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Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction

Sandra M.Zurawski, Felix Vega Jr, Bernard Huyghe and Gerard Zurawski

Department of Molecular Biology, DNAX Research Institute for Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304-1104, USA

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Interleukin-4 (IL-4) and interleukin-13 (IL-13) are two cytokines that are secreted by activated T cells and have similar effects on monocytes and B cells. We describe a mutant form of human interleukin-4 (hIL-4) that competitively antagonizes both hIL-4 and human interleukin-13 (hIL-13). The amino acid sequences of IL-4 and IL-13 are ~30% homologous and circular dichroism (CD) spectroscopy shows that both proteins have a highly α-helical structure. IL-13 competitively inhibited binding of hIL-4 to functional human IL-4 receptors (called hIL-4R) expressed on a cell line which responds to both hIL-4 and IL-13. Binding of hIL-4 to an hIL-4 responsive cell line that does not respond to IL-13, and binding of hIL-4 to cloned IL-4R ligand binding protein expressed on heterologous cells, were not inhibited by IL-13. hIL-4 bound with ~100-fold lower affinity to the IL-4R ligand binding protein than to functional IL-4R. The mutant hIL-4 antagonist protein bound to both IL-4R types with the lower affinity. The above results demonstrate that IL-4 and IL-13 share a receptor component that is important for signal transduction. In addition, our data establish that IL-4R is a complex of at least two components one of which is a novel affinity converting subunit that is critical for cellular signal transduction.

Key words: antagonist/interleukin-4/interleukin-13/mutagenesis/receptor

Introduction

Interleukin-13 (IL-13) is one of a number of protein hormones called cytokines that are secreted by activated T cells (Cherwinski *et al.*, 1987; Brown *et al.*, 1989; Mosmann and Coffman, 1989; McKenzie *et al.*, 1993; Minty *et al.*, 1993; Punnonen *et al.*, 1993). Human IL-13 elicits morphological and cell surface phenotype changes on human monocytes and also facilitates growth and immunoglobulin (Ig) production by human B cells (McKenzie *et al.*, 1993; Minty *et al.*, 1993; Punnonen *et al.*, 1993). All these biological effects are also elicited by human interleukin-4 (hIL-4), another protein hormone secreted by activated T cells (Figdor and te Velde, 1992; Gauchat *et al.*, 1992; Paul, 1992).

The biological actions of IL-4 are mediated by a cell surface receptor that binds IL-4 with high specificity and affinity (dissociation constant $K_d \approx 10^{-10}$ M, reviewed by Harada *et al.*, 1992a). Human and mouse IL-4R have been characterized by cDNA cloning which defined a ~130 kDa glycoprotein (herein referred to as IL-4R ligand binding protein) with a single transmembrane span (Mosley *et al.*, 1989; Galizzi *et al.*, 1990b; Harada *et al.*, 1990; Idzerda *et al.*, 1990). The extracellular domain sequence of IL-4R ligand binding protein is structurally homologous to the extracellular domains of other cytokine receptor proteins (Bazan, 1990a,b, 1991). Several of these other proteins participate in heteromeric interactions where one subunit by itself binds the ligand at a relatively low affinity and the other subunit(s) contribute additional binding affinity and are often important for signaling (Kishimoto *et al.*, 1992; Miyajima *et al.*, 1992). However, the extracellular domain of the IL-4R ligand binding protein alone appears to bind IL-4 at the high affinity that characterizes IL-4R on various IL-4 responsive cells (reviewed by Harada *et al.*, 1992a). Although the intracellular domain is unimportant for binding, it is important for signal transduction (Harada *et al.*, 1992b).

The structural homologies between many of the cytokine receptors is mirrored by structural homologies between their ligands. For example, IL-4 interleukin-2 (IL-2), growth hormone, macrophage colony-stimulating factor and granulocyte macrophage colony-stimulating factor (GM-CSF) are not related at the sequence level, yet all have a similar compact core-bundle structure of four antiparallel α-helices (Diederichs *et al.*, 1991; Bazan, 1992; McKay, 1992; Pandit *et al.*, 1992; Powers *et al.*, 1992). In mouse IL-2, exhaustive mutational analyses led to the discovery that substitution of a residue (Gln141 → Asp) at the C terminus of the fourth α-helix results in loss of receptor activation, but retention of most receptor binding (Zurawski *et al.*, 1990; Zurawski and Zurawski, 1992). This mutant protein is a potent and specific competitive antagonist of IL-2 biological action.

Based on the structural homology between IL-2 and IL-4 (Bazan, 1992; McKay, 1992; Powers *et al.*, 1992), we investigated the importance of residues of hIL-4 that might be analogous to mIL-2 Gln141. In this work we found that substitution of a residue (Tyr124 → Asp) at the C-terminus of the fourth α-helix of hIL-4 specifically abrogates IL-4R activation and renders the protein a competitive antagonist of IL-4 biological action. This property of hIL-4.Tyr124 → Asp (called hIL-4.Y124D) has been described independently (Kruse *et al.*, 1992). Here, we show that this mutant hIL-4 antagonist is specifically defective in interaction with a previously unknown second subunit of the functional IL-4R. In addition, we report that the hIL-4 antagonist blocks IL-13 biological action and we discuss the probable basis of this phenomenon.

Results

Mutant hIL-4 antagonist blocks IL-13 action on TF-1 cells

In a search for mutant hIL-4 antagonists (see Materials and methods), we and others (Kruse *et al.*, 1992) noted that an

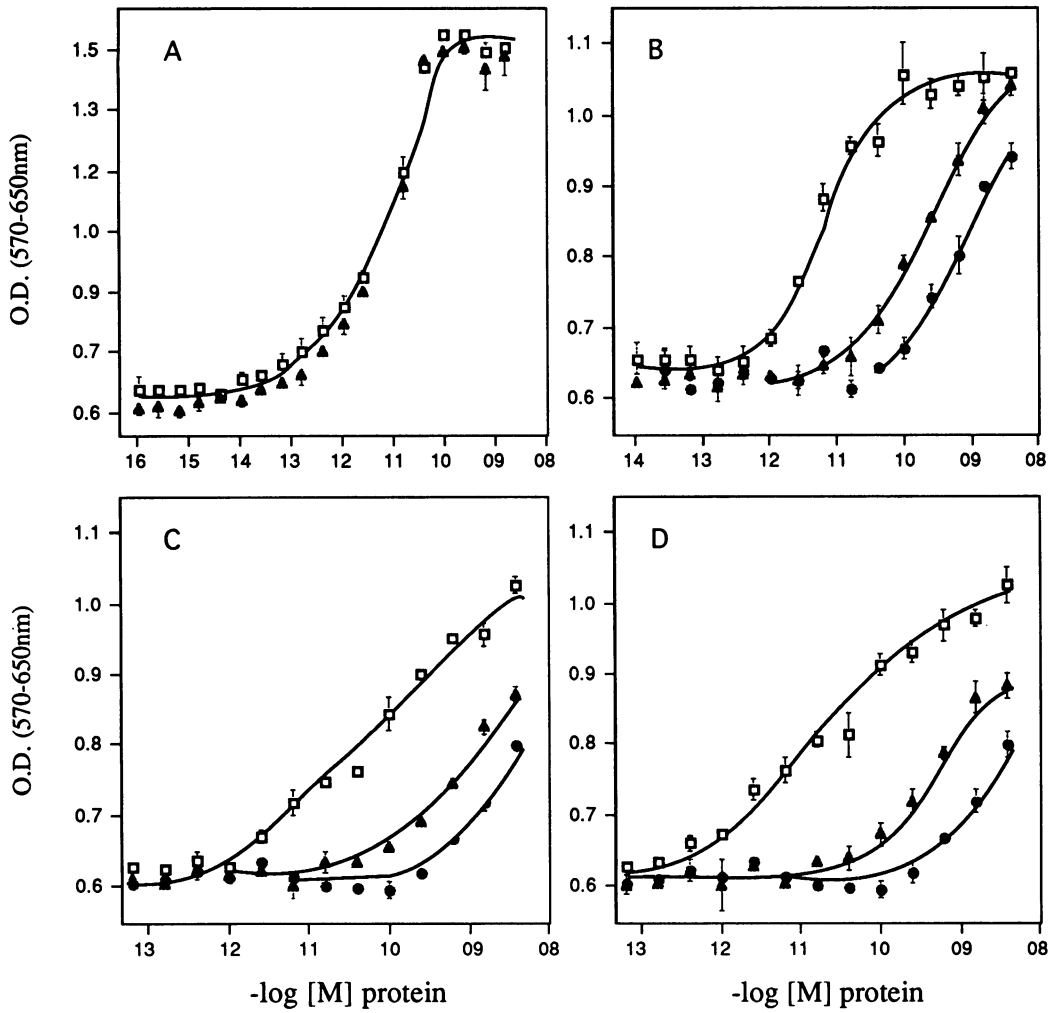

Fig. 1. Mutant hIL-4.Y124D protein antagonizes the action of hIL-4, hIL-13, mIL-13, but not hGM-CSF on TF-1 cells. Dose responses of TF-1 cells to (**A**) hGM-CSF, (**B**) hIL-4, (**C**) mIL-13 and (**D**) hIL-13 were determined in the absence (□) and presence of 2.4×10^{-8} M (●) or 8.0×10^{-9} M (▲) hIL-4.Y124D. Error bars are standard deviations ($n = 3$).

Asp substitution at residues Tyr124 of hIL-4 resulted in loss of receptor activation without significant loss of receptor binding. As expected from these properties, hIL-4.Y124D was a competitive antagonist of the action of native hIL-4 on TF-1 cells (Figure 1B). TF-1 is a human pre-myeloid erythroleukemic cell line which shows a growth response to various human protein hormones, such as GM-CSF, interleukin-3 (IL-3), interleukin-6 (IL-6), IL-4 (Kitamura *et al.*, 1989), and both human and mouse IL-13 (McKenzie *et al.*, 1993). The maximal responses of TF-1 cells to these factors varies widely, but the maximal biological responses of IL-4 and IL-13 are similar. We found that hIL-4.Y124D had no effect on the TF-1 responses to GM-CSF (Figure 1A), IL-3 or IL-6 (data not shown). In contrast, hIL-4.Y124D was a potent antagonist of both mIL-13 and hIL-13 action on TF-1 cells (Figure 1C and D). hIL-4.Y124D was equipotent against hIL-4, mIL-13 and hIL-13 activities on TF-1 cells and inhibited in a dose-dependent manner (Figure 1B–D).

IL-13 competitively inhibits hIL-4 binding to TF-1 cells

Since hIL-4.Y124D antagonizes hIL-4 via competitive inhibition of hIL-4 binding to IL-4R, a similar mechanism was likely for its action against IL-13. Such a mode of hIL-4.Y124D action against IL-13 would imply commonalty between IL-4R and IL-13R. We tested this by comparing the abilities of hIL-4 and hIL-13 to competitively displace binding of [^{125}I]hIL-4 to TF-1 cells. hIL-4 fully competed binding of [^{125}I]hIL-4 to TF-1 cells with the concentration required for 50% inhibition (or IC_{50}) $\approx 8 \times 10^{-13}$ M (Figure 2A). hIL-13 also competed binding of [^{125}I]hIL-4 to these cells (Figure 2A). However, compared with hIL-4, it could not completely displace binding of [^{125}I]hIL-4 ($\sim$63% of the binding was displaced) and its IC_{50} value (5×10^{-11} M) was higher.

Some hIL-4 responsive cell types do not respond to IL-13

The first characterizations of the biological activities of IL-13 have shown concordance between cellular responses to IL-4 and IL-13 (McKenzie *et al.*, 1993; Minty *et al.*, 1993; Punnonen *et al.*, 1993). Human peripheral blood mononuclear cells (PBMNC) activated with phytohemagglutinin (PHA) and certain cloned cell lines derived from human T cells such as SP-B21 proliferate in response to hIL-4 (Spits *et al.*, 1987; Bacchetta *et al.*, 1990). Figure 3 shows that both these hIL-4 responsive cell types did not proliferate in response to hIL-13.

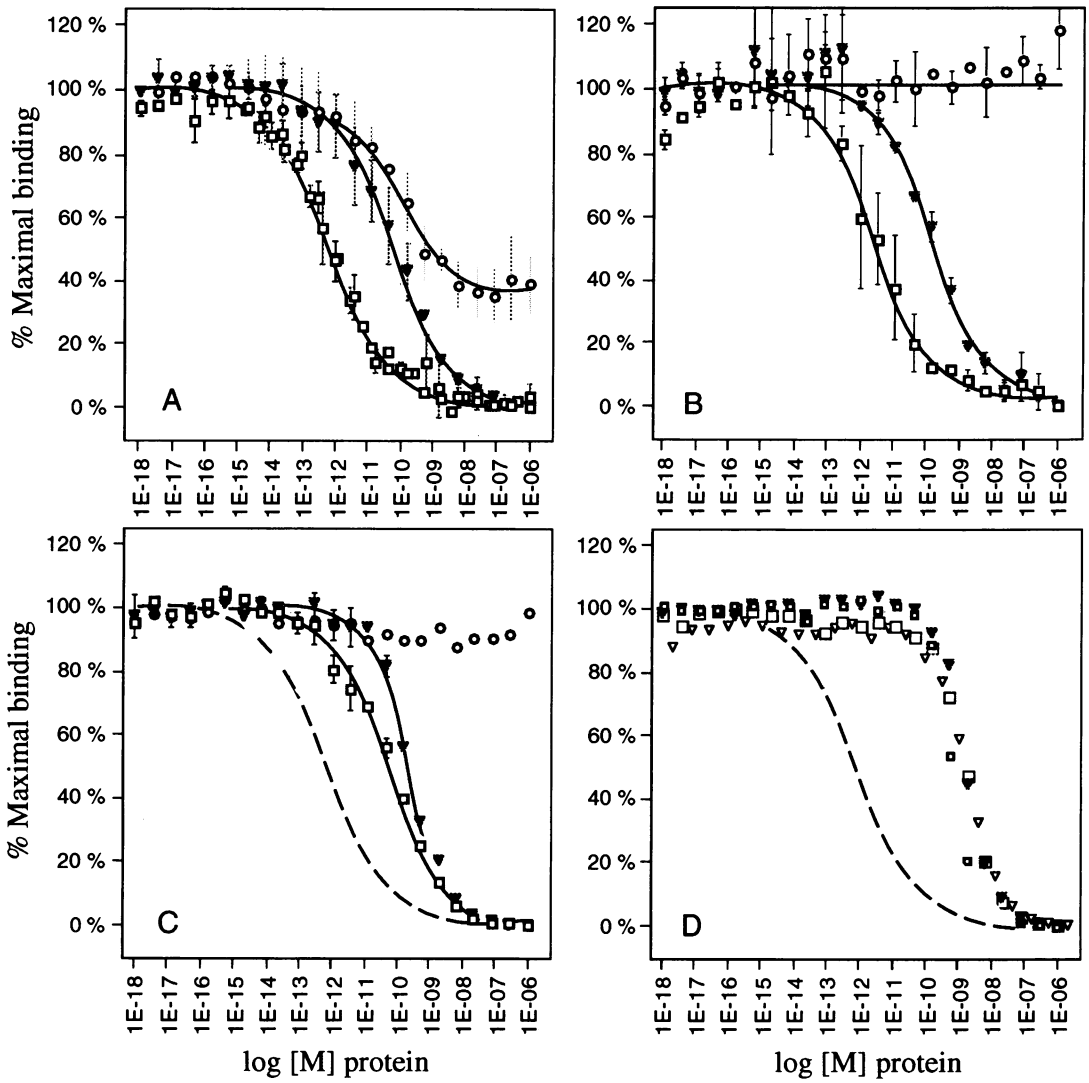

Fig. 2. Competitive displacement of [^{125}I]hIL-4 binding to various cell types. Various amounts of non-labeled hIL-4 (□), hIL-4.Y124D (▼) and hIL-13 (○) were incubated for 2 h at 4°C with 10^{-11} M [^{125}I]hIL-4 and cells. [^{125}I]hIL-4 bound to cells was then determined. (**A**) 1.6 × 10^6 TF-1 cells per point (n = 3). 10^{-6} M hGM-CSF did not compete for binding of [^{125}I]hIL-4 (data not shown). (**B**) 1.0 × 10^6 SP-B21 cells per point (n = 2). (**C**) 1.0 × 10^6 per point Cos-7 cells expressing full-length hIL-4R ligand binding protein (n = 2). The dashed curve is the displacement curve for hIL-4 from (**A**) and is included for reference. (**D**) 1.0 × 10^6 per point Cos-7 cells expressing hIL-4R-S competed with hIL-4 (■) or hIL-4.Y124D (▽) and 1.8 × 10^6 Ba/F3 hIL-4R-S cells per point and 10^{-10} M [^{125}I]hIL-4 competed with hIL-4 (□) or hIL-4.Y124D (▼). 10^{-6} M mIL-3 did not compete for binding of [^{125}I]hIL-4 (data not shown). The dashed curve is the displacement curve for hIL-4 from (A) and is included for reference. Analysis with the Ligand computer program of our combined data for binding to Ba/F3 hIL-4R-S cells estimated K_d = 1.6 × 10^{-10} M (%CV or standard error divided by the value = 15%) for hIL-4 and K_d = 6.2 × 10^{-10} M (%CV = 15%) for hIL-4.Y124D. Error bars are standard deviations. Other experiments, including some with hIL-4.Y124D as the labeled ligand, gave analogous results.

IL-13 does not bind to the IL-4R ligand binding protein

A possible basis for the commonalty between IL-4R and IL-13R on TF-1 cells is that they are the same, but this is unlikely since some hIL-4 responsive cells do not respond to hIL-13. We addressed this issue by comparing the abilities of hIL-4 and hIL-13 to competitively displace binding of [^{125}I]hIL-4 to SP-B21 cells and to the cloned hIL-4R ligand binding protein expressed on monkey Cos-7 cells. Although hIL-4 fully competed binding of [^{125}I]hIL-4 to these cell types, even high levels of hIL-13 (10^{-6} M) did not compete (Figure 2B and C).

The binding properties of hIL-4.Y124D and hIL-4

The binding of hIL-4 to mouse Ba/F3 cells expressing cloned hIL-4R with the cytoplasmic region deleted (hIL-4R-S) corresponded closely (K_d = 1.6 × 10^{-10} M, Figure 2D) to that previously characterized for the high affinity hIL-4 ($K_d \approx 10^{-10}$ M, reviewed in Harada *et al.*, 1992a). Human lymphoma Raji cells have high affinity binding sites for hIL-4 ($K_d \approx 10^{-10}$ M, Kruse *et al.*, 1992) and hIL-4.Y124D protein binds to these cells with only a 3-fold reduced affinity compared with hIL-4 (Kruse *et al.*, 1992). We also found that the avidity of hIL-4 and hIL-4.Y124D for hIL-4R-S expressed on Ba/F3 or Cos-7 cells were within 3-fold of each other (Figure 2D).

TF-1 and SP-B21 cells bound hIL-4 with an apparent affinity that was ~75- to 100-fold higher than the 'high affinity' binding of hIL-4 to Ba/F3 hIL-4R-S cells (compare Figure 2A and B with D). We were surprised by this observation because, while our comparisons were done in parallel and used identical conditions and reagents, TF-1 and

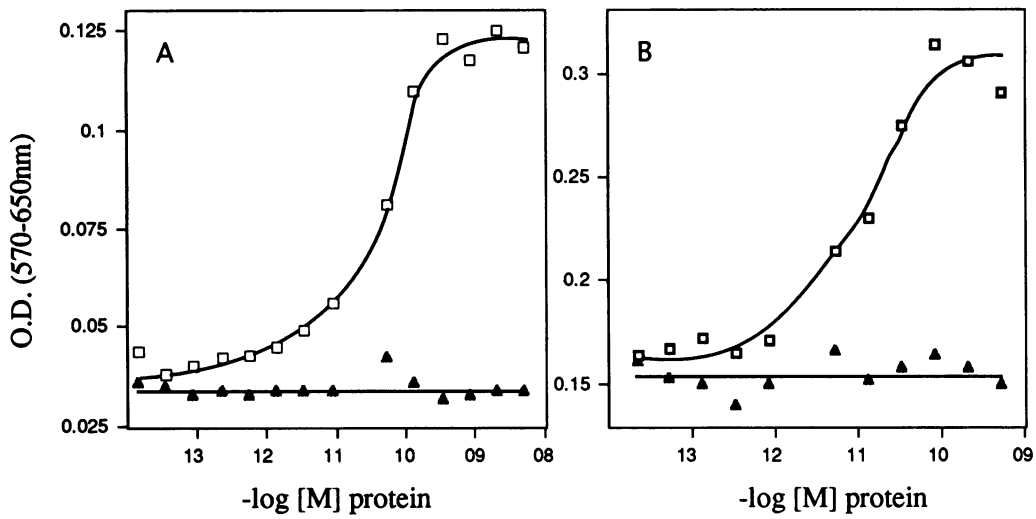

Fig. 3. Some human cell types respond to hIL-4 but not to hIL-13. Dose responses to hIL-4 (□) and hIL-13 (▲) of two cell types were determined. (**A**) Th0 $CD4^+$ B21 T cells. (**B**) PHA-activated PBMNC.

Ba/F3 hIL-4R-S cells have been reported to have similar numbers of binding sites and affinities for hIL-4 as defined by equilibrium binding studies (Harada *et al.*, 1992a,b). This difference does not result from deletion of the hIL-4R cytoplasmic domain as Cos-7 cells expressing the cloned full-length hIL-4R also bound hIL-4 ~50- to 100-fold less avidly than TF-1 and SP-B21 cells (compare Figure 2A and B with C).

In contrast to the widely different binding affinities of hIL-4 on various cell types, there was no significant difference in binding of hIL-4.Y124D to TF-1, SP-B21 and Cos-7 expressing hIL-4R (Figure 2A–C), and its binding to hIL-4R-S on both Cos-7 and Ba/F3 cells was only slightly reduced (Figure 2D).

IL-4 and IL-13 are structural homologues

The commonalty between IL-4R and IL-13R prompted us to examine closely the sequence relatedness of IL-4 and IL-13. We considered only the sequences of the mature human and mouse IL-4 and IL-13 proteins and assumed that known disulfide linkages for IL-4 are preserved for IL-13. The alignment shown in Figure 4 shows that there was significant, although low (~30%), sequence homology between IL-4 and IL-13. The significance of this observation was increased when the known structural features of hIL-4 were considered. All of the 25 residues that contribute to the hIL-4 hydrophobic structural core (Powers *et al.*, 1992) were conserved or had conservative hydrophobic replacements in IL-13. Extensive insertion/deletion differences between IL-4 and IL-13 were, with one exception, confined to loops that connect the four α-helices or two short β-strands. The exception was a shortened α-helix C, although all the α-helix C residues that contribute to the structural core were retained in IL-13 (Figure 4).

The circular dichroism (CD) absorption spectra in the far UV range of mIL-13, hIL-4 and hIL-1α are shown in Figure 5. mIL-13, unlike the β-stranded hIL-1α (Priestle *et al.*, 1988), had a CD absorption spectrum characteristic of a highly α-helical protein such as hIL-4 (Johnson, 1988).

Discussion

IL-13 and IL-4 receptors are functionally related

Our observation that the hIL-4.Y124D antagonist competitively inhibited the biological action on TF-1 cells of both hIL-4 and IL-13 demonstrates a relationship between IL-4R and IL-13R. The ability of hIL-13 to compete for binding of [^{125}I]hIL-4 to TF-1 cells confirmed the commonalty of IL-4R and IL-13R. However, SP-B21 cells respond to IL-4 and not to IL-13 and, on these cells, hIL-13 failed to compete for binding of [^{125}I]hIL-4. hIL-13 also failed to compete for binding of [^{125}I]hIL-4 to Cos-7 cells expressing the cloned hIL-4R ligand binding protein. These data show that IL-4R and IL-13R are different, yet still suggest that IL-4R and IL-13R have a functionally important receptor component in common. Complexity in IL-4R has been previously suggested by studies that find proteins associated with the IL-4R ligand binding protein (Foxwell *et al.*, 1989; Galizzi *et al.*, 1990; Harada *et al.*, 1992b). Also, kinetic studies of soluble natural mouse IL-4R ligand binding proteins indicate that membrane-bound functional IL-4R–IL-4 complexes are more stable than soluble IL-4R–IL-4 (Fernandez and Vitetta, 1991).

Functional IL-4R contains an additional subunit(s) that enhances affinity, helps transduce the signal and is shared with IL-13R

We provide two results from receptor binding analyses which show that functional IL-4R is a complex and can exist in a higher affinity state than previously recognized. First, we observed that the apparent affinity of hIL-4 for functional hIL-4R on TF-1 and SP-B21 cells was ~100-fold greater than for the cloned hIL-4R ligand binding protein expressed on Ba/F3 or Cos-7 cells. Secondly, hIL-4 and hIL-4.Y124D differed only slightly in their binding to IL-4R ligand binding protein expressed on Ba/F3 or Cos-7 cells, but hIL-4.Y124D bound much less avidly than hIL-4 to functional hIL-4R.

A model that accounts for the above observations is that functional IL-4Rs are a complex between the IL-4R ligand

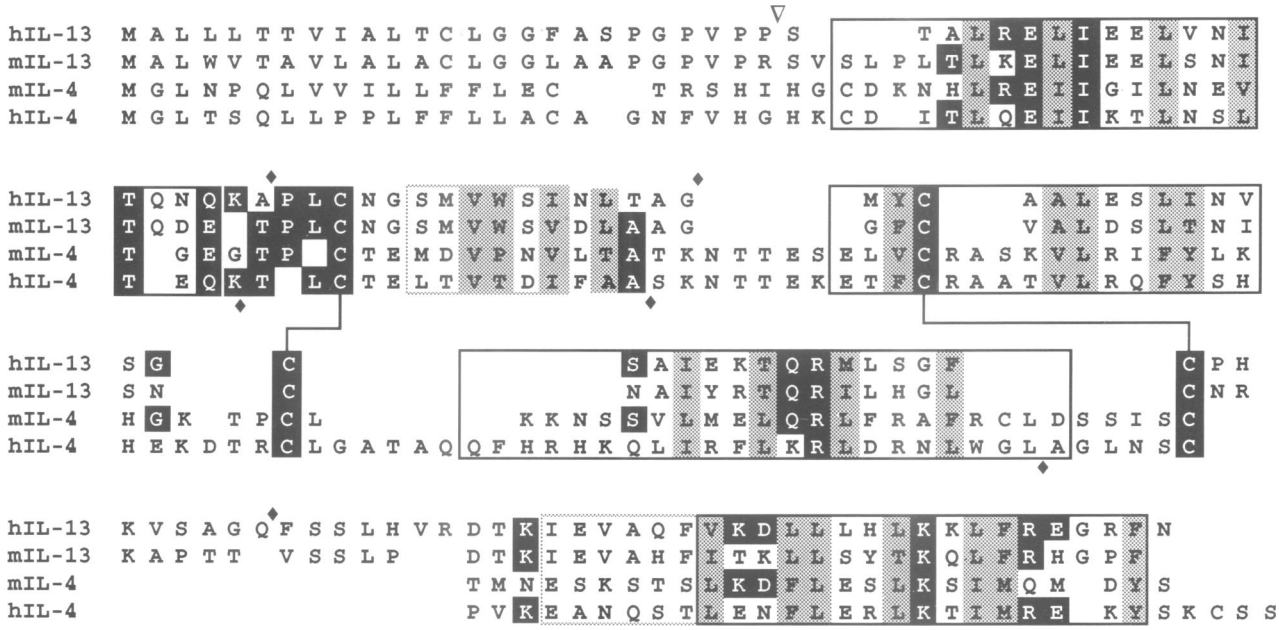

Fig. 4. Sequence relatedness of IL-4 and IL-13. Alignment of the mature protein sequences of human and mouse IL-4 (Lee *et al.*, 1986; Noma *et al.*, 1986; Yokota *et al.*, 1986) and IL-13 (Brown *et al.*, 1989; McKenzie *et al.*, 1993a,b). Residues in black backgrounds are common to at least one IL-4 and one IL-13 sequence. Residues in grey backgrounds have hydrophobic side chains in hIL-4 that are buried in the structural core (Powers *et al.*, 1992). Black boxes delineate the four α-helical regions of hIL-4 and grey boxes delineate the two β-strands of hIL-4 (Powers *et al.*, 1992). △ indicates the hIL-4 leader peptide processing site (Le *et al.*, 1988) and ♦ indicate the gene exon/intron junctions (Otsuka *et al.*, 1987; Arai *et al.*, 1989; McKenzie *et al.*, manuscript submitted). ⌐¬ indicate the disulfide linkages that are known for hIL-4 (Trotta, 1992). A similar alignment has been noted by Minty *et al.* (1993).

binding protein and an additional component (or components) that enhances the affinity of the IL-4R ligand binding protein for IL-4. This additional component(s) may also associate with an IL-13 ligand binding protein present only on a subset of IL-4 responsive cells to form IL-13R. This could account for the ability of hIL-4.Y124D to antagonize IL-13 action on such cells via a mechanism where the additional component(s) are sequestered from IL-13R complexes by forming non-productive hIL-4R−hIL-4.Y124D complexes. This could also account for the ability of hIL-13 to compete for binding of hIL-4 to such cells by decreasing the affinity of hIL-4R via sequestration of the additional component(s) into hIL-13−hIL-13R complexes.

Our recognition of complexity in IL-4R stems from use in biological and receptor binding analyses of IL-13 and hIL-4.Y124D, two reagents that have not previously been available. Failure to recognize previously the 'higher affinity' state of functional IL-4R may result partly from use of radiolabeling of IL-4 via iodination of Tyr residues (Cabrillat *et al.*, 1987; Park *et al.*, 1987). There are only two Tyr residues in hIL-4 and it is possible that such labeled hIL-4 has a reduced affinity for functional hIL-4R. This is not a problem in competition binding analyses which directly measure the affinity of the 'cold' competitor. A second factor that may have hindered the discovery of two affinity states for IL-4R is that the difference between the two affinities may vary and is relatively small (~50- to 100-fold. Thus, if cells have a mixture of IL-4R in both states, especially if the 'lower affinity' state predominates, then two affinities may be difficult to recognize. Direct comparisons between the binding properties of hIL-4 and hIL-4.Y124D now allow meaningful dissection of such situations. For example, our data show slight differences in slopes and affinities of hIL-4 and hIL-4.Y124D binding to full-length compared with cytoplasmically truncated hIL-4R ligand binding protein expressed on Cos-7 cells (Figure 2C and D). This may suggest that the additional IL-4R component(s) requires this intracellular domain for correct association.

Common subunits in other cytokine receptors

The molecular nature of the functionally important receptor component common to IL-4R and IL-13R is unclear. The above model to account for our data is based on the existence of other affinity modulating proteins that are obligatory components shared between several functional cytokine receptors. Such shared components have been discovered in receptors for IL-6, oncostatin-M, leukemia inhibitory factor and ciliary neurotrophic factor, which all share gp130 (Kishimoto *et al.*, 1992), as well as for human IL-3, interleukin-5 (IL-5) and GM-CSF receptors, which all share the β_c protein (Miyajima *et al.*, 1992). This shared β_c receptor subunit accounts for the observed cross-competition of IL-3, IL-5 and GM-CSF binding to certain cell types (Lopez *et al.*, 1991; Nicola and Metcalf, 1991). When assayed on TF-1 cells, hIL-4.Y124D did not antagonize the biological activities of hIL-6, mouse leukemia inhibitory factor, hIL-3 or hGM-CSF and neither hIL-6 nor hGM-CSF competed for hIL-4 binding. Therefore, we feel that gp130 or the β_c protein are not likely candidates for the additional IL-4R component, nor the component shared between IL-4R and IL-13R.

Implications of jointly antagonizing IL-4 and IL-13 responses in vivo

The ability of the hIL-4.Y124D antagonist to act against both hIL-4 and hIL-13 biological responses should provoke a

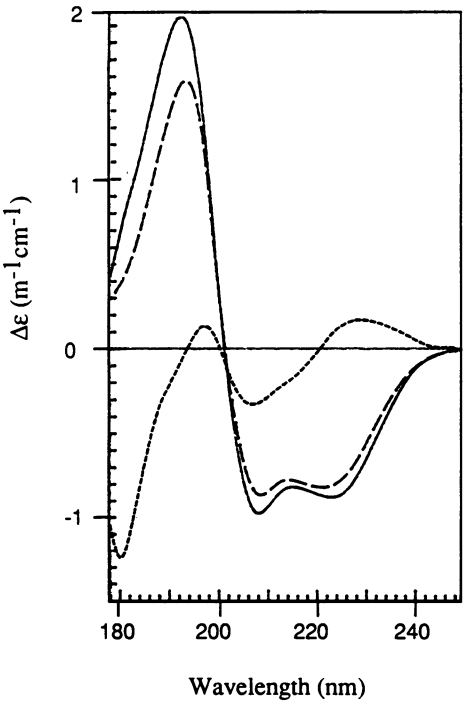

Fig. 5. mIL-13 has a high α-helical content. Far UV CD spectra of mIL-13 (—), hIL-4 (---) and hIL-1α (···).

reappraisal of the therapeutic potential of hIL-4.Y124D. Our results show that, unlike soluble IL-4R ligand binding protein (Garrone *et al.*, 1991) or anti-IL-4 antibodies (Finkelman *et al.*, 1990; Maliszewski *et al.*, 1990), hIL-4.Y124D is not a specific antagonist of hIL-4 action. Inhibitory IL-4 variants have been suggested as potentially useful drugs in the treatment of IgE mediated diseases (Kruse *et al.*, 1992). The ramifications of antagonizing both hIL-4 and hIL-13 responses by hIL-4.Y124D treatment for various disease states cannot be judged fairly until a more complete picture of IL-13 biology emerges. Regarding elucidation of IL-13 biology and the potential efficacy of hIL-4.Y124D, a final point is in order. Our observations of the structural homology between IL-4 and IL-13 and sharing of receptor subunit(s) between IL-4R and IL-13R predict that particular IL-13 residues within α-helix D are specifically important for receptor signaling and that substitutions in these residues may result in IL-13 variants that are antagonists. Our results also predict that such IL-13 antagonists will be effective antagonists against IL-4 responses on cell types that also respond to IL-13.

Materials and methods

Mutagenesis of hIL-4

Based on mutagenesis studies of mIL-2 (Zurawski *et al.*, 1990; Zurawski and Zurawski, 1992), the shared structural frameworks of hIL-2 and hIL-4 (Bazan, 1992; McKay, 1992; Powers *et al.*, 1992), and assuming evolutionary conservation of functionally important residues, we selected hIL-4 residues E114, K117 and Y124 as those most likely to be specifically involved in receptor activation. Substitution mutagenesis at these positions used a synthetically reconstructed hIL-4 coding region (S.Zurawski, unpublished) inserted in the pTacRBS *Escherichia coli* expression plasmid (Zurawski *et al.*, 1986). Double stranded synthetic oligonucleotides (synthesizer and reagents, Applied Biosystems) corresponding to the sequence between *Sal*I and *Hin*dIII recognition sites in the C-terminal coding region and containing equimolar amounts of each deoxynucleotide at the codon selected for randomized substitution were ligated to *Sal*I and *Hin*dIII digested pTac-hIL-4 plasmid. Recombinant plasmids were recovered by transformation and the DNA sequences (Sequenase 2.0 kit, US Biochemical Corp.) of their *Sal*I–*Hin*dIII intervals were determined. Partially pure mutant hIL-4 proteins were prepared as described for mIL-2 proteins (Zurawski and Zurawski, 1989), except that the refolding buffer contained reduced and oxidized glutathione (van Kimmenade *et al.*, 1988), and were assayed using TF-1 cells (see below). We found that substitutions at Y124 resulted in proteins that were partial agonists and that the Y124D substitution had the most drastic defect in cellular activation (data not shown). During the course of this work similar observations were made by others (Kruse *et al.*, 1992) who also showed that hIL-4.Y124D and hIL-4 have similar affinities for hIL-4R. For production of pure hIL-4.Y124D, the pTrpC11-hIL-4 *E.coli* expression plasmid (Zurawski *et al.*, 1986) was subjected to PCR (Geneamp kit, Perkin Elmer Cetus) using the oligonucleotides: CTCCAAGAACACAACTGAGAAGGAAACCTT (proximal to the single *Pst*I restriction site in the coding region) and TTGATTAAGCTT-TCAGCTCGAACACTTTGAATCTTTCTC (a *Hin*dIII recognition site precedes the underlined part which corresponds to the C-terminal coding region containing a GAT codon for residue 124). The PCR product and pTrpC11-hIL-4 plasmid were cleaved with *Pst*I and *Hin*dIII, ligated and pTrpC11-hIL-4Y124D plasmid was recovered and validated by transformation and sequence analysis using previously described methods (Zurawski and Zurawski, 1988).

Purification of proteins

Escherichia coli-derived hIL-4 (van Kimmenade *et al.*, 1988), human interleukin-1α (hIL-1α) (Kronheim *et al.*, 1986) and mIL-13 (McKenzie *et al.*, 1993) were purified as previously described. hIL-4.Y124D was prepared from *E.coli* K12 cells (strain CQ21) harboring the pTrpC11-hIL-4.Y124D plasmid grown overnight at 37°C in 12 l of L-broth containing 50 μg/ml ampicillin in a G53 rotatory shaker (New Brunswick Scientific) at 200 r.p.m. The cells were harvested by centrifugation in a RC-3 centrifuge (all rotors Sorvall) at 4500 r.p.m., 10 min, 4°C. The pellets were resuspended in 450 ml of TE buffer (50 mM Tris–HCl pH 8, 1 mM EDTA) by shaking at 200 r.p.m. for 15 min. Cells were ruptured by four passes through an ice-cooled Microfluidizer model 110 cell disrupter (Microfluidics). Inclusion bodies were collected by centrifugation in a GS-3 rotor at 9000 r.p.m., 40 min, 4°C. The pellet was then washed by resuspension in 450 ml of TE and Triton X-100 was added to a final concentration of 0.5%. Samples were kept at room temperature for 30 min and were then pelleted in a GSA rotor at 8500 r.p.m., 10 min, 4°C. The inclusion bodies were resuspended in 60 ml 5 M guanidine–HCl in PBS (120 mM NaCl, 2.7 mM KCl, 10 mM Na phosphate pH 7.4), 2 mM reduced glutathione, 0.2 mM oxidized glutathione and any remaining insoluble material was removed by centrifugation in a SS-34 rotor at 20 000 r.p.m., 30 min, 4°C. The supernatant was diluted 10-fold into the same buffer without guanidine hydrochloride and stirred gently overnight at 4°C to permit refolding and oxidization. Concentration and exchange into 100 ml 50 mM Na acetate pH 5.0, was then performed using a Millipore Pellicon apparatus (Millipore) equipped with a tangential flow ultrafiltration cassette with a size exclusion of 10 kDa. The sample was subjected to anion exchange chromatography (CM Sepharose 16/100 column, Pharmacia) in the same buffer with elution via a 0–0.7 M NaCl gradient. Fractions containing hIL-4 protein were pooled and subjected to reversed phase chromatography (Poros R 10/100 column, Perseptive Biosystems) with elution via a gradient of 0–50% acetonitrile in 0.1% trifluoroacetic acid/water. Fractions containing hIL-4 were lyophilized, dissolved in 50 mM Na acetate pH 5.0. Proteins were quantified by densitometry (Molecular Dynamics) of stained SDS–PAGE with chicken egg lysozyme (Sigma) as a standard. Independent quantitation by amino acid analysis (Aminoquant, Hewlett-Packard) verified the estimates for hIL-4 (values varied by 15% of their mean) and hIL-4.Y124D (values varied by 3.5% of their mean).

Cell proliferation assays

Colorimetric cell proliferation assays used the human TF-1 cell line at 3×10^4 cells per well for 3 days and were performed as described previously (Mosmann, 1983). Cells were assayed in RPMI medium with L-glutamine and 10% fetal bovine serum (JRH Biosciences), 0.5 μM β-mercaptoethanol (Sigma). Cells were maintained in the above medium containing 1 nM hGM-CSF (Schering-Plough).

PHA blasts were prepared by incubation of 10^6 peripheral blood mononuclear cells per ml with 0.1 mg/ml phytohaemagglutinin (Wellcome Diagnostics) in Yssel's medium (Yssel *et al.*, 1984), supplemented with 1% human AB^+ serum in 24-well Linbro plates (Flow Laboratories) and were used in the proliferative assay after 6 days of incubation. SP-B21, a $CD4^+$ cloned T cell line with unknown antigen specificity (Bacchetta

et al., 1990), was cultured as previously described (Spits *et al.*, 1982). Proliferative responses of both PHA blasts and SP-B21 cells were determined at 5×10^4 cells per well and were performed and developed colorimetrically after 3 days as described above for TF-1 cells.

Ligand binding

Procedures for preparation of cells, separation of bound from free ligand, computer analysis and quantitation have been described previously (Zurawski and Zurawski, 1992). Ba/F3 cells expressing surface hIL-4R-S protein [hIL-4R ligand binding protein deleted for most of the intracellular domain (Harada *et al.*, 1992b)], were grown as for TF-1 cells except that mouse interleukin-3 (IL-3, 100 U/ml) replaced hGM-CSF and 50 μg/ml gentamicin sulfate (Sigma) and 800 μg/ml neomycin G418 (Schering-Plough) were added. ^{125}I-Radiolabeling of *E.coli*-derived hIL-4 and binding conditions were as described (Harada *et al.*, 1992b). Procedures for growth and transient transfection of Cos-7 cells by expression plasmids bearing full-length or cytoplasmically deleted hIL-4 ligand binding proteins have been described previously (Imler and Zurawski, 1992; Harada *et al.*, 1992b).

Circular dichroism spectroscopy

Secondary structural features of hIL-4, hIL-1α and mIL-13 proteins were examined on a J720 spectrophotometer with the 450 W xenon lamp and J700 data analysis software (Jasco). The samples were dialyzed against 20 mM Na phosphate, pH 7. Protein concentrations of the samples were re-determined by UV absorption scanning on a Lambda 6 spectrophotometer (Perkin-Elmer). The absorption maximum at 280 nm was used to calculate the amount of protein using theoretical extinction coefficients based on known molecular weights and expected residue absorption contributions (Fasman, 1976). Samples were diluted to 0.2 mg/ml in a 0.2 mm path length cell. Typical scan parameters for the near UV range were a continuous wavelength scan at 10 mdeg sensitivity, 0.1 nm step resolution at a scan speed of 50 nm/min with a time constant of 2 s. Four accumulations per scan were averaged for an increased signal-to-noise ratio. Phosphate buffer blanks were run and subtracted out from subsequent protein scans and the spectra were noise-reduced using J700 data analysis software.

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