterize the regulation of IL-13R α 2 and IL-13R α 1 in vivo and in vitro. These studies demonstrate that IL-13, IL-4, IL-10, and IFN- γ are potent regulators of the expression and production of IL-13R α 2 and/or IL-13R α 1 in vivo and in vitro.

MATERIALS AND METHODS

Overexpression transgenic mice

Constitutive and inducible overexpression transgenic mice were generated in our laboratories and used in these studies. In these mice the Clara cell 10-kd protein (CC10) promoter was used to target transgene expression to the lungs of C57BL/6 mice. In the constitutive CC10-IL-13, CC10-IL-4, and CC10-IL-10 mice, the CC10 promoter drives the expression of IL-13, IL-4, and IL-10, respectively. These mice were described previously.²³⁻²⁵

To allow IL-13 or IFN- γ to be expressed in a temporally regulated fashion, CC10-rtTA-tTS-IL-13 and CC10-rtTA-IFN- γ mice were used. These are dual transgenic mice that use the reverse tetracycline transactivator (rtTA) and doxycycline (dox) to activate transgene expression. In the CC10-rtTA-tTS-IL-13 mice, the tetracycline-controlled transcriptional suppressor (tTS) is also used to control basal transgene leak.²⁶ The transgenes in these animals were activated by putting dox in the animals' drinking water. In these experiments, 1-month-old mice were randomized to receive normal water or dox water for the desired interval. Dox was administered at 500 mg/L in 4% sucrose and kept in dark brown bottles to prevent light-induced degradation. The constructs have been previously described.^{10,26,27}

mRNA analysis

Each animal was anesthetized, a median sternotomy was performed, the pulmonary vasculature was perfused with PBS, and the heart and lung were removed en bloc. Total cellular RNA was obtained from the lungs through use of Trizol reagent (GIBCO BRL, Grand Island, NY) according to the manufacturer's instructions; this was followed by treatment with DNase I to eliminate DNA contamination. The levels of gene-specific mRNA were then assessed through use of Northern analysis or RT-PCR, as described previously by our laboratories.^{10,28,29} In the Northern blots, 217-, 549-, and 241-bp cDNA, encoding full-length IL-13R α 2, IL-13R α 1, and β -actin, respectively, were used. In the RT-PCR assays, RNA samples were reverse-transcribed and gene-specific primers were used to amplify selected regions of each target moiety. The following primers were used for IL-13R α 2 and IL-13R α 1: for mIL-13R α 2, 5'-CGC-ATT-TGT-CAG-AGC-ATT-GT-3' (sense) and 5'-CCA-AGC-CCT-CAT-ACC-AGA-AA-3' (antisense); for mIL-13R α 1, 5'-GGC-CAT-CCT-GCA-AAA-TAG-TGT-C-3' (sense) and 5'-GGG-TAT-CAT-CAT-TCT-GGT-CTC-C-3' (antisense). In all cases, the optimal numbers of cycles that produce a quantity of gene product that is directly proportional to the quantity of input mRNA was determined experimentally. To verify that equal amounts of undegraded RNA were added in each RT-PCR reaction, β -actin was used as an internal standard through use of primers and conditions previously described by our laboratory.¹⁰ Amplified PCR products were detected through use of ethidium bromide gel electrophoresis, quantitated electronically, and confirmed by nucleotide sequencing.

In situ hybridization

In situ hybridization (ISH) was undertaken as previously described by our laboratory.⁹ The murine IL-13R α 2 and IL-13R α 1 probes that were used were generated by cloning fragments of the mouse genes encoding these moieties into pBS II KS that contains T3 and T7 primer sequences flanking a multiple cloning site (Stratagene, La Jolla, Calif). To obtain the IL-13R α 2 fragment, the primers 5'-GCT-CTA-GAC-GCA-TTT-GTC-AGA-GCA-TTG-T-3' (sense) and 5'-TCG-CTC-GAG-CTT-TGA-GTC-TGG-CCC-TGT-GT-3' (antisense) with XbaI and XhoI restriction enzyme sites were used to amplify a 718-bp fragment from total lung RNA of IL-13 transgene-positive mice. To obtain the IL-13R α 1 fragment, the primers 5'-AGC-TCT-AGA-GTT-GGT-GCT-GCT-ACT-GTG-GA-3' (sense) and 5'-ATC-GCT-CTG-GTC-CCA-GCA-TTA-TCC-TTG-ACC-3' (antisense) with XbaI and XhoI restriction sites were used to amplify a 618-bp fragment from the same RNA pool. Sense and antisense RNA probes in the vector pBS II KS were transcribed in vitro, labeled with digoxigenin, and used in these studies.

Organ culture

Wild-type C57BL/6 mice were anesthetized, a median sternotomy was performed, the pulmonary vasculature was perfused with PBS, and lungs were removed en bloc. The lungs were then inflated with and immersed in Dulbecco modified Eagle medium (DMEM) supplemented with penicillin, streptomycin, and recombinant murine (rm) IL-13 (50 ng/mL) at 37°C for 24 hours. Whole lung RNA was obtained, and the levels of mRNA encoding IL-13R α 2 and IL-13R α 1 were assayed as described above.

Lung cell isolation, radiolabeling, and immunoprecipitation of IL-13R α 2

Lungs from wild-type C57BL/6 mice were obtained as described above and minced into small pieces. The resulting fragments were incubated in collagenase III (150 U/mL; 37°C; Sigma, St Louis, Mo) and DNase I (20 μ g/mL; Sigma) in Hanks' balanced salt solution (HBSS) for 1 hour and triturated through a metal screen into HBSS. This generated a cell suspension that was filtered through nylon mesh and underlaid with 30% Ficoll. After being washed twice, the cells were incubated and allowed to adhere to petri dishes (1×10^6 cells/mL) in RPMI medium for 2 hours. The cells were then rewashed with methionine- and cysteine-free RPMI (Collect, ICN, Costa Mesa, Calif) and incubated in methionine- and cysteine-free RPMI medium with ³⁵S translabel (50 μ ci/mL; ICN) in the presence and absence of rIL-13 (50 ng/mL) for 24 hours. At the end of this incubation period, the cells were lysed in cold RIPA lysing buffer (150 mmol/L NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, and 50 mmol/L Tris [pH 8.0], and protease inhibitor cocktail; Roche, Mannheim, Germany).

The incorporation of radiolabel was evaluated by immunoprecipitation. In these assays, radiolabeled cell lysates were centrifuged at 10,000g for 10 minutes at 4°C and the pellets were discarded. The resulting supernatants were precleared with goat IgG (Santa Cruz Biotechnology, Santa Cruz, Calif) and protein A/G PLUS-agarose conjugates (Santa Cruz Biotechnology). After centrifugation (2500 rpm, 5 minutes, 4°C), the supernatant was incubated with a polyclonal antibody against mIL-13R α 2 (2 μ g/mL; R&D Systems, Minneapolis, Minn) for 1 hour at 4°C; this was followed by incubation on a rocker platform for an additional hour with protein A/G Plus agarose conjugates. The immunoprecipitates were collected by centrifugation (2500 rpm, 5 minutes, 4°C), washed twice with cold PBS and once with iced buffer solution (50 mmol/L Tris-HCl [pH 7.0] and 0.3 mol/L NaCl), and analyzed through use of 12.5% SDS-PAGE gels under reducing conditions. After fixation in 10% acetic acid, the gels were soaked in fluorographic solution, dried, and exposed to radiographic films (hyperfilm-MP, Amersham Life Science, Piscataway, NJ).

In vitro studies

Normal human bronchial epithelial cells (NHBEs) were obtained from Clonetics, Inc (Walkersville, Md) and were cultured in BEGM medium (Clonetics/BioWhittaker) supplemented with penicillin and streptomycin according to the manufacturer's instructions. Once confluent identical cultures were randomized to receive recombinant human (rh) cytokine (rhIL-13, rhIL-4, or rhIFN- γ ; R&D Systems) or control medium. At the end of the incubation period, the cells were mechanically detached and cellular RNA was isolated and evaluated as described above. In selected experiments, confluent cells were incubated in methionine- and cysteine-free RPMI medium with ^{35}S translabel, and radiolabeled IL-13R α 2 was evaluated by immunoprecipitation, as described above.

RAW mouse macrophages were obtained from the American Type Culture Collection (Bethesda, Md) and cultured in DMEM in the presence and in the absence of rmIL-13 (10 ng/mL), rmIL-4 (50 ng/mL), or rmIFN- γ (10 ng/mL) for 4 to 48 hours. At the end of this interval, cellular RNA was isolated as described above.

Breeding of CC10-IL-13 mice with IL-4R α null mutant (-/-) mice

Breeding of CC10-IL-13 mice with IL-4R α null mutant (-/-) mice was accomplished through use of a 2-step protocol. In the first, CC10-IL-13 transgene(+) mice with normal IL-4R α loci (IL-13[+]/IL-4R α [+/+]) were bred with IL-13 transgene(-) mice with a null mutation of IL-4R α (IL-13[-]/IL-4R α [-/-]; Jackson Laboratories, Bar Harbor, Me.). The transgene(+) progeny of this cross were then bred with IL-13(-)/IL-4R α (+/-) mice. This provided the IL-13(-)/IL-4R α (+/+), IL-13(-)/IL-4R α (-/-), IL-13(+)/IL-4R α (+/+), and IL-13(+)/IL-4R α (-/-) mice that were used in these studies. IL-13R α 2 and IL-13R α 1 mRNA were evaluated in these mice as described above.

RESULTS

IL-13 regulation of IL-13R α 2 and IL-13R α 1 mRNA accumulation in vivo

To define the effects of IL-13 on IL-13R α 2 and IL-13R α 1 gene expression, we compared the levels of mRNA encoding these moieties in lungs from IL-13 transgene(+) mice and their transgene(-) sex- and age-matched littermate controls. As shown in Fig 1, levels of IL-13R α 2 and IL-13R α 1 mRNA that were near the limits of detection of our assays were detected in lungs from transgene(-) mice on normal water or dox water. The levels of mRNA encoding these moieties was not significantly altered in lungs from CC10-rtTA-tTS-IL-13 transgene(+) mice that received normal water. In contrast, marked increases in IL-13R α 2 and IL-13R α 1 mRNA were noted when IL-13 was induced through dox administration to these animals. These inductive events were appreciated at the earliest time point that was assessed (1 week). At the 4-week time point, IL-13 caused 7.25- and 4.16-fold increases in IL-13R α 2 and IL-13R α mRNA, respectively, in comparison with what was seen in control animals (Fig 1, B). Similarly, prominent findings were noted after 12 weeks of dox administration (data not shown). In accord with these findings, IL-13R α 2 and IL-13R α 1 mRNA were also markedly induced in CC10-IL-13 transgenic mice in comparison with transgene(-) littermate controls (Fig 1, C).

Localization of IL-13R α 2 and IL-13R α 1 mRNA in vivo

ISH was used to define the sites of IL-13R α 2 and IL-13R α 1 mRNA accumulation in lungs from IL-13 transgene(-) and transgene(+) animals. As shown in Figs 2 and 3, IL-13R α 2 and IL-13R α 1 mRNA were not readily appreciated in lungs from transgene(-) animals. In contrast, IL-13R α 2 and IL-13R α 1 mRNA were readily detected in macrophages and airway epithelial cells in the medium and large airways of CC10-IL-13 transgene(+) animals (Figs 2 and 3). Similar results were obtained with tissues from dox-treated CC10-rtTA-IL-13 transgenic mice (data not shown). These studies demonstrate that macrophages and airway epithelial cells are the major sites of IL-13R α 2 and IL-13R α 1 mRNA expression in lungs from IL-13-producing transgenic animals.

IL-4, IL-10, and IFN- γ regulation of IL-13R α 2 in vivo

To determine whether the ability to stimulate IL-13R α 2 was IL-13-specific, studies were undertaken to determine whether other T_H2 or T_H1 cytokines augmented IL-13R α 2 mRNA accumulation. We first compared the levels of mRNA encoding IL-13R α 2 in the lungs from 2-month-old CC10-IL-4 transgene(+) mice and wild-type littermate controls. This was followed by comparisons of the levels of IL-13R α 2 mRNA in lungs from control mice and mice in which IL-10 or IFN- γ was expressed in an organ-specific fashion. As shown in Fig 4, the levels of IL-13R α 2 were prominently augmented in lungs from transgenic mice expressing IL-4, IL-10, or IFN- γ . Interestingly, IL-4 stimulated, IL-10 did not alter, and IFN- γ inhibited the levels of IL-13R α 1 mRNA in these transgenic lungs (Fig 4). When viewed in combination, these studies demonstrate that IL-4, IL-10, and IFN- γ also augment IL-13R α 2 gene expression. They also demonstrate that IFN- γ , under appropriate circumstances, can simultaneously decrease IL-13R α 1 gene expression in vivo.

Role of IL-4R α in IL-13 stimulation of IL-13R α 2 and IL-13R α 1

To determine whether IL-4R α participates in the stimulation of IL-13R α 2 or IL-13R α 1 by IL-13, we compared the stimulation of IL-13R α 2 and IL-13R α 1 mRNA accumulation in IL-13 transgene(+) animals with (+/+) and (-/-) IL-4R α loci. As noted above, IL-13 was a potent inducer of IL-13R α 2 and IL-13R α 1 mRNA in IL-4R α -sufficient (IL-4R α [+/+]) mice (Fig 5, A). IL-13 did not stimulate IL-13R α 2 mRNA in the absence of IL-4R α (Fig 5, A). Surprisingly, IL-13 did stimulate IL-13R α 1 mRNA accumulation in mice that were deficient in IL-4R α . This induction, however, was not as potent as that in IL-4R α (+/+) mice (Fig 5, A). This indicates that IL-4R α plays a critical role in the pathway by which IL-13 stimulates IL-13R α 2 mRNA accumulation in vivo. It also suggests that IL-13 can stimulate IL-13R α 1 via a pathway that is only partially IL-4R α -dependent.

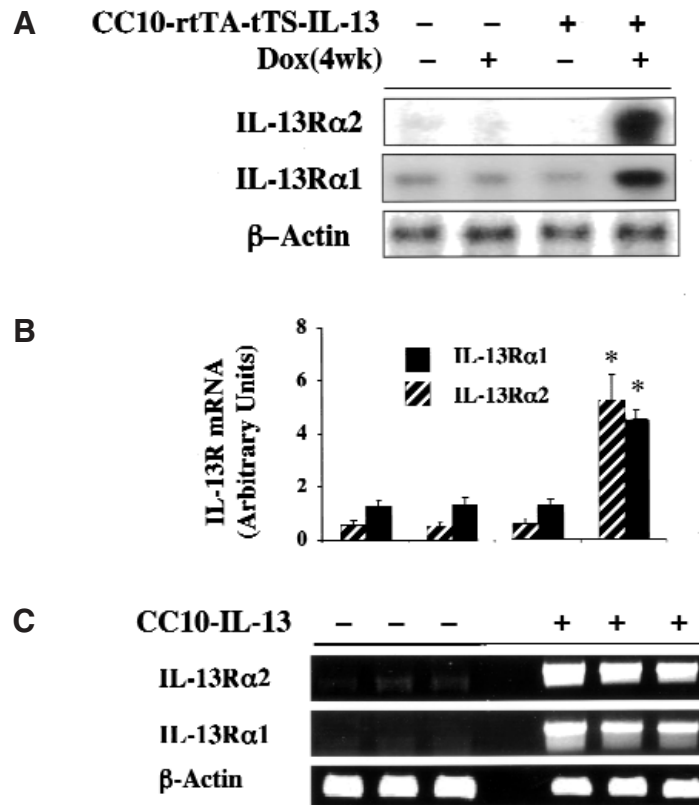


FIG 1. Effects of IL-13 on IL-13Rα2 and IL-13Rα1 mRNA accumulation in vivo. **A**, One-month-old CC10-rtTA-tTS-IL-13 mice were randomized to receive normal water or dox water for 4 weeks, and Northern blot analysis was used to quantitate IL-13Rα2, IL-13Rα1, and β-actin mRNA, as noted. **B**, Densitometric evaluations were undertaken of 5 replicate experiments (* $P < .01$ vs transgene(-) controls; Student t test). **C**, Whole lung RNA was obtained from CC10-IL-13 transgenic mice and transgene(-) littermate controls at 2 months of age, and RT-PCR was used to evaluate the levels of mRNA encoding IL-13Rα2, IL-13Rα1, and β-actin, as noted.

Role of blood-derived leukocytes in IL-13 stimulation of IL-13Rα2 and IL-13Rα1

To determine whether the effects of IL-13 on IL-13Rα2 and/or IL-13Rα1 required blood-derived inflammatory cells, we compared the levels of mRNA encoding these receptor subunits in isolated lung fragments incubated with rmIL-13 (50 ng/mL) or control medium. Recombinant IL-13 was a potent inducer of IL-13Rα2 mRNA accumulation in these tissues (Fig 5, *B*). In contrast, IL-13Rα1 was not similarly stimulated in this organ culture system (Fig 5, *B*).

Effects of IL-13, IL-4, and IFN-γ on NHBE cells and RAW macrophages in vitro

Because our ISH experiments demonstrated that epithelial cells and macrophages are prominent sites of IL-13R expression, studies were next undertaken to determine whether IL-13, IL-4, or IFN-γ stimulated IL-13R mRNA accumulation in NHBE cells or mouse RAW cells in vitro. The levels of IL-13Rα2 and IL-13Rα1 mRNA detected in these cells in the absence of cytokine stimulation was near the limits of detection with this

assay (Fig 6). In accord with our in vivo findings, rIL-13, rIL-4, and rIFN-γ stimulated IL-13Rα2 mRNA accumulation in a dose- and time-dependent fashion in both cell types (Fig 6 and data not shown). The stimulatory effects of IL-13, IL-4, and IFN-γ were seen after 4 hours of cell-cytokine incubation, were noted with doses of cytokine as low as 0.1 to 1 ng/mL, and were nearly maximal with ≥ 1 ng/mL doses of IL-13 or IFN-γ and ≥ 10 ng/mL doses of IL-4 (Fig 6 and data not shown). These effects were at least partially IL-13Rα2-specific, because rIL-13 did not alter the levels of mRNA encoding IL-13Rα1 in NHBE cells, whereas IL-4 and IFN-γ inhibited IL-13Rα1 mRNA accumulation in RAW cells (Fig 6).

Effects of IL-13 on IL-13Rα2 protein production

To determine whether IL-13-induced alterations in IL-13Rα2 mRNA were associated with comparable alterations in IL-13Rα2 protein production, lung mononuclear cells from wild-type mice and NHBE cells were incubated with ^{35} S-methionine and -cysteine for 24 hours in the presence and in the absence of rIL-13 (50 ng/mL). The labeled cells were then lysed, and IL-

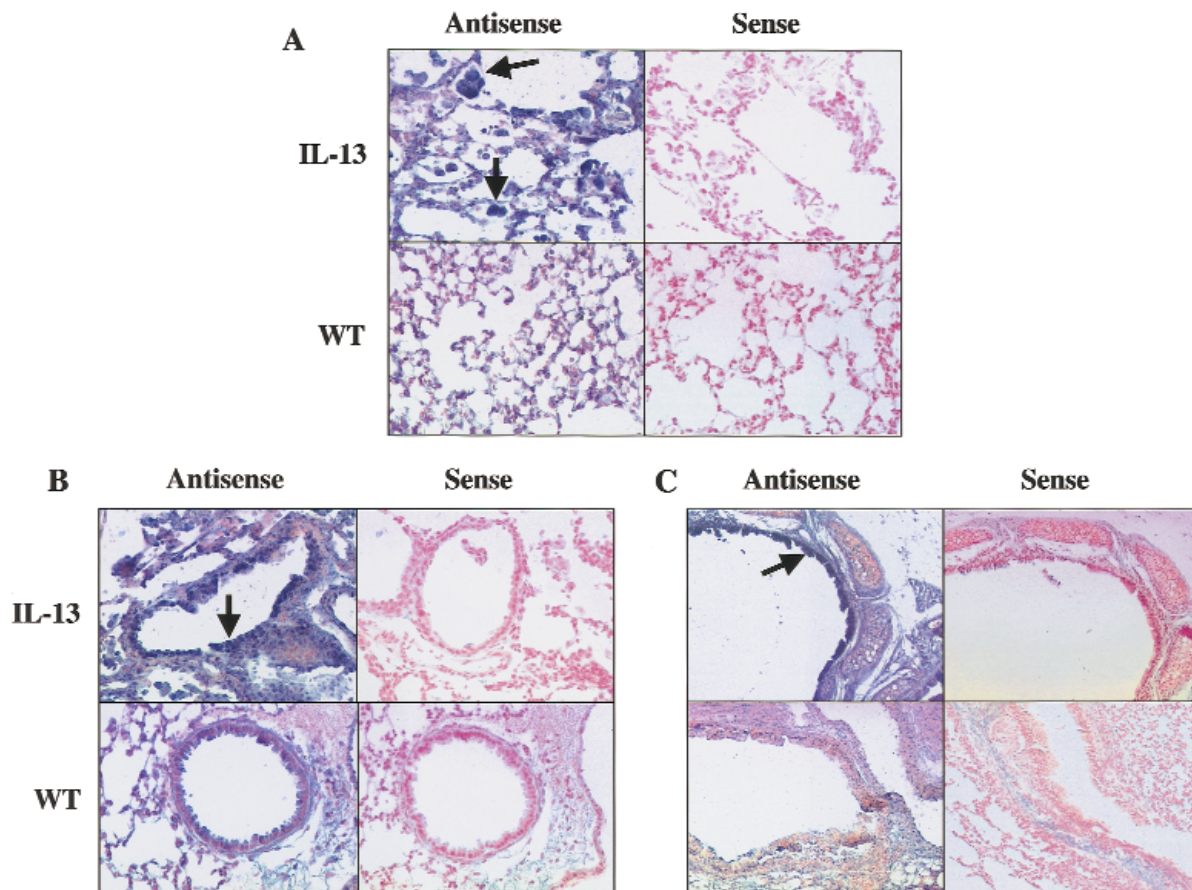


FIG 2. Localization of IL-13R α 2 mRNA accumulation in vivo. In each panel, ISH was used to compare the levels of IL-13R α 2 mRNA in CC10-IL-13 transgene(+) and transgene(-) (wild type [WT]) mice. Sense and antisense probes were used as described in the Methods section. Staining in macrophages (indicated by arrows in A), small airway epithelial cells (B), and large airway epithelial cells (C) can be seen.

IL-13R α 2 protein was analyzed by immunoprecipitation. As can be seen in Fig 7, cell lysates from IL-13-treated lung cells produced more IL-13R α 2 protein (56 kD) than lysates from cells incubated in control medium (Fig 7, A). Enhanced IL-13R α 2 protein (56 kD) production was also noted in NHBE cells treated with rIL-13 (Fig 7, B). When combined with our other findings, these studies demonstrate that IL-13 stimulates IL-13R α 2 mRNA accumulation and protein production.

DISCUSSION

IL-13 is a critical mediator of T_H2-dominated inflammation that has been implicated in the pathogenesis of asthma,^{7,8,25,30} chronic obstructive pulmonary disease (COPD),¹⁰ respiratory syncytial virus infection,³¹ fungal pneumonitis,³² and a variety of malignancies, including Hodgkin's lymphomas, glioblastoma multiforme, and pancreatic cancer.³³⁻³⁵ The protean effects of this cytokine are due, at least in part, to its ability to induce a complex cytokine-driven inflammatory response, cathepsin- and matrix metalloproteinase-mediated tissue proteolysis, and transforming growth factor β ₁-mediated tissue fibrosis.⁹⁻¹¹ The power of this cytokine can also be appre-

ciated in studies from our laboratory that demonstrated that the constitutive lung-specific overexpression of IL-13 causes a fibrodestructive pulmonary response which progresses to respiratory failure and premature death.¹¹ It is clear from these observations that tight control of IL-13 effector pathways is required to allow IL-13 to participate in beneficial host defense and repair responses without causing tissue pathology. This control is achieved, in part, by negative regulators of IL-13 signaling, including Shp-1, SOCS-1, and SOCS-3.³⁶⁻³⁸ In this investigation we tested the hypothesis that components of the IL-13R are dynamic structures whose expression is regulated at sites of inflammation. To test this hypothesis, studies were undertaken to define the cytokine network that regulates the expression of IL-13R α 2 and IL-13R α 1 in the murine lung and in vitro. These studies demonstrate for the first time that IL-13R α 2 is highly regulated in vivo, with IL-13, IL-4, IL-10, and IFN- γ stimulating IL-13R α 2 mRNA accumulation and protein production in macrophages and epithelial cells. They also demonstrate for the first time that IL-13R α 1 is regulated in vivo in the same cell populations in which it is stimulated by IL-13 and IL-4 and inhibited by IFN- γ . Finally, these studies demonstrate that

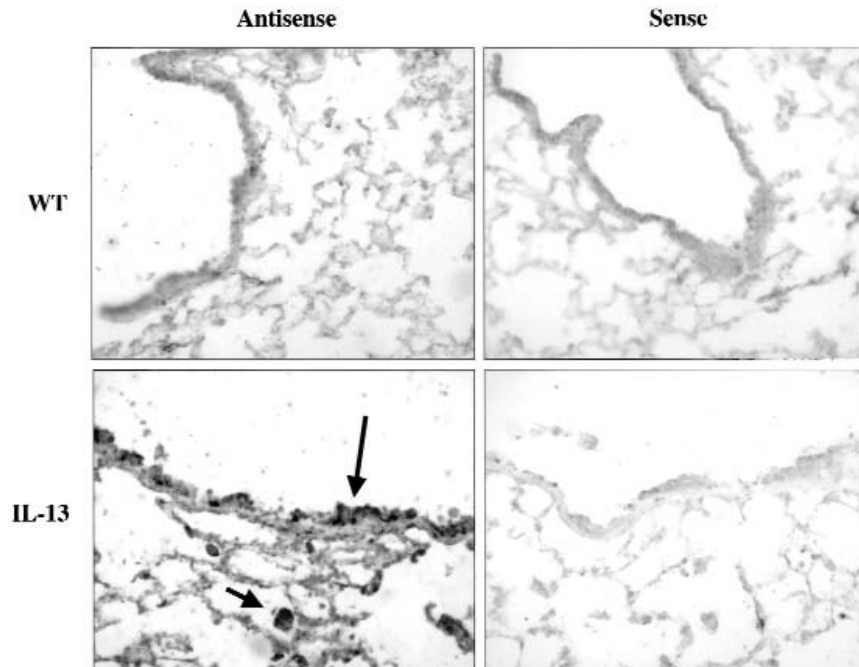


FIG 3. Localization of IL-13R α 1 in vivo. ISH was used to compare the levels of mRNA encoding IL-13R α 1 through use of sense and antisense probes. Epithelial staining (large arrows) and macrophage staining (small arrows) are highlighted.

many of the in vivo effects of IL-13, IL-4, and IFN- γ on IL-13R α 2 and IL-13R α 1 can be recapitulated in vitro through use of epithelial and macrophage culture systems. When viewed in combination, these studies demonstrate that IL-13R α 2 and IL-13R α 1 are highly regulated moieties. They also suggest that the levels of expression of these moieties can be adjusted through these mechanisms to fine-tune IL-13-induced tissue responses.

IL-13R α 2 binds avidly to IL-13 but does not bind IL-4 or other T_H2 cytokine moieties. Thus, at the inception of these studies, we speculated that IL-13 would be unique in its ability to regulate IL-13R α 2 expression. To our surprise, IL-4 and IL-10 also stimulated IL-13R α 2 in vivo. IL-13 and IL-4 both signal via the IL-4R α -IL-13R α 1 receptor complex (which is also called the *type II IL-4 receptor*). In addition, Rahaman et al³⁸ recently demonstrated that IL-13R α 2 controls IL-4 signaling in transformed keratinocytes. These observations suggest that the ability to stimulate IL-13R α 2 is a general property of T_H2 cytokines and a mechanism by which T_H2-dominated responses inhibit IL-13, IL-4, and T_H2 effector pathways. It is important to point out, however, that elevated levels of IL-13R α 2 would effectively compete with the IL-4R α -IL-13R α 1 complex for IL-13 ligand molecules, thereby decreasing the amount of IL-13 that binds to the type II IL-4 receptor and increasing the number of type II IL-4 receptors free to bind IL-4. If IL-13R α 2 does not inhibit IL-4 signaling in nontransformed cells, this could augment IL-4- (and T_H2-) mediated tissue responses. Additional experimentation will be required to define the effects of IL-13R α 2 on IL-4 effector function in complex T_H2-dominated tissue responses.

T_H1 cytokines such as IFN- γ have been demonstrated to inhibit the development of T_H2 tissue responses in a variety of experimental systems.^{39,40} Daines and Hershey²² recently demonstrated that IFN- γ can mobilize intracellular stores of IL-13R α 2 in cells in culture and that this mobilization is a mechanism by which IFN- γ can control IL-13-induced cellular responses. Our studies add to these observations in a number of ways. First, they demonstrate that IFN- γ , in addition to mobilizing pools of preformed IL-13R α 2, also stimulates IL-13R α 2 production through a pretranslational mechanism. This provides a potential mechanism by which IFN- γ can replenish these pools and thereby chronically inhibit IL-13-induced responses in cells and tissues. Second, our studies demonstrate that IFN- γ also inhibits the expression of IL-13R α 1. This would decrease the formation of IL-4R α -IL-13R α 1 receptor complexes and decrease IL-13 and IL-4 signaling (see below).

To obtain an enhanced understanding of the biology of IL-13, we used complex in vivo systems that allowed us to simultaneously assess the effects of cytokines on IL-13R α 1 and IL-13R α 2. These studies demonstrate that IL-13 and other IL-13R α 2-stimulating cytokines simultaneously regulate macrophage and epithelial cell IL-13R α 1, with IL-13 and IL-4 augmenting, IL-10 not altering, and IFN- γ inhibiting IL-13R α 1 gene expression under appropriate circumstances. This suggests that there are multiple ways in which the IL-13R can be modified to control IL-13 effector pathways in the lung. First, the ability of IL-13 and IL-4 to simultaneously augment IL-13R α 2 and IL-13R α 1 would serve to maintain IL-13 signaling and the IL-13 phenotype while ensuring that the magnitude of the

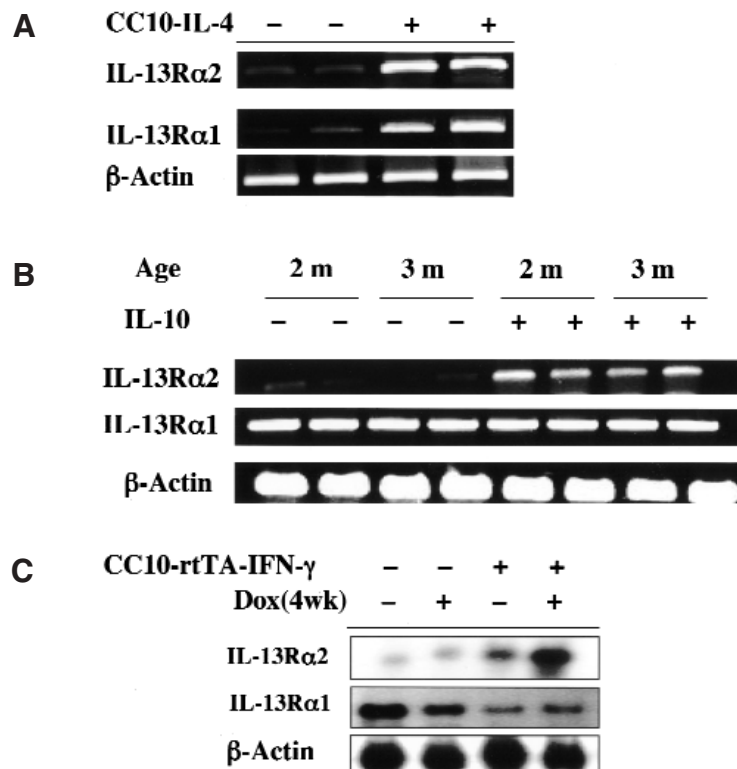


FIG 4. Effects of IL-4, IL-10, and IFN- γ on IL-13R α 2 and IL-13R α 1 mRNA accumulation. These are representative experiments of N = 4. The levels of mRNA encoding IL-13R α 2 and IL-13R α 1 were compared in lungs from 2-month-old CC10-IL-4 transgene(+) and transgene(-) mice (**A**) and 2- and 3-month (m)-old CC10-IL-10 transgene(+) and transgene(-) mice (**B**). Also illustrated are the levels of mRNA encoding IL-13R α 2 and IL-13R α 1 in lungs from CC10-rtTA-IFN- γ mice that have been randomized to receive normal water or dox water at 1 month of age and were maintained on this regimen for 4 weeks (**C**).

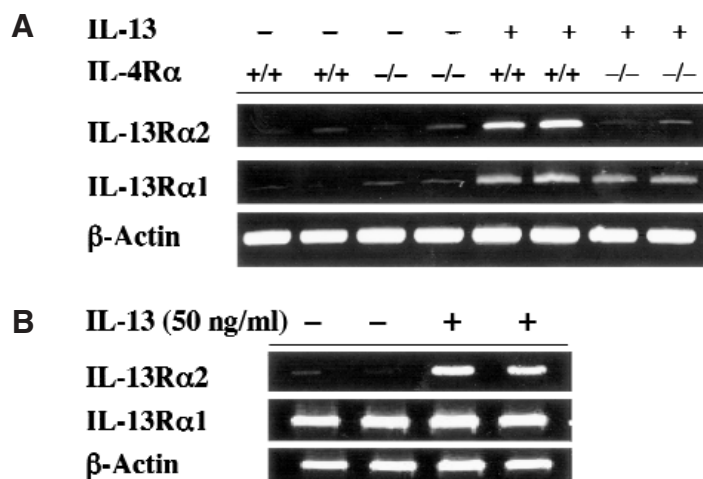


FIG 5. Role of IL-4R α and blood leukocytes in IL-13 regulation of IL-13R α 2 and IL-13R α 1. **A**, The levels of mRNA encoding IL-13R α 2 and IL-13R α 1 in lungs from 2-month old transgene(+) CC10-IL-13 mice that had (+/+) or (-/-) (null mutant) IL-4R α loci were evaluated by means of RT-PCR. **B**, Lung organ cultures were established from lungs of wild-type mice and incubated in the presence and in the absence of rIL-13, as noted. The levels of mRNA encoding IL-13R α 2 and IL-13R α 1 were evaluated by means of RT-PCR.

IL-13 effect is within the bounds of what is physiologic and appropriate. In this setting, IL-13R α 2 does not appear to be designed to "shut off" IL-13 effector pathways. Instead, it might be serving as a braking mechanism or

"surge protector" that minimizes the dangerous effects of high levels of IL-13. Second, the ability of IL-10 to selectively stimulate IL-13R α 2 would serve to dampen IL-13 tissue responses. In contrast, IFN- γ stimulated IL-13R α 2

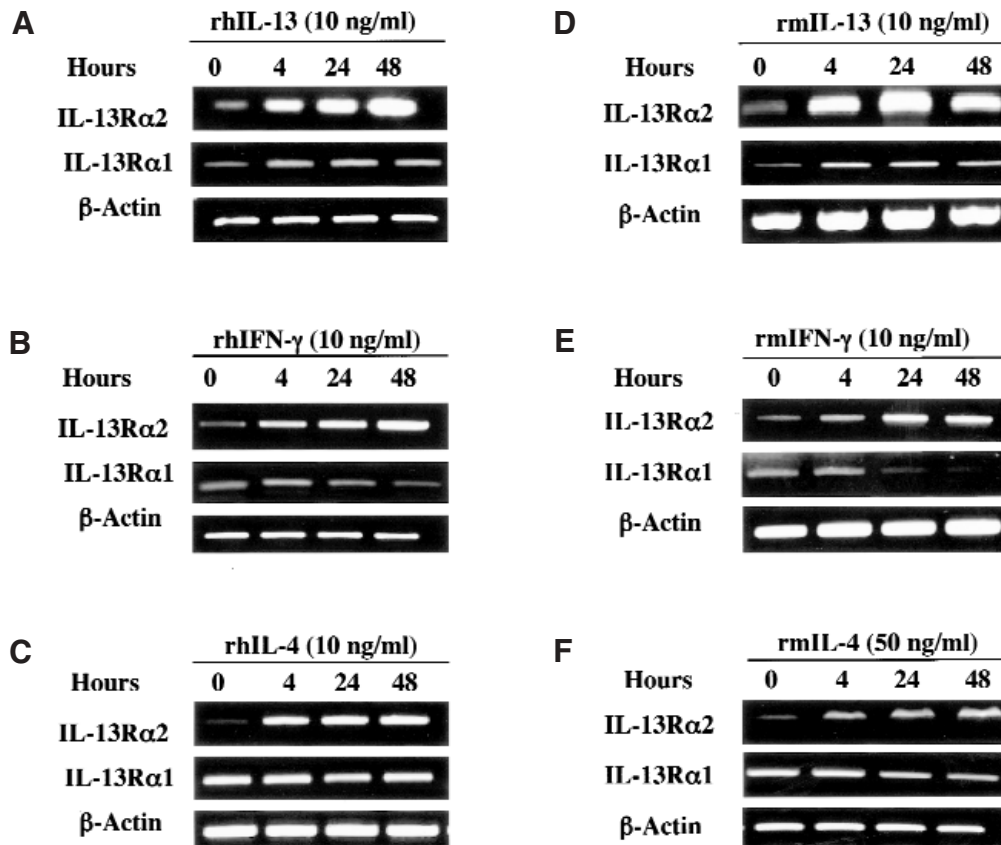


FIG 6. Effects of IL-13, IFN- γ , and IL-4 on the levels of mRNA encoding IL-13R α 2 and IL-13R α 1 in NHBE cells and RAW cells. **A** through **C**, Representative experiments (N = 3) defining the kinetics of the effects of human (h) cytokines on NHBE cells. **D** through **F**, Representative experiments (N = 3) defining the effects of murine (m) cytokines on RAW cells.

while inhibiting the expression of IL-13R α 1. The combined effects of these alterations would be expected to “shut off” or significantly inhibit IL-13 signaling. It is thus easy to see how these regulatory events could contribute to the ability of IFN- γ to control T_H2 inflammation and the anti-inflammatory properties of IL-10.

IL-13 generates an eosinophil-, macrophage-, and lymphocyte-rich inflammatory response in the murine lung.²⁵ To gain insight into the role of this response in the induction of IL-13R α 2, we sought to determine whether IL-13 could stimulate IL-13R α 2 or IL-13R α 1 in isolated organ fragments. IL-13 was a potent stimulator of IL-13R α 2, but not of IL-13R α 1, in lung organ cultures. When combined with our demonstration that the ability of IL-13 to stimulate IL-13R α 1 and IL-13R α 2 was decreased in lungs from animals that were deficient in IL-4R α , these studies demonstrate that IL-13 stimulates IL-13R α 2 and IL-13R α 1 through blood leukocyte-independent/IL-4R α -dependent and blood leukocyte-dependent/partially IL-4R α -dependent mechanisms, respectively.

In summary, our studies demonstrate that IL-13R α 2 and IL-13R α 1 are highly regulated molecules that are controlled in vivo and in vitro by IL-13, IL-4, IL-10, and IFN- γ . These findings highlight mechanisms that can modulate the tissue effects of IL-13 in the setting of T_H2

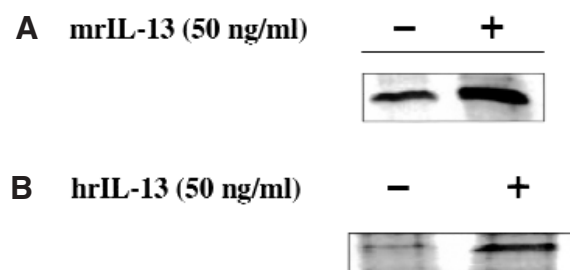


FIG 7. Effects of IL-13 on IL-13R α 2 protein production. These are representative experiments of N = 3. **A**, Mononuclear cells were isolated from lungs from wild-type mice and incubated with ³⁵S-translabel in the presence (+) and in the absence (-) of rmIL-13 (50 ng/mL). **B**, NHBE cells were incubated with ³⁵S-translabel in the presence and in the absence of rhIL-13 (50 ng/mL) for 24 hours. The amount of IL-13R α 2 protein that was produced during these intervals was evaluated by means of immunoprecipitation, as described in the Methods section.

inflammation and mechanisms by which IFN- γ and IL-10 can inhibit local IL-13 and or T_H2 responses. Given that IL-13 has been implicated in the pathogenesis of a variety of diseases, including asthma, COPD, scleroderma, pulmonary fibrosis, cancer, and viral and fungal infections, additional investigation will be required to determine whether the regulatory responses described in this article

or polymorphisms that alter these regulatory responses contribute to the pathogenesis of these disorders.

We thank the investigators and institutions that provided the reagents that were used, and we thank Susan Ardito and Kathleen Bertier for their excellent secretarial assistance.

REFERENCES

- Minty A, Asselin S, Bensussan A, Shire D, Vita N, Vyakarnam A, et al. The related cytokines interleukin-13 and interleukin-4 are distinguished by differential production and differential effects on T lymphocytes. *Eur Cytokine Netw* 1997;8:203-13.
- Zurawski G, de Vries JE. Interleukin-13, an interleukin-4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today* 1994;15:19-26.
- Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-4.
- Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* 1995;154:799-803.
- Urban JF Jr, Noben-Trauth N, Donaldson DD, Madden KB, Morris SC, Collins M, et al. IL-13, IL-4R alpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. *Immunity* 1998;8:255-64.
- Chirramonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest* 1999;104:777-85.
- Grünig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
- Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-60.
- Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Kotliansky V, et al. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating TGF- β_1 . *J Exp Med* 2001;194:809-21.
- Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese R, et al. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000;106:1081-93.
- Zhu Z, Ma B, Zheng T, Homer RJ, Lee CG, Charo IF, et al. IL-13-induced chemokine responses in the lung: role of CCR2 in the pathogenesis of IL-13-induced inflammation and remodeling. *J Immunol* 2002;168:2953-62.
- Aman MJ, Tayebi N, Obiri NI, Puri RK, Modi WS, Leonard WJ. cDNA cloning and characterization of the human interleukin 13 receptor alpha chain. *J Biol Chem* 1996;271:29265-70.
- Caput D, Laurent P, Kaghad M, Lelias JM, Lefort S, Vita N, et al. Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor alpha chain. *J Biol Chem* 1996;271:16921-6.
- Gao PS, Mao XQ, Hopkin JM, Adra CN, Yang X, Shirakawa T. Functional significance of polymorphisms of the interleukin-4 and interleukin-13 receptors in allergic disease. *Clin Exp Allergy* 2000;30:1672-5.
- Hilton DJ, Zhang JG, Metcalf D, Alexander WS, Nicola NA, Willson TA. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc Natl Acad Sci U S A* 1996;93:497-501.
- Miloux B, Laurent P, Bonnin O, Lupker J, Caput D, Vita N, et al. Cloning of the human IL-13R alpha1 chain and reconstitution with the IL4R alpha of a functional IL-4/IL-13 receptor complex. *FEBS Lett* 1997;401:163-6.
- Zhang JG, Hilton DJ, Willson TA, McFarlane C, Roberts BA, Mortiz RL, et al. Identification, purification, and characterization of a soluble interleukin (IL)-13-binding protein. Evidence that it is distinct from the cloned IL-13 receptor and IL-4 receptor alpha-chains. *J Biol Chem* 1997;272:9474-80.
- Andrews R, Rosa L, Daines M, Khurana Hershey G. Reconstitution of a functional human type II IL-4/IL-13 receptor in mouse B cells: demonstration of species specificity. *J Immunol* 2001;166:1716-22.
- Donaldson DD, Elias JA, Wills-Karp M. IL-13 antagonism. In: Hansel T, Barnes P, editors. *Progress in respiratory research: new drugs in asthma, allergy and COPD*. Basel, Switzerland: Karger Press; 2001. p. 260-3.
- Murata T, Obiri NI, Puri RK. Structure of and signal transduction through interleukin-4 and interleukin-13 receptors [review]. *Int J Molec Med* 1998;1:551-7.
- Donaldson DD, Whitters MJ, Fitz LJ, Neben TY, Finnerty H, Henderson SL, et al. The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol* 1998;161:2317-24.
- Daines MO, Hershey GK. A novel mechanism by which interferon-gamma can regulate interleukin (IL)-13 responses. Evidence for intracellular stores of IL-13 receptor alpha -2 and their rapid mobilization by interferon-gamma. *J Biol Chem* 2002;277:10387-93.
- Lee CG, Homer RJ, Cohn L, Link H, Jung S, Craft JE, et al. Transgenic overexpression of IL-10 in the lung causes mucus metaplasia, tissue inflammation and airways remodeling via IL-13- dependent and independent pathways. *J Biol Chem* 2002;277:35466-74.
- Rankin JA, Picarella DE, Geba GP, Temann U-A, Prasad B, DiCosmo B, et al. Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc Natl Acad Sci U S A* 1996;93:7821-5.
- Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities and eotaxin production. *J Clin Invest* 1999;103:779-88.
- Zhu Z, Ma B, Homer RJ, Zheng T, Elias JA. Use of the tetracycline-controlled transcriptional silencer (TTS) to eliminate transgene leak in inducible overexpression transgenic mice. *J Biol Chem* 2001;276:25222-99.
- Wang Z, Zheng T, Zhu T, Homer RJ, Riese RJ, Chapman HA Jr, et al. Gamma interferon induction of pulmonary emphysema in the adult murine lung. *J Exp Med* 2000;192:1587-600.
- Yoon HJ, Zhu Z, Gwaltney Jr. JM, Elias JA. Rhinovirus regulation of IL-1 receptor antagonist in vivo and in vitro: a potential mechanism of disease resolution. *J Immunol* 1999;162:7461-9.
- Zheng T, Nathanson M, Elias JA. Histamine augments cytokine-stimulated interleukin-11 production by human lung fibroblasts. *J Immunol* 1994;153:4742-52.
- Kotsimbos TC, Ernst P, Hamid QA. Interleukin-13 and interleukin-4 are coexpressed in atopic asthma. *Proc Assoc Am Physicians* 1996;108:368-73.
- Johnson TR, Graham BS. Secreted respiratory syncytial virus (RSV) G protein induces IL-5, IL-13 and eosinophilia by an IL-4-independent mechanism. *J Virol* 1999;73:8485-95.
- Blease K, Jakubzik C, Westwick J, Lukacs N, Kunkel SL, Hogaboam CM. Therapeutic effect of IL-13 immunoneutralization during chronic experimental fungal asthma. *J Immunol* 2001;166:5219-24.
- Kawakami M, Leland P, Kawakami K, Puri RK. Mutation and functional analysis of IL-13 receptors in human malignant glioma cells. *Oncol Res* 2001;12:459-67.
- Kawakami K, Kawakami M, Snoy PJ, Husain SR, Puri RK. In vivo overexpression of IL-13 receptor alpha2 chain inhibits tumorigenicity of human breast and pancreatic tumors in immunodeficient mice. *J Exp Med* 2001;194:1743-54.
- Ohshima K, Akaiwa M, Umeshita R, Suzumiya J, Izuhara K, Kikuchi M. Interleukin-13 and interleukin-13 receptor in Hodgkin's disease: possible autocrine mechanism and involvement in fibrosis. *Histopathology* 2001;38:368-75.
- Haque SJ, Harbor P, Tabrizi M, Yi T, Williams BR. Protein-tyrosine phosphatase Shp-1 is a negative regulator of IL-4- and IL-13-dependent signal transduction. *J Biol Chem* 1998;273:33893-6.
- Haque SJ, Harbor PC, Williams BR. Identification of critical residues required for suppressor of cytokine signaling-specific regulation of interleukin-4 signaling. *J Biol Chem* 2000;275:26500-6.
- Rahaman SO, Sharma P, Harbor PC, Aman MJ, Vogelbaum MA, Haque SJ. IL-13R(alpha)2, a decoy receptor for IL-13 acts as an inhibitor of IL-4-dependent signal transduction in glioblastoma cells. *Cancer Res* 2002;62:1103-9.
- Cohn L, Homer RJ, Niu N, Bottomly K. T helper 1 cells and interferon gamma regulate allergic airway inflammation and mucus production. *J Exp Med* 1999;90:1309-18.
- Ford JG, Rennick D, Donaldson DD, Venkayya R, McArthur C, Hansell E, et al. IL-13 and IFN-gamma: interactions in lung inflammation. *J Immunol* 2001;167:1769-77.