cDNA Cloning and Characterization of the Human Interleukin 13 Receptor α Chain*

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We have cloned cDNAs corresponding to the human interleukin 13 receptor α chain (IL-13Rα). The protein has 76% homology to murine IL-13Rα, with 95% amino acid identity in the cytoplasmic domain. Only weak IL-13 binding activity was found in cells transfected with only IL-13Rα; however, the combination of both IL-13Rα and IL-4Rα resulted in substantial binding activity, with a Kd of approximately 400 pM, indicating that both chains are essential components of the IL-13 receptor. Whereas IL-13Rα serves as an alternative accessory protein to the common cytokine receptor γ chain (γc) for IL-4 signaling, it could not replace the function of γc in allowing enhanced IL-2 binding activity. Nevertheless, the overall size and length of the cytoplasmic domain of IL-13Rα and γc are similar, and like γc, IL-13Rα is located on chromosome X.

Interleukin 13 (IL-13) is a T-cell-derived cytokine that exhibits a broad range of activities in the regulation of inflammatory and immune responses. Many of the actions of IL-13 are also exhibited by another T-cell-derived cytokine, IL-4, with which it shares approximately 30% amino acid sequence identity. These shared activities include down-regulation of inflammatory cytokines (1), induction of expression of the IL-1 receptor antagonist (2), induction of surface expression of class II major histocompatibility complex (3), induction of CD23 and IgE expression on B cells (4), costimulation with anti-CD40 antibodies (5), and the inhibition of IL-2-induced proliferation of chronic lymphocytic leukemia cells of B-cell origin (6). Although almost all functions of IL-13 are shared by IL-4, IL-4 additionally exerts unique effects, including the ability to induce responses on certain cell types, including T cells, which do not respond to IL-13 (7). Although the receptor for IL-4 on T cells was found to contain both the 140-kDa IL-4-binding protein (denoted IL-4R or IL-4Rα) and the common cytokine receptor γ chain (γc) (8, 9), it was subsequently demonstrated that IL-4 could also induce actions on nonhematopoietic cells such as COS-7 cells and renal cell carcinoma lines that expressed IL-4Rα but not γc or the γc-associated signaling molecule Janus kinase 3 (10, 12). These data indicated that there are two classes of IL-4 receptor: the type I IL-4 receptor (containing IL-4Rα and γc), which is expressed on a number of lineages, including T cells, B cells, and monocytes; and the type II IL-4 receptor (containing IL-4Rα and another protein originally denoted γ1), which is not expressed on T cells but is expressed on certain other cell types (10). Although it remains unclear whether the exact signals induced by these two types of IL-4 receptors are identical, it is clear that IL-4 can activate signal transducers and activators of transcription 6 via either type of receptor (10). Since antibodies to IL-4Rα could block IL-13 action even though IL-13 could not bind to IL-4Rα (10, 11), we hypothesized that the γ of the type II IL-4 receptor was actually the IL-13R (10). This hypothesis is consistent with biochemical data that IL-13 can partially compete for binding of IL-4 to cells responding to both IL-4 and IL-13 (11), with the ability of both IL-4 and IL-13 to chemically cross-link to ~65 kDa proteins (12, 13), and with the ability of both IL-4 and IL-13 to induce tyrosine phosphorylation of IL-4Rα (14, 15). The ability of IL-4 and IL-13 to act through a shared type II IL-4 receptor (10) was confirmed with the cloning of murine IL-13Rα (16). It was demonstrated that overexpression of murine IL-13Rα in COS cells showed specific binding of 125I-labeled murine IL-13 that could not be competed by murine IL-4 (a finding likely explained by the inability of murine IL-4 to bind to the primed IL-4Rα constitutively expressed on COS cells); however, when CTL-L2 cells were transfected with murine IL-13Rα, both IL-4 and IL-13 could cross-compete with each other for binding to the cells (16). We now report the cloning of a human IL-13Rα cDNA, which together with IL-4Rα allows high affinity IL-13 binding.

MATERIALS AND METHODS

Isolation of cDNA Clones Encoding Human IL-13Rα—A cDNA library in λZap-II (Stratagene, La Jolla CA) was prepared from mRNA from human T-cell lymphotrophic virus-I-transformed MT-2 cells. Based on the sequence of the murine IL-13Rα cDNA (16), primers were selected and evaluated for their ability to generate by PCR from DNA from this library a band that was appropriate in size for IL-13Rα. In this fashion, we found that the MT-2 cDNA library contained human IL-13Rα. A total of 6 × 10⁶ phage clones were plated on LB plates using XL1-Blue MRF+ cells as plating bacteria, and plaques were transferred onto nylon membranes (Amersham Corp.) for hybridization. As described under “Results,” a 431-bp probe was generated by PCR by using primers from two Expressed Sequence Tags clones that appeared to correspond to human IL-13Rα. This probe was labeled with [32P]dCTP by random priming (Boehringer Mannheim DNA labeling kit) and hybridized with membranes in 5 × saline/sodium phosphate/EDTA containing 0.1% SDS, 5 × Denhardt’s solution, and 100 μg/ml salmon sperm DNA at 65°C. Secondary screening was performed with the

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‡ The abbreviations used are: IL, interleukin; IL-13Rα, interleukin receptor α chain; γc, cytokine receptor γ chain; PCR, polymerase chain reaction; bp, base pair.

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eluted primary plaques as described above to obtain pure positive plaques.

The pBluescript phagemid was excised in vivo from the positive phage clones using Ex-Assist helper phage and XL1-Blue bacterial according to the manufacturer's instructions (Stratagene). The phagemids were propagated in XLOLR cells, plasmid DNA was purified, and DNA from each clone was sequenced both on an Applied Biosystems 431 automated sequencer and by manual sequencing using Sequenase (U. S. Biochemical Corp.).

Northern Blot Analysis—Multiple tissue poly(A) Northern blots (Clontech, Palo Alto, CA) were hybridized with a random-primed, labeled 909-bp PCR-amplified probe corresponding to nucleotides 168-1076 of human IL-13Rα, according to the manufacturer's instructions. Blots were hybridized in ExpressHyb solution (Clontech) at 60°C, washed for 40 min in 2×SSC (1×SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 0.6× SDS at room temperature, followed by 40 min at 50°C in 0.1×SSC and 0.1% SDS.

Cell Lines and Transfections—293T and COS-7 cells were cultured in Dulbecco's modified Eagle's medium (Biofluids) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO2. Full-length IL-13Rα and IL-4Rα cDNAs were subcloned into the mammalian expression vector pME18S. 293T cells were transfected at 50% confluence using calcium phosphate precipitation reagents (5 Prime-3 Prime, Inc.). Briefly, 61 μl of 1× CaCl2, was mixed with 5 μg of plasmid DNA plus H2O to 500 μl. DNA precipitation buffer (500 μl) was added gently, and the mixture was added to 293 T cells covered with 30 μl of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The medium was changed after 16 h, and cells were harvested after 24–36 h. COS-7 cells were transfected with DEAE-dextran by standard methods.

Binding and Affinity-labeling Assays—125I-labeled human IL-4 (927 Ci/mmol) and 125I-labeled human IL-2 (885 Ci/mmol) were from Amersham. Human IL-13 was labeled to a specific activity of 1135 Ci/mmol with 125I (Amersham) and IODO-GEN reagent (Pierce) as described previously (12). Binding was performed for the indicated times on ice in RPMI 1640 medium supplemented with 25 mM HEPES, pH 7.4, containing 0.5% bovine serum albumin (binding buffer) and the appropriate unlabeled and iodinated ligands (IL-4, IL-13, or IL-2). For affinity-labeling experiments, cells to which 125I-labeled cytokines had been bound were pelleted and resuspended in phosphate-buffered saline, pH 8.0, containing 1 mM MgCl2. The bifunctional water-soluble chemical cross-linker BS3 (Pierce) was added to a final concentration of 1 μM. Cells were then further incubated for 20 min on ice. After termination of the reaction with 40 mM Tris-HCl, pH 7.5, cells were lysed in lysis buffer.
buffer (10 mM Tris, pH 7.5, containing 1.25% Nonidet P-40, 0.875% Brij, 150 mM NaCl, and 2 mM EDTA) and lysates were analyzed on 7.5% polyacrylamide gels. Dots, identical amino acids; dashes, gaps inserted to improve the alignment.

RESULTS AND DISCUSSION

Isolation of Full-length cDNA for Human IL-13Ra—The sequence for murine IL-13Ra cDNA (16) was used to search for homologous sequences in the data base of the Expressed Sequence Tags, and two partial human clones of unknown function were identified. One clone (GenBank accession number H57074) exhibited an 81% match to nucleotides 406–550 of murine IL-13Ra, whereas another clone (GenBank accession number H89334) was 76% identical to nucleotides 804–985. Primer pairs based on these human sequences were prepared. Using plasmid DNA made from MT-2 and peripheral blood lymphocyte cDNA libraries as templates, we identified primer pairs that could amplify human cDNA fragments of the size anticipated based on the murine IL-13Ra sequence. A 431-bp PCR product was gel purified and sequenced. The sequence showed 80% homology to murine IL-13Ra, indicating that it corresponded to human IL-13Ra. This PCR product was then used to screen a AzaP II library made from MT-2 cells, and 11 hybridizing clones were sequenced. A full-length clone of 1572 bp was identified; it contained an 84-bp-long 5′-untranslated region, an open reading frame encoding 427 amino acids (calculated molecular weight of 48,774), and a 204-bp-long 3′-untranslated region (Fig. 1). The sequence around the predicted ATG start codon had Kozak consensus sequences typical of translation start sites (18). At the N terminus was a hydrophobic region consistent with a signal peptide. We analyzed residues 15–34 as potential signal peptide cleavage sites by summing the weight matrices for positions –12 to +2 for each of these residues; these positions most accurately predict the site of cleavage (19). The highest score was obtained for glycine 21, with a total weight matrix of 7.52. A cleavage site after this glycine also fulfills the other suggested criteria for the signal peptide cleavage site, including a small amino acid in position –1, no aromatic or charged residue at –3, and the absence of proline from positions –3 to +1 (20). Thus, we predict that the first 21 amino acids represent the signal peptide. This prediction is consistent with a hydrophobicity plot of the IL-13Ra amino acid sequence using the Kyte-Doolittle algorithm (data not shown). Based on the hydrophobicity plot, the transmembrane domain was predicted to span amino acids 344–367. Like other members of the cytokine receptor superfamily (21), a WSXWS motif and four conserved cysteine residues were found in the extracellular domain of IL-13Ra (Fig. 1, boxed). A total of 11 potential N-linked carbohydrate addition sites (Asn-X-Ser/Thr) are present in the extracellular domain, including one that is contained within the WSXWS motif. The open reading frame of human IL-13Ra has 81% nucleotide and 76% amino acid identity with the published murine IL-13Ra sequence (16) and contains 3 additional amino acids (Fig. 2). Interestingly, 57 of the 60 amino acids in the cytoplasmic domain (95%) are identical in human and murine IL-13Ra, underscoring the likely significance of this region in signal transduction.

Human IL-13Ra mRNA Is Expressed in Multiple Tissues—The expression pattern of human IL-13Ra was examined by Northern blot analysis (Fig. 3). Two species of mRNA of approximately 4.0 and 2.0 kilobases were detected (Fig. 3). Expression of IL-13Ra mRNA was detected in all tissues examined, with the highest levels in heart, liver, skeletal muscle, and ovary and the lowest levels in brain, lung, and kidney. The high level of expression in skeletal muscle was interesting, since murine IL-13Ra mRNA was not detected in this tissue (16). Like murine IL-13Ra, human IL-13Ra also exhibits two principal transcripts (5.2 and 2.2 kilobases for murine IL-13Ra; Ref. 15; 4.0 and 2.0 kilobases for human IL-13Ra), presumably reflecting alternative polyadenylation in each case. As expected, the human mRNAs are each longer than our full-length cDNA, which did not contain a consensus AATAAA polyadenylation sequence and, therefore, presumably does not contain the entire 3′-untranslated region.

Coexpression of IL-4Ra and IL-13Ra Is Required to Generate High Affinity IL-13 Binding—To examine the binding specificity of human IL-13Ra, we performed affinity-labeling experiments in which 125I-labeled human IL-13 or IL-4 was bound and cross-linked to 293T fibroblasts that had been transfected with IL-13Ra, IL-4Ra, or both IL-13Ra and IL-4Ra. No signal was detected except in cells transfected with both IL-4Ra and IL-13Ra (Fig. 4A). In cells transfected with both cDNAs, a major 75–80-kDa band was seen, presumably reflecting the cross-linking of 125I-labeled human IL-13 (12–15 kDa) to IL-13Ra, thus resulting in an apparent molecular mass of approximately 65 kDa for IL-13Ra (although the calculated molecular weight for this protein is 48,774, the presence of multiple glycosylation sites presumably explains its slower migration on gels). In addition, a larger band corresponding to affinity-labeled IL-4Ra was also seen. Both bands were competed when a 500-fold molar excess of either unlabeled IL-13 and IL-4 were added in the binding phase (Fig. 4A). In contrast, 125I-labeled human IL-4 was cross-linked mainly to the 140-kDa IL-4Ra chain in a fashion that was independent of coexpression of IL-13Ra (Fig. 4B). In cells transfected with both IL-4Ra and IL-13R, in addition to affinity-labeled IL-4Ra, a weak 75–80-kDa band corresponding to affinity-labeled IL-13Ra was also observed. The appearance of both of these affinity-labeled bands was competed by excess unlabeled IL-4 or IL-13 in the binding phase, whereas in cells transfected only with IL-4Ra, only IL-4 and not IL-13 competed the binding of 125I-IL-4 to
IL-4Rα. These results were confirmed in binding assays. Corresponding to the affinity-labeling experiments, significant specific binding of $^{125}$I-IL-13 was observed only when both receptor chains were expressed on 293T cells and either excess IL-4 or IL-13 could compete the binding. The minimal IL-13 binding activity detected when cells were transfected with only IL-13Rα (Fig. 5A) is consistent with the low binding affinity of murine IL-13Rα for murine IL-13 (2–10 nM; Ref. 15). In contrast, expression of IL-4Rα resulted in significant binding of $^{125}$I-IL-4, and additional expression of IL-13Rα only slightly increased this binding (Fig. 5B). Consistent with the affinity-labeling experiments, unlabeled IL-13 could not compete with the binding of $^{125}$I-IL-4 in the absence of IL-13Rα. In cells transfected with both IL-4Rα and IL-13Rα, however, IL-13 displaced approximately 80% of specifically bound $^{125}$I-IL-4 (Fig. 5B), consistent with previously published results in COS cells (11).

**Binding Equilibrium of $^{125}$I-IL-13 and Scatchard Plot Analysis**—To determine the kinetics of binding of IL-13 to its receptor, we incubated 293T cells cotransfected with IL-13Rα and IL-4Rα with $^{125}$I-IL-4 at different times. As shown in Fig. 6A, the specific binding of $^{125}$I-IL-13 steadily increased over 6 h, after which time binding equilibrium was achieved. Based on this slow on rate, displacement experiments for Scatchard analysis were performed after 6 h of binding at 4 °C. Bound and free radioactivity were determined. An experiment representative of four independent experiments is shown.
firm that a receptor complex consisting of human IL-4Rα and IL-13Rα can efficiently bind either IL-4 or IL-13, and that in this context IL-4 and IL-13 can compete for the same binding sites. All known cytokines of the cytokine receptor superfamily use multimerized forms (homodimers, heterodimers, or higher order oligomers) of receptors for signaling. According to the current understanding, one receptor chain functions as a primary ligand-binding protein, and the accessory receptor chain(s) are then recruited to form the functional multimerized receptor complex, which has a higher affinity for the ligand than the primary binding subunit. For IL-4 and IL-13, it is clear from the affinity-labeling experiments that IL-4 more efficiently cross-links to IL-4Rα, whereas IL-13 more efficiently cross-links to IL-13Rα. The weak binding of IL-13 to cells expressing human IL-13Rα was therefore unexpected, since IL-4 shows substantial binding to IL-4Rα. In contrast, human IL-13 needs the expression of both IL-13Rα and IL-4Rα for efficient binding. The relatively poor binding of IL-13 for IL-13Rα suggests that IL-4Rα and IL-13Rα may have certain affinity for each other and that a heterodimer of these chains might exist in the absence of ligand. The development of antibodies will facilitate the ability to address this question. In this regard, it is interesting that in addition to ligand binding sites, the growth hormone receptor also contains a receptor-receptor dimerization surface (site 3) (21). Although site 3 is not sufficient by itself to allow growth hormone receptor dimerization, in the presence of ligand it significantly stabilizes the receptor dimer. It remains to be determined whether IL-4Rα and IL-13Rα can interact in the absence of ligand.

It is useful to also consider the possibility that the receptor

FIG. 6. Binding kinetics and Scatchard plot analysis of binding of 125I-IL-13 to 293T" cells transfected with plasmids containing IL-13Rα and IL-4Rα cDNAs. A, cells were incubated with 200 pM 125I-IL-13 with and without a 500-fold molar excess of IL-13 for the indicated times at 4 °C. Specific binding was calculated by subtracting the bound counts in presence of excess IL-13 from the counts bound in its absence. Binding for Scatchard plot analysis was performed at 4 °C for 6 h; bound and free radioactivity were determined and analyzed using the LIGAND computer program. A displacement curve (B) and Scatchard transformation (C) representative of four experiments are shown. In the experiment shown, the Kd was 400 pM; the range was approximately 300–600 pM.

FIG. 7. IL-13Rα does not cooperate with IL-2Rβ to bind IL-2. COS-7 cells were transfected with the indicated plasmids, incubated with 2 nM 125I-IL-2 in the presence or absence of a 500-fold molar excess of unlabeled IL-2 or IL-13, and cross-linked with BS3, and affinity-labeled proteins were then analyzed on SDS gels. Gels were visualized on a PhosphorImager.

FIG. 8. Localization of the human IL-13Rα gene to chromosome X. A panel of 24 DNA samples from mostly monochromosomal somatic cell hybrids were analyzed by PCR using primers specific for human IL-13Rα. A specific band of 148 bp was amplified reproducibly from two hybrids, one containing only human chromosome X and the other containing both human chromosomes 1 and X, indicating that the gene encoding human IL-13Rα is located on human chromosome X. The human chromosomes contained in each hybrid are indicated. As controls, human genomic DNA (hu.gen.DNA) and murine (Mu) and human (Hu) IL-13Rα cDNAs were included as templates for the PCR reactions.
for IL-13 may contain a third, as of yet unrecognized, receptor component. A precedent for such a model is provided by analogy to the IL-2 receptor system in which IL-2 binds very poorly to IL-2Rβ (22, 23), even though this binding is a prerequisite for interaction of the common γc. However, IL-2 binding to IL-2Rβ is greatly facilitated by its binding to IL-2Ra (24), a receptor-binding protein that is not part of the cytokine receptor superfamily (25) but which has a much faster on rate for IL-2 than IL-2Rβ (26, 27). It is conceivable that such an additional receptor component could also exist for IL-13, although at present, no definitive biochemical data indicate the existence of such a protein.

IL-4Ra can form a functional IL-4 receptor in combination with γc (8, 9). The fact that both IL-13Ra and γc can interact with IL-4Ra to form functional IL-4 receptors implies that IL-13Ra and γc may share at least one epitope for IL-4 binding, even though the extracellular domains of γc and IL-13Ra share only 18% sequence identity (overall homology is 14%). Since IL-2 and IL-4 share related structures containing four α helices in an “up-up-down-down” configuration (28), and it is reasonable to assume that they contact similar regions of γc, we asked whether IL-13Ra might also share an epitope with γc for IL-2 binding. However, affinity-labeling studies showed that IL-2 could not bind to COS cells when these cells were transfected with IL-13Ra together with IL-2Rβ, although, as expected, it could affinity label cells transfected with both IL-2Rβ and γc (Fig. 7). These data show that IL-13Ra binding is specific for IL-4 and IL-13. However, in addition to their being similar in size, it is noteworthy that the genes encoding γc and IL-13Ra are both located on the X chromosome (Fig. 8). In view of the expression of IL-13Ra in multiple tissues, it is conceivable that mutations in IL-13Ra may result in a severe or even lethal phenotype. This will be clarified by targeted deletion of IL-13Ra in mice.

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Addendum—Since submission of this manuscript, Caput et al. (29) have reported the cloning of an IL-13-binding protein that is a member of the cytokine receptor superfamily, providing biochemical proof of the existence of a third receptor component. Thus, we hypothesize that the high affinity human IL-13 receptor contains the protein we have cloned, the one cloned by Caput et al. (29), and IL-4Ra. It may make sense for investigators in the IL-4 and IL-13 fields to reconsider the nomenclature for the receptor subunits, since by analogy to the IL-2 receptor system, the protein cloned by Caput et al. (29) is most similar to IL-2Rβ.