Interleukin (IL)-13 mediates its activities via a complex receptor system. Interleukin-13 receptor α-1 chain (IL-13Rα1) binds IL-13 with low affinity, but does not signal. However, when IL-13Rα1 combines with IL-4 receptor α (IL-4Rα), a signaling high affinity receptor complex for IL-13 is generated. In contrast, IL-13Rα2 alone binds IL-13 with high affinity, but does not signal and has been postulated to be a decoy receptor. Herein, we investigated the cellular localization of IL-13Rα2 and the regulation of its expression by confocal microscopy and flow cytometry in primary and cultured cells. Our results demonstrate that IL-13Rα2 is largely an intracellular molecule, which is rapidly mobilized from intracellular stores following treatment with interferon (IFN)-γ. Up-regulation of IL-13Rα2 surface expression in response to IFN-γ was rapid, did not require protein synthesis, and resulted in diminished IL-13 signaling. These results provide the first evidence that the IL-13Rα2 is predominantly an intracellular molecule and demonstrate a novel mechanism by which IFN-γ can regulate IL-13 responses.

Interleukin (IL)-13 is an immunoregulatory cytokine secreted predominantly by activated Th2 cells (1, 2). IL-4 and IL-13 are related cytokines that belong to the same α-helix superfamily, and their respective genes are located on chromosome 5q31 only 12 kb apart (3). Although they share only 25% homology, IL-13 shares many functional properties with IL-4, including the up-regulation of major histocompatibility complex class II and CD23 antigens on monocytes (1, 3), the induction of IgE synthesis and CD23 expression by B cells (4), and the induction of vascular cell adhesion molecule-1 on endothelial cells (5). Although IL-4 and IL-13 have many overlapping functions, they also have distinct roles. In parasitic infection models, IL-13 has a critical role in the Th2-dependent expulsion of Nippostrongylus brasiliensis (6). Furthermore, IL-13 has recently been shown to be a key mediator of allergic inflammation independent of IL-4 in mouse models whereby IL-13 blockade prevented allergen-induced airway inflammation (7, 8).

IL-13 mediates its effects via a complex receptor system that includes IL-4Rα (IL-4 receptor α chain) and at least two other cell surface proteins, IL-13Rα1 and IL-13Rα2 (9–14). Both IL-13Rα1 and IL-13Rα2 are members of the hematopoietin receptor superfamily and share 37% homology at the amino acid level. Their respective genes have been mapped to the X chromosome, and in vitro expression of IL-13Rα1 and IL-13Rα2 has revealed that they both specifically bind IL-13 (9–14). IL-13Rα1 binds IL-13 with low affinity by itself, but, when paired with IL-4Rα, it binds IL-13 with high affinity and forms a functional IL-13 receptor which signals (12). In cells lacking the common γ chain, this receptor complex also serves as an alternative receptor for IL-4. Consistent with the fact that IL-4 and IL-13 share common subunits, they also share common signaling pathways. Studies in Stat6-deficient mice have revealed that IL-13 signaling utilizes the JAK-Stat pathway and specifically Stat6 (15, 16). Signaling through IL-4Rα/IL-13Rα1 is thought to occur via IL-4Rα, because both IL-4 and IL-13 stimulation of the complex results in activation of signaling intermediates characteristic of IL-4 responses, including phosphorylation of IL-4Rα, insulin-receptor substrate-2, JAK1, and Tyk2 (2, 17).

However, the precise roles of IL-13Rα1 and IL-13Rα2 in IL-13 signaling and responses are not clear. Expression of IL-13Rα1 and IL-4Rα together is sufficient to render cells responsive to IL-13, although both subunits must be derived from the same species (18). Thus, IL-13Rα2 is not required for IL-13 function. Expression of IL-13Rα2 in vitro was insufficient to render cells responsive to IL-13, despite the presence of IL-4Rα (19). IL-13Rα2 has a short cytoplasmic tail (17 amino acids in the human), which contains no box 1 or box 2 signaling motifs, supporting that it has no signaling function. The inability of IL-13Rα2 expression to confer IL-13 responsiveness despite high affinity binding, along with the finding of soluble IL-13Rα2 in vivo (13), has led to the speculation that IL-13Rα2 acts as a decoy receptor. IL-13Rα2 transcripts have been found in the spleen, liver, lung, thymus, and brain (14, 19); however, very little is known about its expression or regulation. IL-13 receptors are expressed on human B cells, basophils, eosinophils, mast cells, endothelial cells, fibroblasts, monocytes, macrophages, respiratory epithelial cells, and smooth muscle; however, functional IL-13 receptors have not been demonstrated on human or mouse T cells. Thus, unlike IL-4, IL-13 does not appear to be important in the initial differentiation of
CD4 T cells into Th2 type cells, but rather appears to be important in the effector phase of allergic inflammation. This is further supported by many observations in vivo, including the following; administration of IL-13 resulted in allergic inflammation (7, 8), tissue-specific overexpression of IL-13 in the lungs of transgenic mice resulted in airway inflammation and mucus hypersecretion (20), IL-13 blockade abolished allergic inflammation independently of IL-4 (7, 8), and IL-13 appears to be more important than IL-4 in mucus hypersecretion (21). Given the importance of IL-13 as an effector molecule, regulation of the expression level of its receptors at the cell surface may be an important mechanism of modulating IL-13 responses. In this report, we examine the cellular localization of IL-13Rα2 and investigate the regulation of its surface expression.

EXPERIMENTAL PROCEDURES

Cells and Reagents—U937 cells were obtained from the ATCC and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Invitrogen), 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Bio-Whittaker, Walkersville, MD). A549 cells, a gift from Dr. James Stark (University of Missouri, Columbia, MO), were maintained in Dulbecco’s modified Eagle’s medium (Bio-Whittaker) supplemented with 8% fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Human nasal epithelial cells were obtained as described previously (22). Briefly, cells harvested from normal human volunteers by gently scraping the inferior nasal turbinate with a sterile cytobrush. The material collected was treated with Sputolysin (Caldon Biotech, Carlsbad, CA) for 5 min, centrifuged for 5 min at 600 g, resuspended in bronchial epithelial growth medium (Bio-Whittaker), and transplanted to collagen-coated tissue culture flasks. Once grown to 80% confluence, cells were liberated from the flasks with trypsin and passed to additional flasks or coverslips for confocal microscopy. Where indicated, cells were incubated with phorbol 12-myristate 13-acetate (PMA, Fisher Scientific, Pittsburgh, PA), ATP (Sigma-Aldrich), or human interferon γ, a gift from Dr. Thomas Shanley (Children’s Hospital Medical Center, Cincinnati, OH).

Elutriated human monocytes, prepared as described previously (23), were kindly provided by Dr. Christopher Karp (Children’s Hospital Medical Center, Cincinnati, OH). They were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Flow Cytometry—U937 cells were washed with cold PBS with 1% fetal bovine serum (FBS) and stained with FITC-conjugated anti-human IL-13Rα monoclonal antibody (Diaclone, Besançon Cedex, France) or a FITC-conjugated murine IgG1 isotype control (PharMin, Conflans, France) or a FITC-conjugated murine IgG1 isotype control (PharMin, Conflans, France). A549 and primary human nasal epithelial cells were liberated from their flasks using Versene (Invitrogen), washed in cold PBS with 1% FBS, and then stained for IL-13Rα2. To assess intracellular IL-13Rα2, cells were permeabilized with PBS containing 1% FBS and 0.2% saponin. Saponin (0.2%) was also added to the antibody and wash solutions. Following the final wash, cells were resuspended in PBS with 1% FBS and analyzed in a FACScan or FACScalibur (Becton Dickinson, San Jose, CA).

For the cycloheximide treatment of U937 cells, U937 cells were cultured overnight (18 h) in the presence and absence of 10 μg/ml cycloheximide in RPMI with 5% FBS. Under these conditions, greater than 85% of protein synthesis was inhibited by [35S]methionine incorporation and over 85% viability is maintained at 48 h by trypan blue exclusion. IFN-γ was then added to a final concentration of 20 ng/ml for the indicated times. Cells were then washed and stained for surface expression of IL-13Rα2 as described above.

Confocal Microscopy—U937 cells in culture or human nasal epithelia cells or A549 cells grown on tissue culture-coated coverslips were washed in cold PBS with 1% FBS, fixed in 1% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA), and then stained with FITC-conjugated anti-human IL-13Rα2 antibody or the FITC-conjugated isotype control in a solution containing PBS, 1% FBS, and 3% cold fish gelatin (Sigma-Aldrich). In the case of permeabilized cells, 0.2% saponin was added to the antibody solution and to all subsequent washes. After the final wash, the U937 cells were resuspended in a minimal volume of Vectashield (Vector Labs, Burlingame, CA), placed on a slide, covered with a coverslip, and the edges sealed with nail polish. Alternatively, the cells on coverslips were inverted onto a slide with a small drop of Vectashield and the edges sealed with nail polish. Cells were observed on a Leica DM IRBC confocal microscope (Leica Microsystems, Heidelberg, Germany).

Electrophoretic Mobility Shift Assay (EMSA)–EMSA analyses were performed as previously described (18, 24). Samples were electrophoresed on a 4% polyacrylamide gel for 100 min. Bands were visualized by autoradiography, and densitometry performed to quantify the Stat6 activity in the samples.

RESULTS

IL-13Rα2 Is Largely Intracellular in U937 Cells—IL-13 treatment of U937 cells results in rapid Stat6 activation and increased CD23 expression (data not shown); thus, U937 cells express functional IL-13 receptors. Although it is known that a functional IL-13 complex requires both IL-4Rα and IL-13Rα1, the function of the IL-13Rα2 is not well understood. We investigated whether U937 cells expressed IL-13Rα2 and determined the relative amount of IL-13Rα2 on the cell surface relative to the intracellular compartment. Intact and permeabilized U937 cells were stained with antibodies specific for IL-13Rα2 and analyzed by flow cytometry (Fig. 1 A). Intact cells expressed modest levels of IL-13Rα2. Strikingly, staining of permeabilized cells was nearly 20 times more intense than the intact cells (Fig. 1 B), demonstrating that IL-13Rα2 is a predominantly intracellular rather than a membrane protein in U937 cells.

To further investigate the cellular location of IL-13Rα2, confocal microscopy was performed on intact or permeabilized U937 cells (Fig. 1 C). Intact cells demonstrated rim staining with the IL-13Rα2 antibody consistent with a low level of surface expression. In contrast, permeabilized cells showed bright intracellular staining, confirming the predominant intracellular location of IL-13Rα2. There was no significant staining with the isotype control in intact or permeabilized cells.

IFN-γ Stimulation of U937 Cells Results in Up-regulation of IL-13Rα2 Surface Expression—Having established that IL-13Rα2 is present mostly intracellularly in U937, we next wanted to determine whether this intracellular pool or receptors could be mobilized to the surface. IL-13 has been shown to modulate the IL-13Rα1 expression in several cell types (25), so we first examined whether IL-13 had similar effects on IL-13Rα2 expression. However, IL-13 did not modulate IL-13Rα2 expression in U937 or A549 cells (data not shown). We next examined whether nonspecific activation of cells with PMA would alter IL-13Rα2 expression. As shown in Fig. 2 A, PMA treatment of U937 cells resulted in a dose-dependent increase in IL-13Rα2 surface expression. IFN-γ acts as a counter-regulator of IL-13 in many systems and down-regulates IL-13 responses (26, 27), although the mechanism for this modulation is not well understood. One possible mechanism is that IFN-γ modulates IL-13 receptor expression. If IL-13Rα2 is a decoy receptor as has been postulated, then we reasoned that IFN-γ may up-regulate this receptor to counteract the IL-13 response. IFN-γ treatment of U937 cells resulted in increased surface expression of IL-13Rα2 in a dose-dependent manner (Fig. 2 B). Maximal up-regulation following stimulation with IFN-γ was seen with physiologic doses of 20 ng/ml. Thus, IFN-γ stimulation leads to up-regulation of surface IL-13Rα2 in U937 cells.

Up-regulation of IL-13Rα2 on U937 Cells Following IFN-γ Stimulation Is Rapid and Persistent—We next examined the kinetics of IFN-γ induced up-regulation of surface IL-13Rα2 (Fig. 2 C). IFN-γ treatment resulted in a rapid increase in the surface expression of IL-13Rα2. The increase was detectable at 4 h and reached maximum levels at 24 h. Longer incubation periods revealed that IL-13Rα2 surface expression returned to near base-line levels after 8 days (data not shown). There was interexperimental variation noted in the magnitude of en-
enhanced IL-13Rα2 surface expression induced by IFN-γ with a range of 60–250% maximal increase in IL-13Rα2 surface expression.

We investigated whether IFN-γ-dependent IL-13Rα2 cell surface trafficking was accelerated by stimulating rapid calcium-dependent exocytic mechanisms in U937 cells. Cells were treated with ATP, a known agonist for G-protein-coupled calcium mobilization on U937 cells (28), in the presence or absence of IFN-γ. Treatment with ATP had no effect on the kinetics or magnitude of IL-13Rα2 mobilization (Fig. 3). Similar results were obtained with a calcium ionophore (data not shown).

IFN-γ-dependent Up-regulation of IL-13Rα2 Does Not Require Protein Synthesis—Although the IFN-γ effect on IL-13Rα2 surface expression was rapid, it remained possible that the observed IFN-γ-dependent increase in IL-13Rα2 surface expression was not because of mobilization of preformed molecules in intracellular compartments, but rather because of novel protein synthesis. To address this, we utilized the protein synthesis inhibitor, cycloheximide. Cycloheximide had no effect on the magnitude (Fig. 4A) or kinetics (Fig. 4B) of IFN-γ-dependent up-regulation of surface IL-13Rα2. Thus, IFN-γ results in mobilization of preformed IL-13Rα2 molecules and does not require protein synthesis.

Up-regulation of Surface IL-13Rα2 by IFN-γ Results in Diminished IL-13-dependent Stat6 Activation—We next examined what effect IL-13Rα2 trafficking to the cell surface had on IL-13 signaling. Cells were treated with IFN-γ for 24 h to achieve maximum cell surface trafficking of IL-13Rα2, and then cells were stimulated with IL-13 for 20 min and assayed for Stat6 activation (Fig. 5). Cells pre-treated with IFN-γ displayed markedly diminished Stat6 activation and decreased sensitivity to IL-13 when compared with IL-13-treated cells that had not received IFN-γ. Thus, increased surface IL-13Rα2 expression correlates with decreased magnitude and sensitivity of IL-13 signaling.

IL-13Rα2 Is Largely Intracellular in A549 Respiratory Epithelial Cells and Primary Nasal Epithelial Cells—We next investigated whether the predominance of IL-13Rα2 in intracellular compartments was unique to the U937 cell line by investigating the cellular localization of IL-13Rα2 in another cell type.
Mobilization of Intracellular IL-13Rα2 by IFN-γ

Fig. 3. ATP has no effect on the kinetics or magnitude of IL-13Rα2 mobilization alone or with IFN-γ. Panel A, U937 cells were incubated with 250 units/ml IFN-γ alone (solid circles, dashed line) or with 250 units/ml IFN-γ and 100 nm ATP (empty circles, solid line) for the indicated times, and surface IL-13Rα2 expression determined by flow cytometry. Means and error bars are from three independent experiments. Panel B graphically depicts results from cells incubated with IFN-γ or media alone for 24 h, followed by the addition of 100 nm ATP for the indicated times. Surface expression of IL-13Rα2 was then determined by flow cytometry. MCF, mean channel fluorescence.

Fig. 4. IFN-γ-dependent increased IL-13Rα2 surface expression is not dependent on protein synthesis. Panel A, U937 cells were pretreated overnight in the presence of medium alone or medium containing 10 μg/ml cycloheximide, and then 20 ng/ml IFN-γ was added to the cultures in the continued presence or absence of cycloheximide (CHX). After 18 h, surface expression of IL-13Rα2 was determined by flow cytometry. For both cycloheximide-treated and untreated cells, the dotted line represents autofluorescence, the solid line represents the isotype matched control, IL-13Rα2 staining is depicted by the bold line, and the dashed line represents enhanced IL-13Rα2 expression following stimulation with IFN-γ. Panel B demonstrates the kinetics of IFN-γ-dependent IL-13Rα2 up-regulation in the presence (triangles) or absence (circles) of 10 μg/ml cycloheximide. Data point averages and error bars are derived from three experiments. MCF, mean channel fluorescence.

Fig. 5. IFN-γ pretreatment of U937 cells results in diminished IL-13-dependent STAT-6. U937 cells were treated with media containing 250 units/ml IFN-γ (filled circles, dashed line) or media alone (empty circles, solid line) for 24 h. They were then stimulated with IL-13 at the indicated concentrations for 20 min. Nuclear extracts were analyzed for Stat6 activity by EMSA. Autoradiographs from three separate experiments were analyzed by densitometry. Densitometric units were plotted, and statistically significant differences were noted by asterisks.

Cell line, A549. IL-13 has been shown to have effects on epithelial cells (2, 20, 29), and, in atopic disease, epithelial cells are the target of some of its critical effector functions, such as up-regulation of chemokine expression (30). A549 cells are derived from human bronchial epithelium. We investigated the relative amount of IL-13Rα2 on the cell surface relative to the intracellular compartment (Fig. 6A). Staining of permeabilized cells was ~8-fold more intense compared with intact cells, demonstrating that the receptor is a predominantly intracellular rather than cell surface protein in A549 cells, similar to our findings in U937 cells. Confocal microscopy of intact or permeabilized A549 confirmed its localization in intracellular compartments (Fig. 6C). The intact cells demonstrated no significant staining in response to either the IL-13Rα2 antibody or its isotype-matched control, consistent with the low surface expression detected by flow cytometry. In contrast, permeabilized cells showed bright intracellular staining with the IL-13Rα2 antibody, but no significant staining with the isotype control. Because both U937 and A549 cells are immortalized cell lines, it remained a possibility that our observations demonstrating that IL-13Rα2 is a largely intracellular molecule were peculiar to immortalized cell lines. To directly address this, we examined primary human nasal epithelial cells for the presence of IL-13Rα2 by confocal microscopy (Fig. 6). The results were identical to those observed in A549 and U937 cells. Intact human nasal epithelial cells demonstrated no significant staining for IL-13Rα2. However, permeabilized primary human nasal epithelial cells revealed bright intracellular staining. No significant staining was seen with the isotype control.

Primary Human Monocytes Contain a Large Intracellular Pool of IL-13Rα2 That Is Mobilized to the Surface with IFN-γ Treatment—We next wanted to examine whether IFN-γ treatment resulted in cell surface trafficking of IL-13Rα2 receptors in primary cells. For these studies we chose human monocytes because we had seen an effect in U937 monocyteic cells. We obtained fresh elutriated primary monocytes and stained permeabilized and non-permeabilized cells for IL-13Rα2 expression following treatment with media alone or media containing IFN-γ (Fig. 7A). Primary monocytes contained a large intracellular pool of IL-13Rα2 and IFN-γ up-regulated IL-13Rα2 on the
surface of these cells. Although U937 cells subjected to IFN-γ stimulation demonstrated approximately a 2-fold increase in surface IL-13Rα2 in response to IFN-γ, the primary human monocytes showed a nearly 6-fold induction in surface expression (Fig. 7). Furthermore, there was no significant change in IL-13Rα2 staining in the permeabilized cells treated with IFN-γ. This supports that IFN-γ mobilizes the intracellular pool of IL-13Rα2 because further up-regulation was not observed in cells stained for both the surface and intracellular fractions by permeabilization. This demonstrates that the IFN-γ induced up-regulation of IL-13Rα2 observed in U937 cells also occurs in primary human cells. Furthermore, in primary cells, the magnitude of receptor up-regulation is much greater than in immortalized cell lines, further highlighting its biologic relevance.

**DISCUSSION**

In this report, we provide the first evidence that the IL-13Rα2 is predominantly an intracellular molecule and that IFN-γ leads to increased surface expression of IL-13Rα2 by mobilization of preformed IL-13Rα2. Furthermore, this observed IFN-γ-dependent up-regulation of IL-13Rα2 was associated with diminished IL-13 signaling supporting the hypothesis that IL-13Rα2 acts as a decoy receptor that can regulate IL-13 responses. This represents a novel mechanism by which IFN-γ can regulate IL-13 responses. Large pools of IL-13Rα2 were found intracellularly in cultured monocytes, respiratory epithelial cells, primary respiratory epithelium, and primary human monocytes, supporting that this observation is not restricted to a given cell type, but rather appears to be widespread. In primary monocytes, IFN-γ resulted in a 6-fold increase in surface
IL-13Rα2 expression. Although confocal microscopy is not sufficient to determine the subcellular localization of the intracellular IL-13Rα2, it is interesting to note that, in the primary human nasal epithelial cells, along with the diffuse cytoplasmic staining, there was some bright localized staining that may represent distinct vesicles containing IL-13Rα2. Further studies are under way to address this possibility.

The inability of IL-13Rα2 expression to confer IL-13 responsiveness despite high affinity binding, along with the finding of soluble IL-13Rα2 in vivo (13), has led to the hypothesis that IL-13Rα2 acts as a decoy receptor, and our data support this. Modulation of IL-13Rα2 expression would allow a mechanism for fine-tuning of IL-13 responses. Analogous observations have been made in the IL-1 receptor system. IL-1 has two cognate receptors, IL-1R and IL-1RII (31). IL-1R binds IL-1 and results in a functional signal. In contrast, IL-1RII has a short cytoplasmic domain of only 29 amino acids and has no signaling role (32). Overexpression of IL-1RII on cell surfaces was shown to impair IL-1 responses (33, 34). Interestingly, IL-13 has been shown to modulate the expression of the IL-1RII decoy receptor (35). Another receptor system, which utilizes a decoy receptor, is the T lymphocyte receptors for CD80 and CD86, CD28 and CTLA-4. CTLA-4 is an important negative regulator of T cell activation. It is not required for ligand induced cellular activation, but acts as a decoy receptor for the B7 molecules. CTLA-4 exists in intracellular pools, which are rapidly mobilized upon activation resulting in an ~100% increase over baseline surface staining for CTLA-4 (36). This magnitude of change in the surface expression of a decoy receptor was sufficient to alter cellular responsiveness. In our experiments, IL-13Rα2 surface expression was increased by IFN-γ treatment of cells. The effect was evident within hours and did not require protein synthesis, confirming that it resulted from the mobilization of preformed IL-13Rα2 stores. By confocal microscopy it appeared that there was a large excess of intracellular IL-13Rα2 versus on the cell surface, and permeabilized U937 cells had nearly 20-fold more IL-13Rα2 staining compared with intact cells by flow cytometry. However, the magnitude of IL-13Rα2 mobilization by IFN-γ resulted in only a 60–250% increase over the basal level in U937 cells. Although this approximates the changes seen in other systems, such as CTLA-4, it appears that the mobilization of IL-13Rα2 represents a small portion of the total available intracellular IL-13Rα2 in some cells. In contrast, in primary monocytes, IFN-γ treatment resulted in a nearly 6-fold induction in surface expression. This appeared to represent the majority (approximately two-thirds) of the intracellular IL-13Rα2 pool because increased staining was not noted in the permeabilized IFN-γ-treated cells relative to the IFN-γ-treated non-permeabilized cells. These differences may represent cellular differences between primary and immortalized cells. In addition, confocal microscopy and flow cytometry are different readouts, and the data obtained by these two different methods cannot be compared quantitatively.

Considerable effort is taking place to understand the biologic role of IL-13, but, thus far, little is known about the regulation of IL-13 responses. IL-13 and IL-4 share many structural and functional characteristics; however, they each have distinct biologic functions. IL-4 directly promotes the differentiation of naive T cells to Th2 cells (37, 38) and, thus, plays a well recognized role in Th2 development. In contrast, IL-13 receptors have not been found on T cells (3); consequently, IL-13 does not support the proliferation of mouse or human T cells. In mouse models, specific blockade of IL-13 using a soluble IL-13Rα2 resulted in a reversal of airway hyperresponsiveness (8), underscoring the importance of IL-13 independent of IL-4. Furthermore, overexpression of IL-13 in the lungs of mice resulted in several characteristics of the asthmatic phenotype (20). It has been postulated that IL-4 is more important for Th2 deviation and the induction of the allergic response, whereas IL-13 may be more important in the effector phase of allergic inflammation (2). In this case, regulation of IL-4 may be more critical at the transcriptional or production level, whereas regulation of IL-13 may be more important in later phases of the immune response. IFN-γ-dependent up-regulation of IL-13Rα2 and subsequent dampening of IL-13 signaling described herein provide one mechanism for rapid fine-tuning of the IL-13 response during the later phases of the allergic cascade. Alternatively, modulation of IL-13Rα2 expression may provide a mechanism whereby the IL-4 and IL-13 response can be differentially regulated. Because IL-13Rα2 binds IL-13 with high affinity, but it does not bind IL-4, increased expression of IL-13Rα2 would lead to increased binding of available IL-13. Thus, less IL-13 would be available for binding to the functional IL-13Rα2/IL-4Rα complex, which could then be available for IL-4 binding. Consequently, modulation of IL-13Rα2 expression would likely regulate both IL-4 and IL-13, and in opposite directions. Differential IL-13Rα2 expression during different phases of the allergic immune response may account for some of the distinct effects of IL-4 and IL-13.

The biologic roles of the two known IL-13-binding proteins, IL-13Rα1 and IL-13Rα2, are still not clear. IL-13Rα1 is part of the type II IL-4/IL-13 receptor complex that signals via the JAKStat pathway. However, it is unclear whether IL-13Rα1 itself has any signaling role, positive or negative. The cytoplasmic domain of IL-13Rα1 has been shown to be required for IL-13 signaling, suggesting that IL-13Rα1 may signal through a distinct pathway (39). This could provide an alternative mechanism for differential effects between IL-4 and IL-13. IL-13Rα2 has a very short cytoplasmic domain (17 amino acids in the human) with no known signaling motifs, supporting that it has no signaling function. However, this remains to be strictly proven, and it is possible that IL-13Rα2 has a distinct and unique signaling capacity. The generation and characterization of gene-targeted mice for IL-13Rα1 and/or IL-13Rα2 will be critical to understand the roles of these receptors in IL-13 and IL-4 responses.

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