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The Murine IL-13 Receptor α2: Molecular Cloning, Characterization, and Comparison with Murine IL-13 Receptor α1

Debra D. Donaldson,* Matthew J. Whitters,* Lori J. Fitz,* Tamelyn Yee Neben,* Heather Finnerty,* Sheryl L. Henderson,* Richard M. O’Hara Jr.,* David R. Beier,† Katherine J. Turner,* Clive R. Wood,* and Mary Collins2*

Two components of a receptor complex for IL-13, the IL-4R and a low affinity IL-13-binding chain, IL-13Rα1, have been cloned in mice and humans. An additional high affinity binding chain for IL-13, IL-13Rα2, has been described in humans. We isolated a cDNA from the thymus that encodes the murine orthologue of the human IL-13Rα2. The predicted protein sequence of murine IL-13Rα2 (mIL-13Rα2) has 59% overall identity to human IL-13Rα2 and is closely related to the murine low affinity IL-13-binding subunit, IL-13Rα1. The genes for both mIL-13-binding chains map to the X chromosome. A specific interaction between mIL-13Rα2.Fc protein and IL-13 was demonstrated by surface plasmon resonance using a BIACORE instrument. Ba/F3 cells that were transfected with mIL-13Rα2 expressed 5000 molecules per cell and bound IL-13 with a single Kd of 0.5 to 1.2 nM. However, these cells did not proliferate in response to IL-13, and the IL-4 dose response was unaffected by high concentrations of IL-13. In contrast, the expression of mIL-13Rα1 by Ba/F3 cells resulted in a sensitive proliferative response to IL-13. Consistent with its lower affinity for IL-13, IL-13Rα1.Fc was 100-fold less effective than IL-13Rα2.Fc in neutralizing IL-13 in vitro. These results show that mIL-13Rα2 and mIL-13Rα1 are not functionally equivalent and predict distinct roles for each polypeptide in IL-13R complex formation and in the modulation of IL-13 signal transduction.


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3 The GenBank accession no. for murine IL-13Rα2 is U65747.

4 Abbreviations used in this paper: m, murine; h, human; CHO, Chinese hamster ovary; KSCP, single-strand conformation polymorphism; RU, resonance unit; Agn2, angiotensin receptor-2; 125I-mIL-13; 125I-labeled mIL-13; HA, hemagglutinin.

mIL-3 and mIL-6 were obtained from titered COS-1 and Chinese hamster ovary (CHO) cell supernatant. All other cytokines were purchased from R&D Systems (Minneapolis, MN). The Ba/F3 cell line (11) was maintained in RPMI 1640, 10% (v/v) FBS, and 0.05% (v/v) CHO mIL-3-conditioned medium. The B9 cell line was cultured in RPMI 1640, 10% (v/v) FBS, 20 nM of β-mercaptoethanol, and 0.01% (v/v) COS-1 mIL-6-conditioned medium (12).

Isolation of mIL-13Rα2

Poly(A)+ RNA was prepared from the thymii of 6- to 8-wk-old C3H/HeJ mice, and a cDNA library of 1.5 × 106 primary recombinant phage was prepared according to the manufacturer’s instructions using the Zap Express Kit from Stratagene (La Jolla, CA). A total of 200,000 plaques were screened with a degenerate 17-base oligonucleotide probe of the sequence 5′-dKSRCTCCABK CRCTCCA-3′ (K = G or T; S = C or G; B = A or G; and C or T) using standard tetramethylammonium chloride hybridization conditions (13).
Isolation of hIL-13Rα2

A partial fragment of the human homologue of this receptor was isolated by PCR using oligonucleotides that had been derived from the mouse sequence. cDNA was prepared from human testis poly(A)⁺ RNA that had been obtained from Clontech (Palo Alto, CA). A DNA fragment of 274 base pairs (bp) was amplified from this cDNA by PCR with the oligonucleotides 5'-dATAGTTAACCAGTTCCACC-3' and 5'-CTCCATTCGGCTCAAAATTC-3' using Taq polymerase in 1× Taq buffer containing 1.5 mM MgCl₂ for 30 cycles (94°C for 1 min, 42°C for 1 min, and 72°C for 1 min). The DNA sequence of this fragment was determined, and two oligonucleotides were radiolabeled with [³²P]ATP using polynucleotide kinase, and genomic DNA from a mouse strain was amplified using standard protocols (anneal at 55°C for 1 min, extend at 72°C for 2 min, and denature at 94°C for 1 min for 40 cycles, with a final extension at 72°C). A volume of 2 μl of the amplified reaction was added to 85 μl of stop solution (United States Biochemical, Cleveland, OH), denatured at 94°C for 5 min, and immediately placed onto ice. An aliquot of 2 μl of each reaction was loaded on a nondenaturing 6% acrylamide-sequencing gel and electrophoresed in 0.5× Tris-borate/EDTA buffer for 2 to 3 h at 40 mA in a cold room. A primer pair with the sequence 5'-dCCCAACATTCCTGAGATAGCAGGC-3' (forward) and 5'-dATGGCTTTT-3' (internal) followed by autoradiography. Expression vectors for mIL-13Rα2 and mIL-13Rα1 RNA in tissues

Northern blots of poly(A)⁺ RNA from various tissues (Clontech) were performed as recommended by the manufacturer. An RT-PCR analysis of cDNA was conducted according to the manufacturer’s instructions. Following DNase inactivation, each sample was divided into two tubes; random hexamer-primed first strand cDNA synthesis (Superscript II Preamplification System; Life Technologies, Gaithersburg, MD) was conducted according to the manufacturer’s instructions, except that the reverse transcriptase was omitted from one tube in each sample pair. Reaction products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) as described by the manufacturer and recovered in 50 μl of double-distilled H₂O. We conducted 50-μl PCRs using 5 μl of the recovered material and Advantage cDNA Polymerase Mix (Clontech) according to the manufacturer’s instructions. A total of 25 reaction cycles were performed at 94°C for 1 min, at 60°C for 1 min, and at 72°C for 2 min for actin and at 96°C for 1 min, at 56°C for 1 min and at 72°C for 1 min for mIL-13Rα2 in a Perkin-Elmer Cetus Thermal Cycler (Branchburg, NJ). The oligonucleotide primers that were used for the amplification of mIL-13Rα2 were 5'-dATGCGTCTATTTACTTTTACCTG-3' (forward) and 5'-dATGCGTCTATTTACTTTTACCTG-3' (reverse), which generate an 800-bp fragment; the primers used for γ-actin were 5'-dATGCGTCTATTTACTTTTACCTG-3' (forward) and 5'-dATGCGTCTATTTACTTTTACCTG-3' (reverse), which generate a 900-bp fragment. An aliquot of each reaction was electrophoresed on a 0.8% agarose (w/v) gel and transferred to nitrocellulose. The membranes were hybridized at 55°C for 18 h to end-labeled oligonucleotides that were specific for mIL-13Rα2 or mIL-13Rα1. Filters were exposed to autoradiography for 2 days.

Transfection of Ba/F3 cells

Expression vectors for mIL-13Rα1 (6) and mIL-13Rα2 were constructed in pLNCX (17). Ba/F3 cells were transfected with 20 μg of either mIL-13Rα2 or mIL-13Rα1 linearized expression plasmids by electroporation using the Gene Pulsar (Bio-Rad, Richmond, CA) at 270 V and 960 μF. Pools of stable transfectants (Ba/F3-IL-13Rα2 or Ba/F3-IL-13Rα1) were isolated by selection in 1.0 mg/ml of G418 (Life Technologies).

SDS-PAGE analysis of mIL-13Rα2.T7 and mIL-13Rα1.HA protein

Expression vectors were also constructed that encode either full-length mIL-13Rα2 or mIL-13Rα1 fused in frame at the C terminus to sequences encoding either T7 or hemagglutinin (HA) epitope tags and inserted into the COS-1 expression vector, pED. COS monolayers that had been transiently transfected with mIL-13Rα2 or mIL-13Rα1 expression plasmids or mock transfected were radiolabeled with [³²P]methionine and [³¹S]cysteine (Amersham, Arlington Heights, IL). Proteins containing the T7 epitope tag were precipitated from detergent-solubilized cell extracts by incubation with a murine T7.Tag mAb that had been conjugated to agarose (Novagen, Madison, WI). Proteins containing the HA epitope tag were precipitated by incubation with an antibody against the HA-peptide epitope (Boehringer Mannheim, Indianapolis, IN) and with goat anti-mlgG that had been conjugated to Sepharose 4B (Zymed, San Francisco, CA). Immunoprecipitated material was examined by reducing SDS-PAGE that was followed by treatment with Amplify (Amersham) and fluorography.

Production of mIL-13Rα2.Fc or mIL-13Rα1.Fc protein

Expression vectors encoding either the extracellular domain of IL-13Rα2 (amino acids(aa) 1–332) or IL-13Rα1 fused to a Gly-Ser-Gly spacer and the DNA sequence encoding the hinge-CH2-CH3 regions of the human antibodies were also constructed in pED. The mIL-13Rα2.Fc or mIL-13Rα1.Fc protein was produced from stably transfected CHO cells and purified via protein A-Sepharose chromatography (Pharmacia, Uppsala, Sweden) (18).

BIACORE binding assay

A BIACORE 2000 instrument (Biacore AB, Uppsala, Sweden) was used to show IL-13 binding to IL-13Rα2.Fc (19). Purified IL-13Rα2.Fc, IL-11Rα2.Fc, or hIL-17A (The Binding Site, Birmingham, U.K.) in 10 mM sodium acetate (pH 7.4), 100 mM sodium chloride, 3.4 mM EDTA, and 0.005% (v/v) Tween 20. Purified IL-13, IL-4, IL-7, and IL-15 (R&D Systems) were separately injected at 10 μg/ml in series over the immobilized IL-13Rα2.Fc, IL-11Rα2.Fc, and IgG1 for 10 min at 2 μl/min. Binding was quantified as the increase in RU at 60 s after the end of injection compared with a baseline established at 20 s before injection.

Affinity measurements

Carrier-free mrIL-13 was purchased from R&D Systems and radiiodinated with [³¹I]-labeled N-succinimidyl p-iodobenzoyl (New England Nuclear, Boston, MA), according to the manufacturer’s instructions. 125I-labeled mIL-13 (13) was purificated by fractionation over a PD-10 column (Pharmacia) followed by ultrafiltration on a Centricron 3 microcentrifugation system and resuspended in saline. The specific activity of the final iodinated mIL-13 was greater than 1.0 × 10⁶ counts per minute (cpm) per μg of iodine.

Production assays

The stimulation of proliferation of B9, Ba/F3, Ba/F3-IL-13Rα2, and Ba/F3-IL-13Rα1 cells in response to IL-13 or IL-4 was measured by [³¹H]thymidine (New England Nuclear) incorporation into DNA. Cells (0.5–1 × 10⁵/well) were seeded into 96-well plates in 200 μl of media with and without growth factors. After incubation for 3 days, 1 μCi/well of [³¹H]thymidine was added, and the cells were incubated for an additional 4 h.
incorporated radioactivity was determined using an LKB 1205 plate reader (LKB Pharmacia, Gaithersburg, MD).

Results

Isolation of mIL-13Rα2 cDNA

The hemopoietin receptor superfamily is characterized by a conserved aa motif, WSXWS, in the extracellular domain (21). We screened an adult murine thymus cDNA library using a degenerate 17-base oligonucleotide that encodes a subset of sequences containing this motif. Clone A25 was identified as a positive that did not hybridize to probes derived from known cytokine receptors. Sequencing of the 1.5-kilobase (kb) murine A25 cDNA revealed a 1149-bp open reading frame that was predicted to encode a 383-aa protein. The predicted sequence contains a putative signal peptide, an extracellular domain with four potential N-linked glycosylation sites, a transmembrane domain, and a short cytoplasmic domain (Fig. 1). The extracellular domain contains an N-terminal fibronectin type III domain followed by a typical cytokine receptor module at residues 139 to 321. This cytokine receptor module includes a WSEWS motif and the four cysteine residues that are the most conserved features of the hemopoietin receptor superfamily. The A25 domain structure is shared with the murine receptors for granulocyte-macrophage CSF, IL-3, IL-5, and mIL-13Rα1 (NR4). The extracellular region of A25 is most closely related to mIL-13Rα1, with 29% identical aa residues (Fig. 1). However, in contrast to mIL-13Rα1, the short cytoplasmic tail of A25 contains no box 1 or box 2 signaling motifs (22). A25 shows 59% overall aa identity with hIL-13Rα2 (10), suggesting that it is the murine orthologue (Fig. 1).

Mapping of mIL-13Rα2 and mIL-13Rα1

The genes encoding both mIL-13Rα2 and mIL-13Rα1 were mapped by SSCP analysis (14). IL-13Rα2 and IL-13Rα1 were both found to map to chromosome X, with log of the odds likelihood scores of 26.8 and 18.5, respectively. No recombinants were found between IL-13Rα2 and DXMit34 in the 89 progeny that were scored. The position of IL-13Rα2 with respect to flanking microsatellite markers is: DXMit426.425 (IL-13Rα2, FIGURE 1. Alignment of the deduced aa sequence of mIL-13Rα2 cDNA (A25) with mIL-13Rα1 (NR4), hIL-13Rα2, and mL-5R. The predicted signal peptide and transmembrane domain for the mIL-13Rα2 is underlined, and potential N-linked glycosylation sites are marked with #. aa that are characteristic of the hemopoietin receptor superfamily are marked by *.

![Image](https://example.com/image.png)
No recombinants were found between IL-13Rα1 and angiotensin receptor-2 (Agtr2) in the 80 progeny that were scored. The position of IL-13Rα1 with respect to flanking markers is: DXMit85 – 3.8 ± 2.1 cM – (IL-13Rα1, Agtr2) – 3.8 ± 2.1 cM – DXMit49.

Expression of mIL-13Rα2 and hIL-13Rα2 RNA in tissues

We also examined the expression of murine and human IL-13Rα2 RNA by Northern blotting and RT-PCR (Fig. 2). Two transcripts of 1.5 and 2.1 kb were detected in the murine spleen, brain, and 7-day-old embryo. The presence of mIL-13Rα2 RNA was further confirmed in the spleen and brain by RT-PCR. A single transcript of 2.1 kb was seen in the human liver, lung, and thymus, and a smaller 1.5-kb transcript was observed in the placenta, brain, and heart. An intensely labeled transcript in human testis was detected and was shown to be a single band of 2.1 kb upon shorter exposure. Testis RNA from a second pooled source showed the same intensity of signal. Apparent differences in transcript size in the RNA preparations of different human tissues did not correlate with changes in actin transcript mobility. The sequence comparisons of four independent cDNA clones from human testis were identical in the coding region and ranged in size from 1.1 to 1.3 kb.

Comparison of mIL-13Rα2 and mIL-13Rα1 protein by SDS-PAGE

Results from cross-linking studies of hIL-13 and hIL-13R have shown specific binding proteins of similar molecular masses from a variety of tissue sources. Therefore, we wished to directly compare the observed molecular masses of noncross-linked mIL-13Rα1 and mIL-13Rα2. The mIL-13Rα2 and mIL-13Rα1 cDNA were modified to encode epitope tags at the carboxyl terminus of each protein and inserted into a COS expression vector. COS cells that had been transiently transfected with these plasmids were biosynthetically labeled with [35S]methionine and [35S]cysteine and examined by immunoprecipitation with mAb specific for the T7 (for IL-13Rα2) or HA (for IL-13Rα1) epitopes. SDS-PAGE and fluorography resulted in the detection of both polypeptides; each polypeptide had a similar apparent molecular mass of ~56 kDa (Fig. 3).

Binding studies

The specificity of the interaction between IL-13 and mIL-13Rα2 was investigated by surface plasmon resonance using a BIACORE 2000 instrument. IL-4, IL-5, IL-7, IL-9, IL-15, and IL-13 were screened for binding to immobilized mIL-13Rα2.Fc, and only IL-13 binding was detected. None of these cytokines were found to interact with IL-11Rα.Fc or human IgG1 (Fig. 4). Similar results were obtained for hIL-13Rα2.Fc protein; these results were consistent with results reported previously (10).

To determine the affinity of IL-13 for mIL-13Rα2, we used Ba/F3 cells that had been stably transfected with the mIL-13Rα2 expression plasmid. IL-13 was iodinated and bound to the transfected cells. A Scatchard analysis of the binding data from three separate experiments revealed that these cells expressed ~5000 IL-13-binding sites of a single Kd of 0.5 to 1.2 nM (Fig. 5). No binding was detected on untransfected Ba/F3 cells.
Biologic responsiveness of transfected cell lines

Hilton and colleagues have reported that the expression of mIL-13Rα1 (NR4) by their Ba/F3 subline was not sufficient to allow a proliferative or survival response to IL-13 (6). In contrast, the expression of mIL-13Rα1 in CTLL cells did confer a proliferative response to IL-13 (6). The potential signaling functions of hIL-13Rα2 have not yet been investigated. Therefore, we examined the proliferative responses of IL-3-dependent Ba/F3 cells that had been transfected with mIL-13Rα2 or mIL-13Rα1 to assess the relative contributions of each protein to receptor function. Ba/F3 cells expressing mIL-13Rα2 were unable to proliferate in response to IL-13, even though they expressed high numbers of receptors per cell (Figs. 5 and 6). Surprisingly, Ba/F3 cells that had been transfected with mIL-13Rα1 responded to IL-13 and proliferated with a half-maximal response of 200 pg/ml. The half-maximal proliferation to IL-4 occurred at ~1.2 ng/ml and was unchanged from untransfected Ba/F3 cells even in the presence of high levels of IL-13.

mIL-13Rα2 is a potent inhibitor of IL-13 in vitro

The effect of a dimeric form of mIL-13Rα2, the IL-13Rα2.Fc fusion protein, on the proliferative response of Ba/F3 cells to IL-13 was examined. mIL-13Rα2.Fc was added at various concentrations to cultures of Ba/F3 cells that contained a saturating concentration (3 ng/ml) of mIL-13. Figure 7 shows that the concentration of fusion protein giving a 50% level of inhibition with a half-maximal response of 200 pg/ml. The half-maximal proliferation to IL-4 occurred at ~1.2 ng/ml and was unchanged from untransfected Ba/F3 cells even in the presence of high levels of IL-13.

A. Ba/F3 cells expressing IL13Rα2

B. Ba/F3 cells expressing IL13Rα1

FIGURE 3. SDS-PAGE analysis of immunoprecipitated, epitope-tagged, 35S-labeled, mIL-13Rα2 or IL-13Rα1 protein expressed by COS cells. COS monolayers were transfected with expression constructs encoding mIL-13Rα2 or IL-13Rα1. Cells were radiolabeled and lysed, and proteins that had been immunoprecipitated with anti-HA or anti-T7 were examined by SDS-PAGE and fluorography.

FIGURE 4. Analysis of cytokine binding to IL-13Rα2.Fc by BIACORE. The specificity of the interaction between IL-13 and IL-13Rα2.Fc was investigated by surface plasmon resonance using a BIACORE 2000 instrument. IL-4, IL-5, IL-7, IL-9, IL-15, and IL-13 were screened for binding to immobilized IL-13Rα2.Fc. The binding as reflected by changes in RU is shown.

FIGURE 5. Scatchard analysis of 125I-labeled IL-13 to Ba/F3 cells expressing IL-13Rα2. The inset shows equilibrium binding. r² = 0.91 kDa = 0.56 nM. The data shown are from one of three experiments. The Kd values from each of the three experiments were 0.56, 0.88, and 1.2 nM.
of IL-13-stimulated proliferation is 8 ng/ml. Similar results were obtained using B9 cells (data not shown). In contrast, mIL-13Ra1Fc was at least 100-fold less effective in neutralizing the proliferative effect of IL-13 than mIL-13Ra2Fc (Fig. 7). This difference in potency is consistent with the lower affinity (Ka = 2–10 nM) of IL-13 for mIL-13Ra1 (6) when compared with the higher affinity of IL-13 for mIL-13Ra2 (Ka = 0.5 nM).

Discussion

Two distinct members of the hemopoietin receptor family have been shown to specifically bind IL-13 with differing affinities. The lower affinity subunit, IL-13-Rα1, has been shown to participate in the formation of an IL-13R complex in combination with the IL-4R (6–9). A higher affinity subunit, which had only been described previously in humans, is designated hIL-13-Rα2 (9). However, the contribution of this IL-13-binding chain to receptor function has not been elucidated.

We have used motif-based cloning to identify a novel member of the hemopoietin receptor family, designated mIL-13Ra2, which encodes the murine orthologue of hIL-13-Rα2. Comparisons of the human and murine receptors show that they are structurally conserved, share 59% aa identity, and specifically bind IL-13 with high affinity. Within the hemopoietin receptor family, we show that the extracellular region of mIL-13Ra2 is most closely related to mIL-13Ra1. In contrast, the cytoplasmic domains of mIL-13Ra2 and mIL-13Ra1 are dissimilar. Importantly, the cytoplasmic domains of both the murine and human IL-13Ra2 are short and devoid of box 1 or box 2 signaling motifs. However, hIL-13Ra2 does contain a putative consensus phosphorylation site, Y369PKM (23), which is potentially suitable as a docking site for an SH2-containing signaling molecule.

We also show that two IL-13Ra2 mRNA transcripts of 1.5 and 2.1 kb are expressed in the murine brain, spleen, and day 7 embryo, although lower levels were detected in the liver, lung, and testis with longer exposure (data not shown). Isolation of IL-13Ra2 from a thymus cDNA library also indicated that this gene is expressed in the murine thymus at a low level (1/200,000 cDNA clones). Interestingly, the transcript is also expressed in RNA isolated from mouse embryos, with strong expression at day 7. This observation suggests that IL-13 may have an important role in embryogenesis.

An hIL-13Ra2 mRNA transcript of either 1.5 or 2.1 kb was detected and was found to have a broad range of expression in primary tissues. A particularly high level of expression was observed in human testis. The cDNA clones that were isolated by us from the testis were identical in sequence to the cDNA that was isolated by Caput et al. (10) from human renal carcinoma cells as well as to several partial cDNA clones that were isolated from human brain RNA (data not shown). The difference in tissue-specific transcript expression between mice and humans may be related to differences in IL-13 function between the two species. For example, IL-13 has been shown to induce IgE class switching in human but not in murine B cells. Another observation is that this receptor is also expressed in tissues that are not typically associated with immune function, such as the testis and brain. It will be interesting to determine which cells in these tissues are expressing IL-13Ra2, and whether they are cells that might be associated with immune surveillance.

The full-length murine and human cDNAs isolated by us correspond to the smaller 1.5-kb transcripts. Zhang et al. have determined the partial sequence of a soluble form of IL-13-binding protein in mouse serum and urine that matches that of the predicted N terminus of mIL-13Ra2 (2A). This soluble form may be generated by proteolytic processing. Alternatively, the larger 2.1-kb mIL-13Ra2 transcript may encode this form.

Using SSCP analysis, we localized the genes for mIL-13Ra1 and IL-13Ra2 to the murine X chromosome. This finding is consistent with the reported chromosomal locations of hIL-13Ra1 and hIL-13Ra2 on the X chromosome (7, 25). Although the hIL-13Ra2 gene has been precisely mapped to Xq24 (25), the precise location of the hIL-13Ra1 on the X chromosome has not been determined. We have found that mIL-13Ra1 is nonrecombinant with mAgtr2. Since linkage relationships in this subchromosomal region are highly conserved between mice and humans, our data predict that the hIL-13Ra1 would also be tightly linked to hAgtr2, which maps to human chromosome Xq22–23 (26). The presence of these two genes on the X chromosome suggests that they should be considered as candidate genes in X-linked immune disease.

Our BIACORE data demonstrate that mIL-13Ra2 specifically binds IL-13. In particular, we were unable to detect the binding of IL-4 at high concentrations to mIL-13Ra2. Our affinity measurements for the mIL-13Ra2 that is expressed in Ba/F3 cells ranged from 0.5 nM to 1.2 nM and are similar but not identical with the 0.25 and 0.44 nM affinity that was determined for the hIL-13Ra2 in COS and Caki cells, respectively (10). This discrepancy may reflect a true difference between the human and murine proteins or may reflect experimental variation (e.g., differences due to methods of iodination). These affinities are in contrast to the lower
affinity that was measured for cells expressing either mIL-13Rα1 (2–10 nM) (6) or hIL-13Rα1, which could not be detected unless both IL-13Rα1 and IL-4Rα were coexpressed (7).

The structural differences between the cytoplasmic domains of the high and low affinity IL-13-binding chains suggest that these chains are functionally distinct. To test this possibility, we examined the proliferative responses to IL-13 in Ba/F3 cells that had been transfected with either the low affinity mIL-13Rα1 chain or the high affinity mIL-13Rα2 chain. These cells express the IL-4Rα, and the mitogenic response to IL-4 was unchanged by the heterologous expression of these IL-13-binding proteins. We found that the expression of the IL-13Rα1 chain led to a proliferative response to IL-13, presumably by the formation of an IL-13Rα1/IL-4R complex. In contrast, expression of the IL-13Rα2 was not sufficient to generate a mitogenic response to IL-13.

The extracellular region of mIL-13Rα2 has been fused to the Fc portion of hIgG1 to generate a soluble form of this IL-13-binding protein. We have demonstrated that this molecule is a specific inhibitor of IL-13 in vitro and can block the IL-13-stimulated proliferation of Ba/F3 cells that have been transfected with the IL-13Rα1. We have also made an IL-13Rα1Fc fusion protein and examined the ability of this fusion protein to inhibit the proliferation of an IL-13-dependent cell line. This inhibition requires 100-fold more IL-13Rα1Fc fusion protein than IL-13Rα2Fc, which is consistent with its lower affinity for IL-13. We have found that soluble IL-13Rα2Fc fusion protein can inhibit the action of IL-13 in murine models in vivo (27).

The effects of the IL-13Rα2Fc fusion protein and the relative affinities of the two IL-13-binding proteins raise the possibility that the IL-13Rα2 is a dominant negative inhibitor of or a decoy for IL-13. This function would be paralleled by IL-1, the IL-1 type II inhibitor of IL-13 in vitro and can block the IL-13-stimulated proliferation of Ba/F3 cells that have been transfected with either the low affinity mIL-13Rα1 chain or the high affinity mIL-13Rα2 chain. These cells express the IL-4Rα, and the mitogenic response to IL-4 was unchanged by the heterologous expression of these IL-13-binding proteins. We found that the expression of the IL-13Rα1 chain led to a proliferative response to IL-13, presumably by the formation of an IL-13Rα1/IL-4R complex. In contrast, expression of the IL-13Rα2 was not sufficient to generate a mitogenic response to IL-13.

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