Cloning of the human IL-13Rα1 chain and reconstitution with the IL-4Rα of a functional IL-4/IL-13 receptor complex

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Abstract The human homologue of the recently cloned murine IL-13 binding protein (IL-13Rα1) was cloned from a cDNA library derived from the carcinoma cell line CAKI-1. The cloned cDNA encodes a 427 amino acid protein with two consensus patterns characteristic of the hematopoietic cytokine receptor family and a short cytoplasmic tail. The human protein is 74% identical to the murine IL-13Rα1, and 27% identical to the human IL-13Rα2. CHO cells expressing recombinant hIL-13Rα1 specifically bind IL-13 ($K_d \approx 4$ nM) but not IL-4. Co-expression of the cloned cDNA with that of IL-4Rα resulted in a receptor complex that displayed high affinity for IL-13 ($K_d \approx 30$ pM), and that allowed cross-competition of IL-13 and IL-4. Electrophoretic mobility shift assay showed that IL-13 and IL-4 were able to activate Stat6 in cells expressing both IL-4Rα and IL-13Rα1, while no activation was observed in cells expressing either one or the other alone.

Key words: IL-13 binding protein; IL-13 signal transduction; IL-4 receptor complex

1. Introduction

Interleukin-13 (IL-13) is a cytokine secreted by activated T-lymphocytes which regulates inflammatory and immune responses [1,2]. It shares several biological activities with IL-4, another T-cell derived cytokine, in a variety of cell types such as B-cells, monocytes, fibroblasts and endothelial cells [3].

The functional redundancy of IL-4 and IL-13 suggested very early on that both cytokines probably shared receptor components [4–6]. The IL-4 receptor comprises two chains, the IL-4Rα and the γc [7–10]. Neither of these two chains binds IL-13 [5], but recent reports have shown that IL-4Rα contributes to the IL-13 receptor [11–13].

Recently, two proteins that bind specifically IL-13 have been cloned, one from murine tissue [14] and the other from human cells [15]. Since both proteins are most probably responsible for the initial interaction of IL-13 with the receptor complex(es) we propose to call them IL-13Rα1 and IL-13Rα2. IL-13Rα1 and IL-13Rα2 are distantly related (27% identity and 46% homology), but both proteins have short cytoplasmic domains, and two consensus patterns, four conserved cysteines in the amino-terminal half of the extra cellular domain and the WSXWS motif located in the C-terminal region of the extra cellular domain, considered signatures of the hematopoietic cytokine receptor family (for review see [19]). Interestingly, both proteins bind IL-13 with very different affinities, $K_d \approx 10$ nM and 50 pM for IL-13Rα1 and IL-13Rα2, respectively. We describe here the cloning of the human IL-13Rα1, and the pharmacological and functional characterization of the recombinant protein expressed alone or with IL-4Rα in stably transfected CHO cells.

2. Materials and methods

2.1. Growth factors and cells

Recombinant hIL-13 was produced and purified in our laboratory as previously described [2]. Human IL-4 was obtained from Tebu (Le Perray en Yvelines, France).

CAKI-1 cells (ATCC HTB 46), the B9 hybridoma cell line, and CHO cells were cultured as described [15].

2.2. cDNA library construction, isolation of cDNAs and sequence analysis

Total RNA from B9 hybridoma cells was used to synthesize cDNA [2]. A specific DNA fragment of the murine IL-13Rα1 was obtained by PCR using this cDNA and the following primers: 5′-AGAGGAATTACCCCTGGATG-3′ (sense) and 5′-TCAAGGAGCTGCTGCTTTCTTCA-3′ (anti-sense) corresponding to the nucleotides 249–268 and 1256–1275, respectively, of the mIL-13Rα1 sequence described by Hilton et al. [14].

The PCR product obtained (1027 bp) was purified, labelled (specific activity 2.4×10^9 dpm/μg) using the Random Primers DNA labelling kit (BRL), and used as a probe to screen a CAKI-1 cDNA library [15].

2.3. Binding and biological activity assays

Binding experiments on transfected CHO cells were performed using radiolabelled hIL-13 as described [5].

For the electrophoretic mobility shift assay (EMSA), 2×10^6 CHO cells or recombinant cell lines were plated onto 10 cm dishes and transfected 24 h later with 6 μg of plasmid DNA. After 48 h, the cells were washed and incubated in the presence of hIL-13 or hIL-4 (10 nM) for 30 min at 37°C, then rinsed twice with cold PBS containing 0.5 mM EDTA, harvested with a cell scraper in 1.2 ml PBS and finally transferred into 1.5 ml microcentrifuge tubes. Cellular extracts were prepared as described by Jiang and Eberhardt [16]. Gel shift assays were performed as described by Kölher et al. [17] with 10–20 μg of proteins and 5×10^4–1×10^5 cpm of the ^{32}P-labelled probe corresponding to the human Cε element from the human Cε control region [18] (5′-GATCCACTTCCCAAGAACAGA-3′, the core sequence is underlined). Stat6 containing complexes were confirmed by supershifting with 2 μg of a monoclonal antibody anti-Stat6, M20 (Santa Cruz, CA), added to the binding reaction before EMSA.

3. Results

3.1. Cloning and sequencing of the human IL-13Rα1

A DNA fragment of the murine IL-13Rα1 [14] was derived from B9 total RNA and used to screen by hybridization a CAKI-1 cDNA library. Homologous sequences were relatively abundant (1/5000). The homologous full length cDNA is 3999 bases long, excluding the poly-A tract, and has a long 3′ untranslated region of 2145 bases. A canonical AATAAA polyadenylation signal is found at the predicted location. The open reading frame between nucleotides 34 and 1851 defines a

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  1  MEWPARLCGL WALLLCAGGG GGGGGAAPTE TQPPVTNLSV SVENLCTVIW
 51  TWNPPEGASS NCSLWYFSHF GDKQDKKIAP ETRRSIEVPL NERICLQVGS
101  QCSTNESEKP SILVEKCISP PEGDPESAVT ELQCIWHNLS YMKCSWLPGR
151  NTSPDTNYTL YYWHRSLEKI HQCENIFREG QYFGCSFDLT KVKDSSFEQH
201  SVQIMVKDNA GKIKPSFNIV PLTSRVKPDP PHIKNLSFHN DDLYVQWENP
251  QNFISRCLFY EVEVNNSQTE THNVFYVQEA KCENPEFERN VENTSCFMVP
301  GVLPDTLNTV RIRVKTNKLC YEDDKLWSNW SQEMSIGKKR NSTLYITMLL
351  IVPVIVAGAI IVLLLYLKRL KIIIFPPIPD PGKIFKEMFG DQNDDTLHWK
401  KYDIYEKQTK EETDSVVLIE NLKKASQ
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Fig. 1. Amino acid sequence of human IL-13Rα1. The amino acids corresponding to the predicted signal peptide are indicated with dashes. Potential *N*-glycosylation sites (Asn-X-Ser/Thr) are labelled with asterisks. Conserved cysteines in the hematopoietic cytokine receptor family are labelled with solid circles. The WSXWS and PXXPXP motifs are boxed. And the transmembrane domain is underlined. The human IL-13Rα1 cDNA sequence has been submitted to the EMBL Data Library (accession number Y09328).

polypeptide of 427 amino acids. The sequence codes for a membrane protein with a putative signal peptide, a single membrane-spanning domain and a short cytoplasmic tail (Fig. 1). Ten sites for potential N-linked glycosylation are located in the extracellular region. Importantly, two consensus patterns considered signatures of the hematopoietic cytokine receptor family (for review see [19]) are also found, four conserved cysteines in the amino-terminal half of the extra cellular domain, and the WSXWS motif located in the C-terminal region of the extra cellular domain. Furthermore, a proline-rich motif (PXXPXP) is located in the cytoplasmic region near the transmembrane domain. Alignment studies reveal homologies with the murine IL-13Rα1 (74% identity and 84% similarity) and to a lesser extent with the human IL-13Rα2 (27% identity and 51% similarity) and with the human IL-5Rα (26% identity and 46% similarity).

3.2. Expression and characterization of the IL-13 binding protein

CHO cells transfected with the isolated cDNA encoding the IL-13Rα1 showed specific binding of labelled IL-13. Scatchard analysis of the saturation curve showed a single component site with a K_d value of 4.5 ± 0.5 nM and a maximal binding capacity of 2.6×10^4 receptors/cell (Fig. 2A). The affinity displayed by the recombinant receptor is much lower than that displayed by the IL-13Rα2, with a K_d of 57 ± 10 pM [15]. However, when the saturation experiments were performed on CHO cells co-expressing IL-13Rα1 with IL-4Rα, the Scatchard analysis clearly showed the presence of two sites for IL-13 (Fig. 2B). One exhibited a dramatic increase in affinity (K_d: 32 ± 8 pM), and the other had a K_d similar to the one observed in the cells expressing IL-13Rα1 alone, 4.2 ± 1.4 nM. The high affinity binding site was not detected if the saturation experiments were performed in the presence of a large excess of IL-4 (not shown). No modification in IL-13 affinity resulted from the co-expression of IL-13Rα1 and IL-13Rα2 (not shown) and IL-13 did not bind to IL-4Rα, as previously described [5].

In competition studies, IL-13 was effective in inhibiting the labelled IL-13 binding to the cells expressing the IL-13Rα1. Labelled IL-4 neither bound to the IL-13Rα1, nor inhibited the binding of labelled IL-13 to this receptor (Fig. 3A). Several other cytokines (IL-2, IL-3, IL-5, IL-7, GM-CSF) were not able to displace IL-13 binding (not shown). However, when IL-13Rα1 and IL-4Rα were co-expressed in CHO cells a high affinity binding site for IL-13 was reconstituted, as shown in Fig. 2B, and this high affinity IL-13 binding was fully displaced not only by IL-13 but also by IL-4 (Fig. 3B). Co-expression of the IL-13Rα1 and IL-4Rα did not change the affinity of the IL-4 receptor for IL-4 (not shown) but allowed displacement of labelled IL-4 by IL-13 (Fig. 3C). These results show that both receptor chains interact in the cell membrane to reconstitute a receptor complex that displays high affinity for IL-13 and that is shared by both IL-13 and IL-4.

3.3. Biological activity

To examine whether IL-13Rα1 is able to transduce a signal to the cell we analyzed the activation of Stat6 because this regulator of gene transcription is activated by IL-13 and IL-4 [17]. Stable transfectants expressing IL-13Rα1 either alone or in combination with IL-4Rα were stimulated with IL-13 or IL-4 and the nuclear extracts were analyzed for binding to an oligonucleotide probe containing the Cε Stat response element from the Cε human control region [18]. The results (Fig. 4) showed that no activation was detected in non-transfected CHO cells incubated with IL-4 or IL-13. Similar negative results were observed on IL-4 or IL-13 stimulation of CHO cells expressing either IL-4Rα or IL-13Rα1. However, in CHO cells expressing both chains, IL-4Rα and IL-13Rα1, stimulation with IL-4 or IL-13 clearly resulted in a binding activity to the oligonucleotide probe in the nuclear extracts. The presence of Stat6 in the complexes was confirmed by supershifting experiments as described in Section 2 (not shown).

4. Discussion

We describe here the cloning and characterization of the human IL-13Rα1. The protein, homologous to the IL-13 binding protein recently cloned from murine tissue (IL-13Rα1) [14], recognizes IL-13 with much lower affinity than the other IL-13 binding protein cloned from human cells (IL-13Rα2) [15]. IL-13 binding to CHO cells expressing hIL-13Rα1 cannot be displaced by IL-4. Co-expression of IL-4Rα with IL-13Rα1 resulted in the reconstitution of a receptor complex that bound IL-13 with higher affinity than the IL-13Rα1 alone, and that allowed cross-competition between IL-

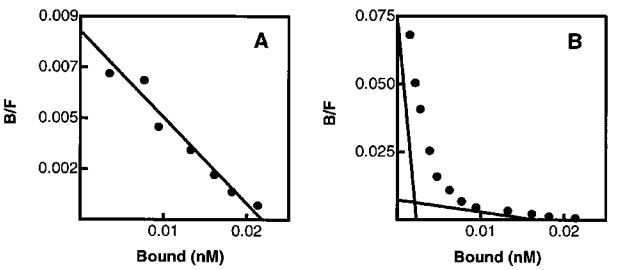

Fig. 2. Characterization of the recombinant IL-13Rα1 expressed in CHO cells. Scatchard analysis of the [^{125}I]IL-13 saturation curve with cells expressing (A) IL-13Rα1, which indicated the presence of ~26 000 sites/cell with a K_d of 4.5 ± 0.5 nM and (B) IL-13Rα1 and IL-4Rα, which indicated the presence of ~4000 sites/cell with a K_d of 32 ± 8 pM and of ~20 000 sites/cell with a K_d of 4.2 ± 1.4 nM.

13 and IL-4 as previously described for the murine IL-13Rα1 [14]. The experiments of activation of Stat6, as assayed by its property to bind to a specific sequence from the Cε promoter, complete and extend the binding results. IL-13Rα1 by itself is not capable of transducing a signal either for IL-13 or for IL-4, but when co-expressed with IL-4Rα it is capable of reconstituting a receptor complex that is able to transduce a signal for both cytokines. It should be noted that CHO cells expressing only IL-4Rα do not respond to IL-4 as measured by Stat6 activation. Since CHO cells do not express γc (unpublished results), the results are in line with previous reports that indicated the need for γc for the reconstitution of a functional IL-4 receptor [20]. The activation of Stat6 by IL-4 in cells co-expressing IL-4Rα and IL-13Rα1 clearly show that IL-13Rα1 can replace γc for the reconstitution of an active IL-4 receptor. The fact that IL-13Rα1 can replace γc for the reconstitution of an active IL-4 receptor explains, as previously suggested [6], the conflicting reports describing the need for γc for an active IL-4 receptor [9,20], and the description of active IL-4 receptors in the absence of γc [21,22]. Since the cytoplasmic domain of IL-13Rα1 is 26 amino acids shorter than that of γc we are currently investigating whether IL-13Rα1 contributes to the recruitment of Jak3, as described for γc [23], and/or to other signaling events as recently suggested [24]. In this context, it is important to emphasize the presence in the IL-13Rα1 of a proline-rich motif located in the cytoplasmic region near the transmembrane domain suggesting that IL-13Rα1 can associate with some kinases of the Jak family [25]. Together, these results show that IL-13Rα1 and IL-4Rα are sufficient to reconstitute a functional receptor for IL-13 and IL-4, and they do not exclude the possibility that other protein(s) may be associated in some cell types with the natural IL-4/IL-13 receptor complex as recently described for γc [24,26]. Two recent reports describe the homodimerization of IL-4Rα and, as a result, the intracellular signaling that finally leads to Stat6 activation. In both reports chimeric receptors were used in which the cytoplasmic and transmembrane domains of IL-4Rα were fused to the extracellular domain of the erythropoietin receptor [27] and γc [28], and dimerization was induced either with erythropoietin or with a monoclonal antibody. The apparent contradiction of these reports with our observation that CHO cells expressing IL-4Rα alone do not respond to IL-4 may indicate that if two IL-4Rα cytoplasmic domains are brought together they are able to transduce a signal to the cell, but that IL-4 does not induce dimerization of natural IL-4Rα. In line with this hypothesis are the results of Hoffman et al. who showed that IL-4 forms a 1:1 complex with the soluble portion of IL-4Rα [29]. Alternatively, the dimerization and activation of IL-4Rα by IL-4 may depend on the density of the receptor in the cell membrane, and/or on the presence of other subunit(s) of the receptor complex that are absent in CHO cells.

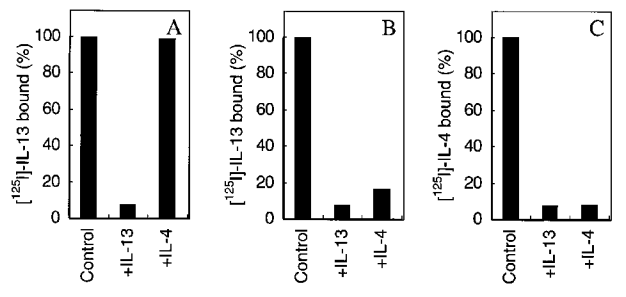

Fig. 3. Cross-competition of IL-13 and IL-4 on CHO cells expressing IL-13Rα1 alone or with IL-4Rα. A: Displacement of labelled IL-13 to cells expressing IL-13Rα1 by IL-13 (20 nM) and IL-4 (20 nM). B: Displacement of labelled IL-13 by IL-13 (20 nM) and IL-4 (20 nM) on cells expressing IL-13Rα1 and IL-4Rα. C: Displacement of labelled IL-4 by IL-13 (20 nM) and IL-4 (20 nM) on cells expressing IL-13Rα1 and IL-4Rα.

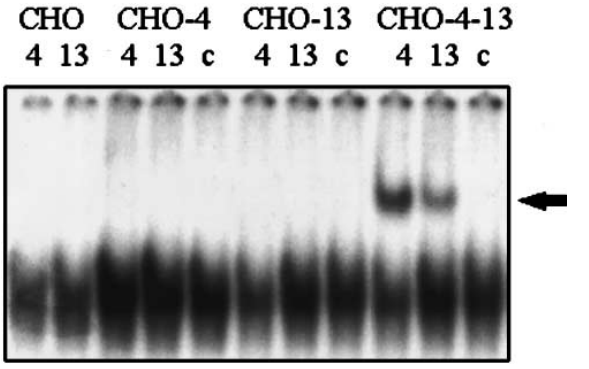

Fig. 4. Signal transduction of IL-13 and IL-4 in CHO cells expressing IL-13Rα1 alone or with IL-4Rα. The different cell lines, CHO, CHO expresssing IL-4Rα (CHO-4), IL-13Rα1 (CHO-13), and IL-4Rα and IL-13Rα1 (CHO-4-13) were incubated in the absence (c) or in the presence of 5 nM of IL-4 (4) or IL-13 (13) as indicated and then the nuclear extracts were analyzed for Stat6 activation as described in Section 2.

In conclusion, our results demonstrate that IL-13Rα1 and IL-4Rα in the absence of γc are sufficient for the reconstitution of an active IL-13 and IL-4 receptor. The availability of the human IL-13Rα1 and IL-4Rα should allow the design of experiments to better assess the stoichiometry and the role played by each protein, and the relationship with γc and human IL-13Rα2, in the functional IL-4/IL-13 receptor complex.

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