

Molecular Basis for Shared Cytokine Recognition Revealed in the Structure of an Unusually High Affinity Complex between IL-13 and IL-13R α 2

Patrick J. Lupardus,^{1,2} Michael E. Birnbaum,^{1,2} and K. Christopher Garcia^{1,*}

¹Howard Hughes Medical Institute, Department of Molecular and Cellular Physiology, Department of Structural Biology, and Program in Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

²These authors contributed equally to this work

*Correspondence: kcgarci@stanford.edu

DOI 10.1016/j.str.2010.01.003

SUMMARY

Interleukin-13 is a cytokine important for development of T helper cell type 2 (Th2) responses and plays a critical role in asthma and allergy. The IL-13 Receptor α 2 (IL-13R α 2) is a receptor for IL-13 lacking canonical Jak/STAT signaling functions. Here we present the crystal structure along with a mutational and biophysical analysis of the IL-13/IL-13R α 2 complex. While retaining a similar mode of IL-13 binding to its related signaling receptor, IL-13R α 1, IL-13R α 2 uses peripheral receptor residues unused in the IL-13/IL-13R α 1 complex to generate a larger and more complementary interface for IL-13. This results in a four orders of magnitude increase in affinity, to the femtomolar level, compared to IL-13R α 1. Alanine scanning mutagenesis of the IL-13 interface reveals several common “hotspot” residues important for binding to both receptors, but also identifies a prominent IL-13R α 2-specific contact. These results provide a framework for development of receptor subtype-selective IL-13 antagonists and indicate a decoy function for IL-13R α 2.

INTRODUCTION

Cytokines are soluble proteins that mediate the growth, homeostasis, development, and effector functions of the immune response, as well as provide critical crosstalk between adaptive and innate immunity. Cytokines are key mediators of T cell-mediated immunity and drive the differentiation of activated CD4⁺ T cells into the Th1, Th2, and Th17 T helper subtypes by dimerization of cognate cytokine receptors and activation of Jak/STAT signaling (Leonard, 1999). The four-helix bundle cytokines IL-4 and IL-13 have important roles in the differentiation and effector functions of T helper cell type 2 (Th2)-polarized T cells, which are necessary for the antiparasitic immune response (Wynn, 2003). Largely produced by activated Th2 cells, IL-13 causes alternative activation of macrophages, induces class switching of B cells to the IgE and IgG4 antibody isotypes, stimulates mast cells and eosinophils, and can also induce changes in the respi-

ratory and vascular epithelium (Hershey, 2003). Dysregulation of IL-13-mediated responses has been implicated in the pathology of asthma and allergic disease (Barnes, 2002; Wills-Karp, 2004).

IL-13 signaling is initiated through heterodimerization of the IL-13 receptor family members IL-13R α 1 and IL-4R α in what is termed the type II complex (Figures 1A and 1B) (Aman et al., 1996; Hilton et al., 1996). IL-13 is first recruited by IL-13R α 1, followed by binding of the binary IL-13/IL-13R α 1 complex to IL-4R α to form the ternary signaling complex (LaPorte et al., 2008). Importantly, receptor dimerization is mediated by a composite IL-13/IL-13R α 1 interface, and IL-13 alone has no measurable affinity for IL-4R α (Andrews et al., 2002). IL-4 can also signal through a type II receptor heterodimer, but assembles the receptor complex in the reverse order, which may partially explain the different signaling potencies of IL-4 versus 13 (Junttila et al., 2008; LaPorte et al., 2008). Upon dimerization, receptor-associated Jak kinases recruit and activate STAT6 to induce effector gene transcription (Kelly-Welch et al., 2003).

IL-13 also has the ability to form a second complex with its high-affinity receptor, IL-13R α 2. Discovered contemporaneously with IL-13R α 1, IL-13R α 2 shares 21% sequence identity with IL-13R α 1 (Caput et al., 1996) and, like IL-13R α 1, contains an unusual top-mounted S-type Ig fold, followed by two fibronectin type III-like domains and a WSXWS box that make up the prototypical “cytokine-binding homology region” (CHR) (Donaldson et al., 1998) (Figure 1A). The biological role of IL-13R α 2 has been a matter of debate, due to its high affinity for IL-13 (reported to be ~500 pM) and short intracellular domain (~20 amino acids) that lacks a canonical Box I/II Jak binding site and that does not activate STAT6 upon cytokine binding (Caput et al., 1996; Donaldson et al., 1998). IL-13R α 2 is upregulated during certain types of infections (Chiaromonte et al., 2003; Mentink-Kane et al., 2004; Morimoto et al., 2009) and is expressed on multiple cell types outside of the hematopoietic system (Donaldson et al., 1998). IL-13R α 2 has been found intracellularly, on the cell surface, and as a shed-soluble receptor in mice (Daines and Hershey, 2002; Daines et al., 2006), although it has not been found as a soluble protein in human studies (O’Toole et al., 2008). IL-13R α 2 knockout mice exhibit a phenotype corresponding to increased IL-13 production, including higher systemic concentrations of IL-13 and increased IgE and IgG4 production (Wood et al., 2003). This has led to a general consensus that the primary role of IL-13R α 2 is as a decoy receptor and dominant-negative regulator of IL-13 (Hershey, 2003; O’Toole et al.,

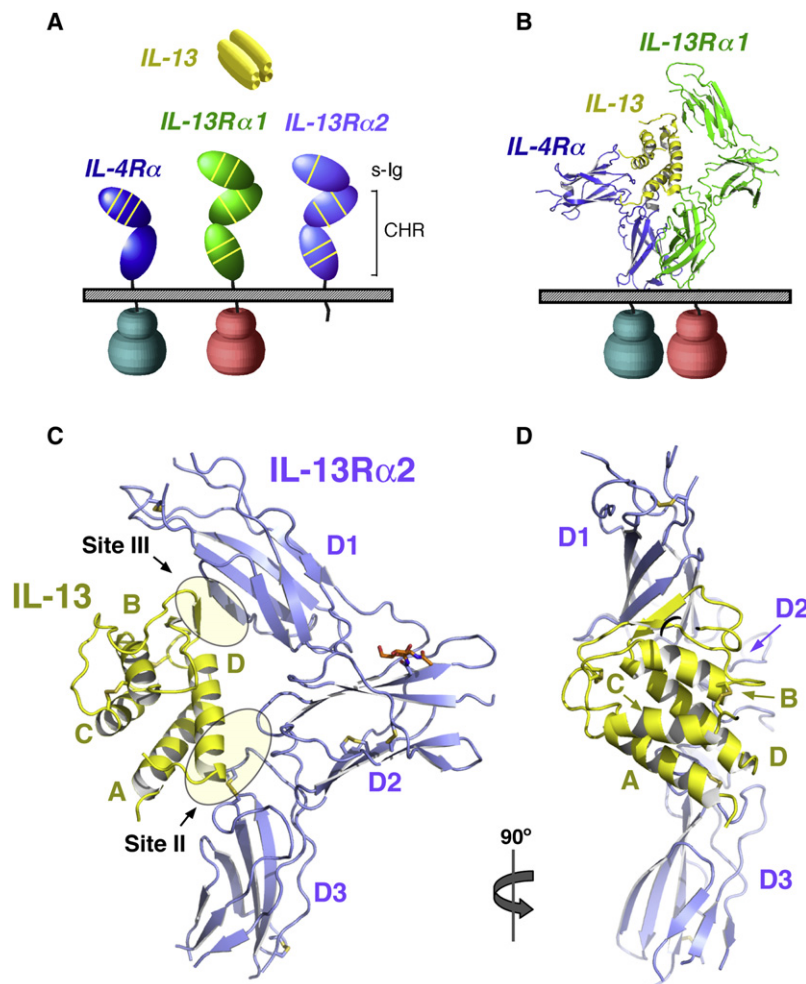


Figure 1. The Overall Structure of the IL-13/IL-13R α 2 Complex

(A) Schematic representation of the IL-13 receptor family. S-Ig stands for s-type Ig domain. CHR stands for cytokine-binding homology region. Disulfide bonds in the receptors are represented by yellow lines.

(B) The structure of the IL-13/IL-13R α 1/IL-4R α signaling complex (PDB ID 3BP0).

(C and D) The structure of the IL-13/IL-13R α 2 complex. Side (C) and face-on (D) views of IL-13/IL-13R α 2. IL-13 helices A-D are colored yellow and IL-13R α 2 domains 1-3 (D1-D3) are colored blue. The cytokine-receptor interaction sites II and III are highlighted in ovals. The single N-acetylglucosamine sugar residue attached to Asn215 is colored orange and disulfide bonds are colored yellow.

through homo- or heterodimerization. Here, we present the crystal structure of IL-13 in complex with IL-13R α 2 and a comparative biophysical analysis of the IL-13 interaction with IL-13R α 1 and IL-13R α 2 by alanine scanning and affinity measurement. We discover that while the IL-13 receptor-interacting residues are almost completely conserved, IL-13R α 2 has evolved an interlocking site II interface to bind IL-13 with an extraordinarily high affinity rarely seen in nature, suggesting an important role for this receptor in blocking IL-13 sensitivity in IL-13R α 2-expressing cells.

RESULTS

The Structure of IL-13 Bound to IL-13R α 2

To express the IL-13/IL-13R α 2 complex for crystallization, we co-infected insect HiFive cells with recombinant baculoviruses encoding

IL-13 and the cytokine binding domains (D1-D3) of IL-13R α 2. As IL-13 and IL-13R α 2 are both heavily glycosylated (with four predicted Asn-linked glycosylation sites on IL-13 and three on IL-13R α 2), the insect cells were grown in the presence of kifunensine to render Asn-linked glycans endoglycosidase-H (Endo-H) sensitive. IL-13 and IL-13R α 2 viruses were then coinfecting with Endo-H virus to cleave the kifunensine-sensitized glycans in situ, leaving a single N-acetylglucosamine residue attached to the Asn side chain (Lupardus and Garcia, 2008). After purification, surface lysines were reductively methylated (Walter et al., 2006) and the complex was purified by size exclusion chromatography. Deglycosylated and methylated IL-13/IL-13R α 2 complex behaved identically to the unmodified complex, while crystal diffraction was improved from ~ 9 to 3.1 Å (Table 1).

The overall architecture of the IL-13/IL-13R α 2 complex is similar to the structure of IL-13 in complex with IL-13R α 1 (Figures 1B-1D) (LaPorte et al., 2008). Preservation of the general "two-site" binding mode, in which IL-13 appears to be "pinched" between the top and bottom domains of the receptor, is not surprising given IL-13R α 1 and IL-13R α 2 evolved from a common ancestor and have a similar domain structure (Arima et al., 2005; Boulay et al., 2003). Compared to the more common two-domain cytokine receptors, the cytokine-binding region of

IL-13 and the cytokine binding domains (D1-D3) of IL-13R α 2. As IL-13 and IL-13R α 2 are both heavily glycosylated (with four predicted Asn-linked glycosylation sites on IL-13 and three on IL-13R α 2), the insect cells were grown in the presence of kifunensine to render Asn-linked glycans endoglycosidase-H (Endo-H) sensitive. IL-13 and IL-13R α 2 viruses were then coinfecting with Endo-H virus to cleave the kifunensine-sensitized glycans in situ, leaving a single N-acetylglucosamine residue attached to the Asn side chain (Lupardus and Garcia, 2008). After purification, surface lysines were reductively methylated (Walter et al., 2006) and the complex was purified by size exclusion chromatography. Deglycosylated and methylated IL-13/IL-13R α 2 complex behaved identically to the unmodified complex, while crystal diffraction was improved from ~ 9 to 3.1 Å (Table 1).

The overall architecture of the IL-13/IL-13R α 2 complex is similar to the structure of IL-13 in complex with IL-13R α 1 (Figures 1B-1D) (LaPorte et al., 2008). Preservation of the general "two-site" binding mode, in which IL-13 appears to be "pinched" between the top and bottom domains of the receptor, is not surprising given IL-13R α 1 and IL-13R α 2 evolved from a common ancestor and have a similar domain structure (Arima et al., 2005; Boulay et al., 2003). Compared to the more common two-domain cytokine receptors, the cytokine-binding region of

Table 1. Data Collection and Refinement Statistics for the IL-13/IL-13R α 2 Complex

	IL-13/IL-13R α 2 Native
Data collection	
Space group	I 2
Cell dimensions	
a, b, c (Å)	73.37, 86.57, 166.79
α , β , γ (°)	90, 96.8, 90
Wavelength (Å)	1.0
Resolution (Å)	82.8–3.05 (3.21–3.05)
R _{merge}	0.124 (0.581)
I/ σ I	7.6 (2.0)
Completeness (%)	100.0 (100.0)
Redundancy	3.8 (3.8)
Refinement	
Resolution (Å)	82.8–3.05
No. reflections (total/test)	18755/954
R _{work} /R _{free}	21.9/26.9
No. atoms	
Protein	6027
Sugar	28
Calcium	2
Water	31
B factors	
Protein	55.9
Sugar	75.5
Calcium	77.4
Water	38.7
Rmsd	
Bond lengths (Å)	0.012
Bond angles (°)	1.42
Highest resolution shell is in parentheses.	

IL-13R α 2 consists of three domains: N-terminal S-type Ig domain (D1) and two fibronectin III-like domains (D2 and D3) that make up the canonical CHR. The IL-13 receptor binding paradigm, established by the interaction of IL-4 and IL-13 with IL-13R α 1, is characterized by two binding sites, termed “site II” and “site III” (LaPorte et al., 2008). Site II is the canonical “growth hormone-like” interaction of the D2-D3 “elbow” region of the CHR with the A and D helices of IL-13 (Figures 1C and 2B), while site III is comprised of the A-B and C-D loops of the cytokine contacting the D1 Ig-like domain through an extended anti-parallel β sheet interaction (Figures 1C and 2A). Both of these interactions are preserved in the IL-13/IL-13R α 2 complex, burying a total of approximately 1840 Å² of surface area (1190 Å² and 650 Å² in site II and III, respectively).

The site II interface in the IL-13/IL-13R α 2 complex is formed by an unusual splayed, “spider-like” arrangement of four side chains on the D helix of IL-13 (K104, K105, F107, and R108) (Figure 2B). Looking top-down onto the D helix, each of these four residues is offset by $\sim 90^\circ$, with three of the four (K104, F107, and R108) inserting into depressions created by the D2-D3 CHR loops on IL-13R α 2. K104 fits in a groove created

by the D3 FG loop of IL-13R α 2, while R108 inserts into a canyon created by the BC loop of D3 and the AB and EF loops of D2. K104 and R108 also form polar bonds to the receptor at the periphery of the interface, with K104 forming a salt bridge with D318 and R108 participating in a hydrogen bond with the backbone carbonyl of P266. F107 occupies a pocket lined by the IL-13R α 2 residues Y315, the BC-FG loop disulfide (C269–C316), and R268, which also forms two hydrogen bonds with the peptide carbonyls of F107 and R108 in IL-13. The A helix contributes R11, I14, and E15 to the interface, with R11 and E15 forming a pocket for and hydrogen bonding with the IL-13R α 2 FG loop residue Y315.

The defining feature of the site III interface is the extension of the antiparallel β sheet of the IL-13R α 2 D1 Ig domain by two strands formed by the AB and CD loops of IL-13 (Figure 2A). The IL-13R α 2 interface is dominated by the backbone of the D1 c' β strand along with the side chains of residues T83, I84, I85, and T86. These opposing side chains form a flat, nonpolar interface complementary to the IL-13 site III epitope, which is formed by the CD loop strand and a hydrophobic patch generated by M33, W35, and the methylene chain of K89. Each end of the c' strand of IL-13R α 2 is “capped” with lysines (K82 and K89). Overall, the site III interaction is primarily driven by the backbone β sheet hydrogen bonding with nonpolar van der Waals interactions between the side chains on each side.

IL-13 Binding Energetics

To biophysically characterize the interaction between IL-13 and IL-13R α 2 and compare the binding energetics to that of the IL-13/IL-13R α 1 complex, we used two methods to measure the affinity of the IL-13/IL-13R α 2 complex: thermodynamic analysis by isothermal titration calorimetry (ITC) and kinetic analysis by surface plasmon resonance (SPR). We performed ITC as described previously (LaPorte et al., 2008), using fully glycosylated IL-13 and soluble extracellular domains for IL-13R α 1 and IL-13R α 2. When we measured the thermodynamics of IL-13 binding to IL-13R α 2 by ITC, we found the reaction to be strongly exothermic ($\Delta H = -20.0$ kcal/mol), but the high affinity of the interaction produced a steep titration transition that could not be accurately fit to determine K_D (Figure 3A). To accurately determine the dissociation constant, we performed displacement ITC, a method developed to reduce the apparent K_D of an interaction by pre-complexing one of the binding partners with a ligand of lower affinity (Sigurskjold, 2000). We were able to take advantage of the relatively lower affinity of IL-13R α 1 for IL-13 (Figure 3B) and use it as a competitor for IL-13R α 2 binding. By this method, we were able to accurately fit the titration transition and measure a dissociation constant of 4.7 pM for the IL-13/IL-13R α 2 interaction (Figure 3C). As measured by ITC, IL-13R α 2 therefore has over 5000-fold higher affinity for IL-13 than IL-13R α 1. This interaction is enthalpically driven and entropically unfavorable. Although one cannot unambiguously assign energetics simply by inspection of structure, the ITC results suggest that the interaction between IL-13 and IL-13R α 2 is, on balance, driven primarily by formation of polar bonds, which tend to have strong exothermic signatures, and to a lesser extent desolvation, which tends to be entropically favorable, although the site II interface does contain several clearly hydrophobic interactions.

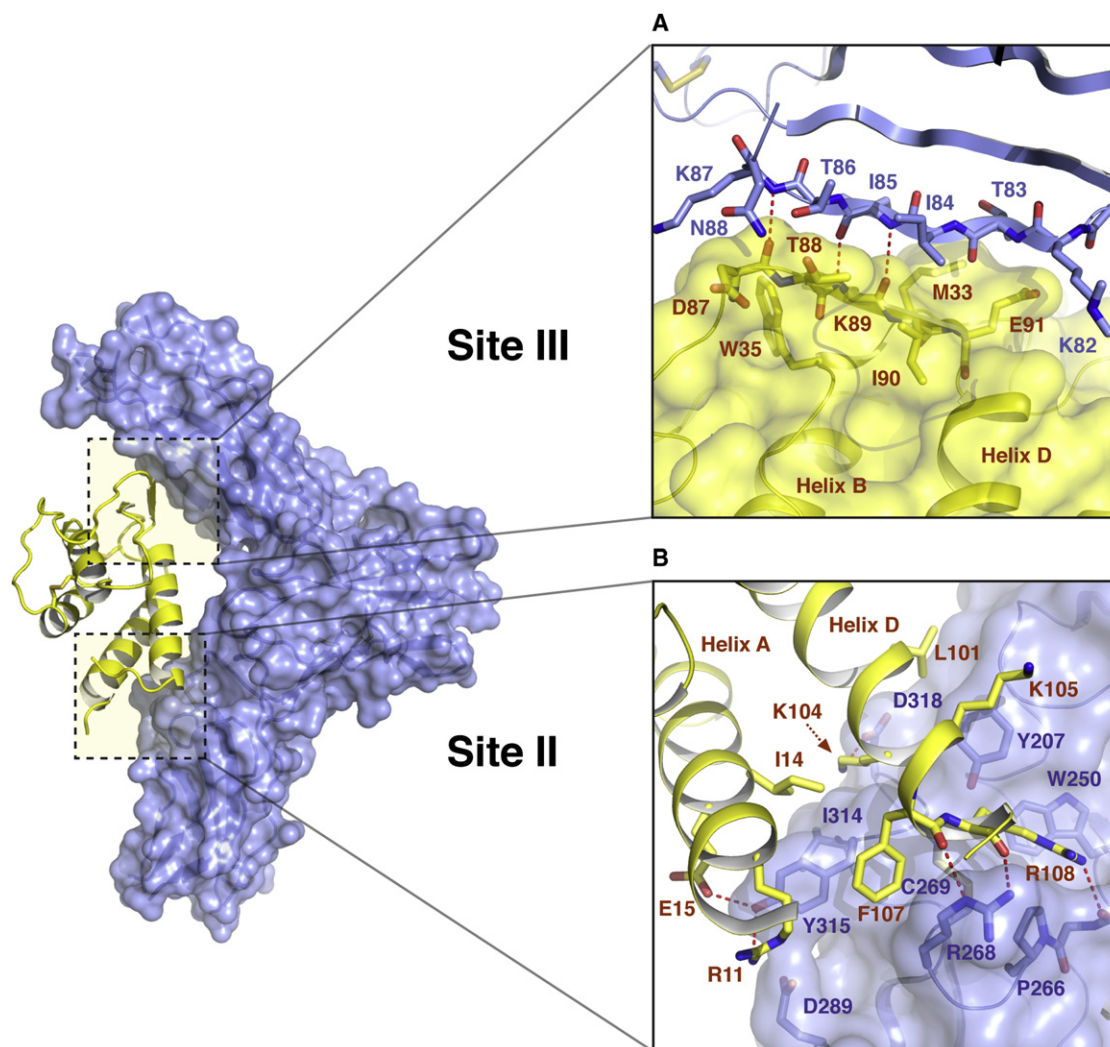


Figure 2. The Interactions of IL-13R α 2 with IL-13

The overall structure to the left is a ribbon depiction of IL-13 bound to a molecular surface of IL-13R α 2.

(A) View of the “site III” interface showing the backbone β sheet interactions between the c' strand of IL-13R α 2 D1 and the CD loop strand of IL-13.

(B) View of the “site II” interface, showing the interactions between the D2 and D3 loops of IL-13R α 2 and the A and D helices of IL-13. Interacting residues are shown as sticks and polar interactions are drawn as dashed red lines.

We next measured the kinetics of binding for both the IL-13/IL-13R α 1 and IL-13/IL-13R α 2 interactions. C-terminally biotinylated IL-13R α 1 or IL-13R α 2 were immobilized on a streptavidin SPR surface, with a range of IL-13 concentrations passed over the surface to measure rates of association and dissociation. We found the affinity between IL-13 and IL-13R α 1 to be 1.7 nM by SPR (Figure 3D), consistent with previously reported values (Andrews et al., 2002), albeit with 10-fold higher affinity than observed via ITC. We then attempted to determine the affinity between IL-13 and IL-13R α 2 using SPR and found the complex had an exceptionally slow dissociation rate. Even when allowed to dissociate for 180 min (compared to 1 min for IL-13/IL-13R α 1 dissociation measurements), there was essentially no dissociation of IL-13 from IL-13R α 2 observed (Figure 3E). Our measurements indicate a dissociation rate slower than $2 \times 10^{-6} \text{ s}^{-1}$ and an interaction half-life

of several days ($t_{1/2} = \ln 2/k_{\text{off}}$). While an exact K_D cannot be determined with high confidence due to limitations in accurately measuring the complex dissociation, these kinetic readings indicate IL-13R α 2 has sub-100 fM affinity for IL-13 by SPR. These results indicate the IL-13/IL-13R α 2 interaction is among the highest affinity protein-protein complexes reported in the literature.

Comparison of the IL-13/Receptor Complexes

These extraordinary differences in affinity led us to carefully compare our structure to the structure of IL-13 in complex with IL-13R α 1 (PDB ID 3BPO) (LaPorte et al., 2008) to determine the molecular basis for the greater than 20,000-fold affinity differential observed between the complexes. Overlay of the IL-13 molecules indicates that the structure of the cytokine is nearly unchanged upon binding to its receptors (see Figure S2 available

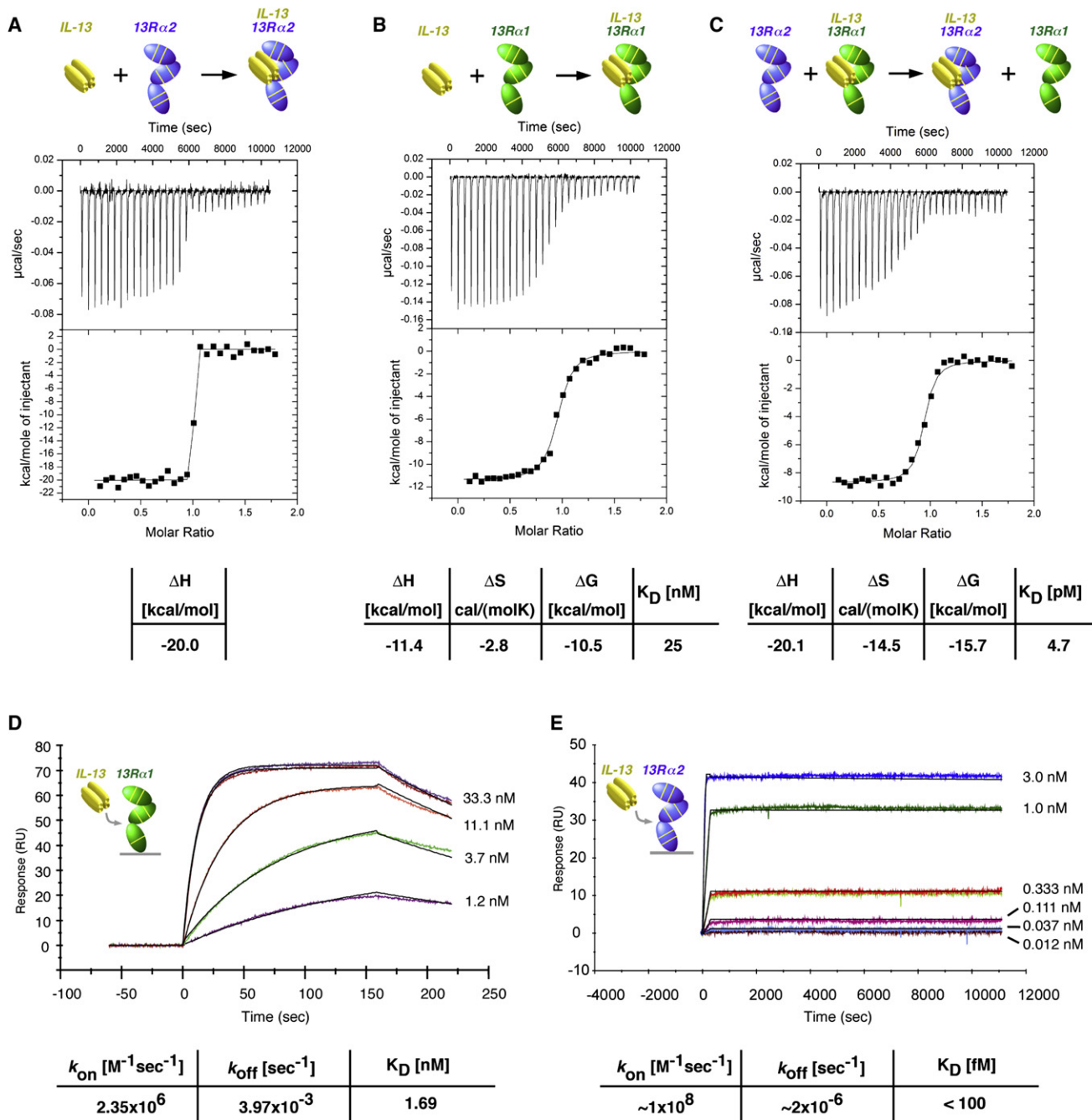


Figure 3. Biophysical Characterization of the IL-13 Interaction with IL-13R α 1 and IL-13R α 2

(A–C) Thermodynamic measurements of binding performed by ITC. Parameters measured for binding of IL-13 to IL-13R α 2 (A), IL-13 to IL-13R α 1 (B), and displacement of IL-13R α 1 from IL-13 by IL-13R α 2 (C) are summarized in the tables below each binding isotherm. Only ΔH could be calculated from the IL-13/IL-13R α 2 titration; the data could not be fit for K_D due to the steepness of the curve.

(D and E) SPR measurement of binding kinetics for the IL-13/IL-13R α 1 and IL-13/IL-13R α 2 interactions.

online), with a C α backbone root mean square deviation of ~ 1.1 Å. Clearly, the cross-reactivity of IL-13 for IL-13R α 1 and IL-13R α 2 is not mediated by significant changes in the contact residues or side chain positions on the A and D helices of IL-13 (Figure S2). Comparison of rotamer positions of IL-13 for sites II and III show surprisingly few differences between the

IL-13R α 1 and IL-13R α 2 complexes, with one major exception being the site II D helix residue R108. Instead, the difference in affinity seems to be almost entirely mediated by sequence and structural differences of the contact surfaces presented by the two receptors (Figures 4A–4F) and, in particular, the site II interface (Figures 4C–4F). Perhaps most prominently, in site II, the D2

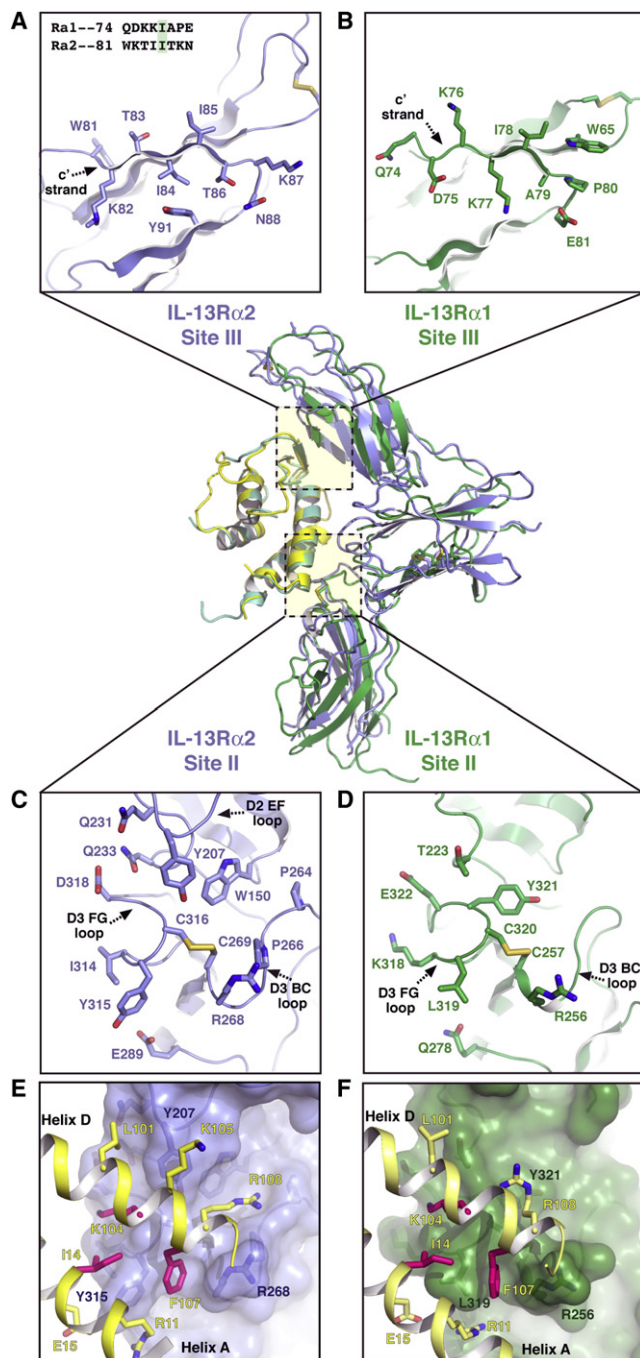


Figure 4. Comparison of the Site II and Site III Interfaces for IL-13 in Complex with IL-13R α 1 and IL-13R α 2

To make comparisons between the two IL-13/receptor complexes, the IL-13 molecules were structurally superimposed.

(A–D) Comparison of residues that comprise the site III and site II interfaces for IL-13R α 2 (A and C, in blue) and IL-13R α 1 (B and D, in green). Cytokine-interacting residues are shown as sticks.

(E and F) “Projection” view of IL-13 helices A and D depicted as ribbons and sticks interacting with semi-transparent molecular surfaces of IL-13R α 2 (E) and IL-13R α 1 (F). Interacting residues are shown as sticks, with the key hot-spot residues I14, K104, and F107 colored pink.

EF loop of IL-13R α 2 protrudes toward the D helix of IL-13, and Y207 at the tip of this loop makes a number of important contacts with L101, K105, and R108 of IL-13 (Figures 4C and 4E). The D2 EF loop is disordered in the structure of IL-13/IL-13R α 1 (Figure 4D), and the absence of cytokine contacts leaves a large empty space between IL-13 helix D and IL-13R α 1 (Figure 4F); this empty space is occupied by the D2 EF loop in the IL-13R α 2 complex (compare Figure 4E to 4F). Due to the usage of the EF loop by IL-13R α 2, comparison of the site II interfaces shows a significant increase in buried surface area (1190 Å² versus 880 Å²) compared to IL-13R α 1 (Figure 5). In fact, the IL-13R α 2 Y207 contact with K105 of IL-13 is distinct in the IL-13R α 2 complex (Figure 5) and denotes a clear site of specificity between the two complexes that we interrogated by mutagenesis (discussed below).

The differences between the site III interfaces are less striking (Figures 4A and 4B), with 660 Å² and 650 Å² buried for IL-13R α 1 and IL-13R α 2 (Figure 5), respectively, and the interfaces dominated by backbone β sheet hydrogen bonds and nonpolar van der Waals interactions. There is little sequence conservation between the IL-13-interacting residues in IL-13R α 1 and IL-13R α 2 (Figure 4A, inset), suggesting that, as for site II, each complex has evolved a highly distinct set of receptor-ligand contacts.

Alanine-Scanning Mutagenesis of the IL-13 Interface

Our discovery that IL-13 uses primarily the same residues to bind IL-13R α 1 and IL-13R α 2 with dramatically different affinities led us to investigate the relative energetic contributions that each side chain makes to the overall affinity of each complex. We identified the fifteen residues in IL-13 that made significant side chain interactions in either or both of the receptor-binding interfaces (Table S1 and Figure S3) and created a panel of alanine point mutants for SPR affinity measurements against IL-13R α 1 and IL-13R α 2. Due to the extremely slow dissociation rate between IL-13 and IL-13R α 2, we first performed a single-concentration binding screen by flowing 3 nM of each IL-13 mutant over immobilized IL-13R α 2. While there was some variation in observed association rate for several of the IL-13 mutants, only four mutants showed any appreciable dissociation within the 10 min window used for the screen: I14A, K104A, K105A, and F107A (Figure S4). We performed complete kinetic analysis of these four mutants and found the K_D values for these mutants to be 1 pM, 9 pM, 70 pM, and 289 pM for K105A, I14A, F107A, and K104A, respectively (Figure S4; Table 2). Each mutant showed an approximately 10-fold decrease in association rate ($\sim 10^8$ M⁻¹s⁻¹ to $\sim 10^7$ M⁻¹s⁻¹), but a 10- to 10,000-fold increase in the dissociation rate (Table 2). ITC measurements for IL-13 K104A and F107A binding to IL-13R α 2 were also performed, and these interactions were found to have K_D values of 2.8 and 1.3 nM, respectively (Figure S6). These results are between two and three orders of magnitude lower affinity than the wild-type IL-13 binding to IL-13R α 2 by ITC (4.7 pM), corresponding to the change in affinity seen in our SPR measurements.

We next made affinity measurements via SPR for IL-13 binding to IL-13R α 1 to identify the energetically important interacting residues for this complex. We found that unlike IL-13R α 2, mutation of both site II and site III residues could disrupt the affinity of

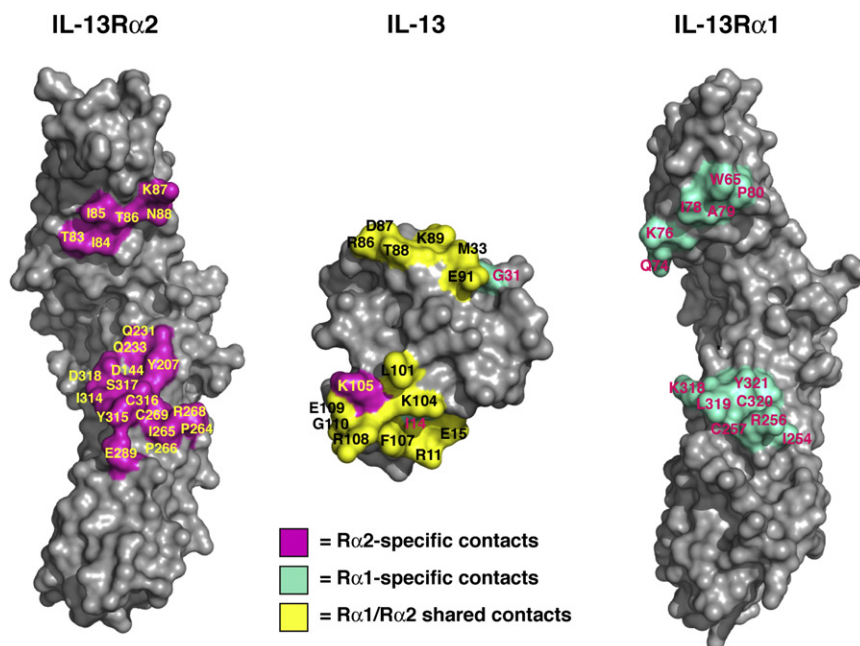
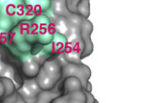


Figure 5. Shared and Distinct Contact Surfaces in the IL-13/IL-13R α 2 and IL-13/IL-13R α 1 Interfaces

Surface representation of IL-13 (middle panel), with residues interacting with IL-13R α 2 (pink), IL-13R α 1 (teal), or both receptors (yellow) highlighted. The IL-13-interacting surfaces of IL-13R α 2 and IL-13R α 1 are highlighted in pink and yellow, respectively (left and right panels).

IL-13R α 1 for IL-13. In site III of IL-13, mutation of residues K89, I90, and E91 each decrease affinity for IL-13R α 1 by $\sim$ 10-fold, while M33A has a more significant effect ($\sim$ 50-fold decrease) and D87A and T88A had no effect (Figure S5; Table 2). In contrast, none of these IL-13 point mutants appreciably disrupted IL-13R α 2 binding. Each of these side chains contribute significant surface area to both site III binding interfaces (Figure 5; Figure S3), so it appears that the IL-13/IL-13R α 2 site III interface is more tolerant to point mutations, with the high affinity of the site II interface possibly able to compensate for site III disruption.

The site IL-12 mutants that most severely disrupt IL-13R α 2 binding (I14A, K104A, and F107A) also significantly weaken the IL-13/IL-13R α 1 interaction, with binding being reduced ~200-fold for I14A and K104A (Table 2; Figure S4). IL-13 F107A disrupts affinity for IL-13R α 1 by at least 50-fold; the actual extent of disruption is likely larger, as the IL-13 F107A mutant was poorly behaved at concentrations higher than 1 μ M, which caused the SPR sensorgrams to show severe perturbations at equilibrium and not approach the theoretical R_{max} for the surface (Figure S4). In addition to those shared residues, each IL-13 complex also has distinct IL-13 D helix residues that disrupt binding upon mutation to alanine. In the IL-13/IL-13R α 2 complex, mutation of IL-13 K105 to alanine reduces binding over 10-fold (Table 2), likely due to contacts the K105 side chain makes with Y207 in the D2 E-F loop (Figures 4C and 4E). In the IL-13/IL-13R α 1 complex, IL-13 K105 does not contact the receptor due to the lack of involvement of the IL-13R α 1 EF loop in IL-13 binding; instead, IL-13 R108 packs into the space on IL-13R α 1 occupied by K105 and the D2 E-F loop in the IL-13R α 2 structure (Figures 4E and 4F). Predictably, mutation of K105 to alanine does not affect IL-13R α 1 binding due to its lack of contact in the structure, while changing R108 to alanine reduces affinity for IL-13R α 1 by 100-fold (Table 2). Interestingly, even though R108 appears



to make extensive contacts with IL-13R α 2 (Figure 4E), mutation of this residue to alanine has no appreciable effect on complex affinity. Therefore, while there are slight differences in IL-13 residues energetically important for IL-13R α 1 and IL-13R α 2 complex formation that could serve as targets to produce IL-13 variants with more or less selectivity for IL-13R α 1 versus IL-13R α 2 (such as K105), the cluster of residues I14, K104, and F107 compose a mutually shared “hotspot” surface and functional epitope essential for binding of IL-13 to both receptors.

Specific versus Degenerate Binding in the IL-13 Receptor Family

Prior studies have shown that while IL-13 binds to both IL-13R α 1 and IL-13R α 2, IL-4 can only bind IL-13R α 1 after first forming a complex with IL-4R α and has no apparent affinity for IL-13R α 2 (Andrews et al., 2002, 2006). An important question that we can now address is how IL-13R α 2 has evolved high affinity for IL-13 without also gaining additional affinity for IL-4. An alignment of the A and D helices of IL-4 bound to IL-13R α 1, IL-13 bound to IL-13R α 1, and IL-13 bound to IL-13R α 2 shows that the IL-13 hotspot residues I14, K104, and F107 correspond to IL-4 residues I11, R121, and Y124, respectively (Figure 6). This high level of conservation, including similar rotamer conformations, indicates the core IL-13 receptor binding hotspot on IL-4 and IL-13 is preserved among the complexes. Importantly, a number of peripheral IL-13 amino acids that participate in the IL-13R α 2 complex but neither of the IL-13R α 1 complexes are quite divergent, including two charge reversals (K105/E122 and E15/K12 on IL-13 and IL-4, respectively) (Figure 6). This leads us to conclude that IL-13R α 2 has evolved to specifically interact with IL-13 via increased shape complementarity and additional peripheral contacts with the cytokine, while IL-13R α 1 has remained promiscuous by primarily interacting with the shared core of IL-4/IL-13 hotspot residues and minimizing peripheral contacts.

DISCUSSION

IL-13R α 2 is a high affinity IL-13-specific receptor thought to function as a “decoy” and regulator of IL-13 signaling. Here we report the crystal structure of IL-13 in complex with IL-13R α 2, which reveals the molecular basis for the extraordinary affinity

Table 2. Summary of Kinetic and Thermodynamic Binding Measurements for IL-13 Mutants Binding to IL-13R α 1 and IL-13R α 2

SPR Kinetics/Equilibrium Affinity						
IL-13 Mutant	IL-13R α 1 Immobilized			IL-13R α 2 Immobilized		
	k_{on} (M $^{-1}$ s $^{-1}$)	k_{off} (s $^{-1}$)	K_D (M $^{-1}$)	k_{on} (M $^{-1}$ s $^{-1}$)	k_{off} (s $^{-1}$)	K_D (M $^{-1}$)
IL-13WT	2.35×10^6	3.97×10^{-3}	1.69×10^{-9}	1.22×10^8	1.93×10^{-6}	1.58×10^{-14}
IL-13 R11A	1.87×10^6	4.87×10^{-3}	2.60×10^{-9}			
IL-13 I14A			3.17×10^{-7}	1.79×10^7	1.52×10^{-4}	8.47×10^{-12}
IL-13 E15A	2.82×10^6	4.45×10^{-3}	1.58×10^{-9}			
IL-13 V18A	3.26×10^6	4.43×10^{-3}	1.36×10^{-9}			
IL-13 M33A			8.65×10^{-8}			
IL-13 D87A	2.74×10^6	7.51×10^{-3}	2.74×10^{-9}			
IL-13 T88A	2.80×10^6	4.88×10^{-3}	1.75×10^{-9}			
IL-13 K89A			1.28×10^{-8}			
IL-13 I90A			1.70×10^{-8}			
IL-13 E91A			1.35×10^{-8}			
IL-13 L101A	3.53×10^6	4.63×10^{-3}	1.31×10^{-9}			
IL-13 K104A			3.93×10^{-7}	9.64×10^6	2.71×10^{-3}	2.89×10^{-10}
IL-13 K105A	1.31×10^6	7.71×10^{-3}	5.89×10^{-9}	9.16×10^6	1.06×10^{-5}	1.16×10^{-12}
IL-13 F107A			$>1.0 \times 10^{-7a}$	1.15×10^7	9.45×10^{-4}	8.91×10^{-11}
IL-13 R108A			2.11×10^{-7}			
ITC Thermodynamics						
Titrand	Titrant	ΔH (kcal/mol)	ΔS (cal/mol*K)	ΔG (kcal/mol)	K_D (M $^{-1}$)	n
IL-13	IL-13R α 1	-11.4	-2.8	-10.5	2.5×10^{-8}	0.937
IL-13	IL-13R α 2	-20.0	ND	ND	ND	0.980
IL-13/ IL-13R α 1	IL-R13 α 2	-20.1	-14.5	-15.7	4.7×10^{-12}	0.943
IL-13 K104A	IL-R13 α 2	-20.4	-28.2	-11.9	2.78×10^{-9}	0.884
IL-13 F107A	IL-R13 α 2	-17.1	-15.7	-12.3	1.27×10^{-9}	0.949

^aAccurate K_D could not be determined due to non-specific binding of IL-13F107A to the SPR control channel at concentrations $>1\mu\text{M}$.

of the receptor for IL-13. An alanine scan of the IL-13 interface to determine the amino acids most important for binding to IL-13R α 1 and IL-13R α 2 indicates that both receptors use a central set of site II hotspot residues for binding to IL-13. Yet in contrast to IL-13R α 1, IL-13R α 2 makes a number of peripheral interface contacts to gain specificity and a four orders of magnitude increase in binding affinity.

Our structure and the accompanying biophysical analysis of the IL-13/IL-13R α 2 complex raise several important questions related to the in vivo function of IL-13R α 2. The femtomolar affinity of IL-13R α 2 for IL-13 is unprecedented in cytokine/receptor interactions and ranks among the highest affinity non-covalent protein-protein interactions as yet identified. Why is such tight binding necessary for IL-13 regulation? It has been previously suggested that IL-13R α 2 functions at least in part as a decoy receptor, reducing levels of IL-13 in the cellular microenvironment and downregulating IL-13 signaling on cells expressing the receptor. This has been suggested by experiments in which downregulation of IL-13 signaling after parasitic infection was dependent on IL-13R α 2 (Chiaramonte et al., 2003; Mentink-Kane et al., 2004) and in which an IL-13 overexpression phenotype is seen in an IL-13R α 2 mouse knockout model (Wood et al., 2003). A number of decoy receptors involved in cytokine regulation have been characterized, including IL-1

receptor II (IL-1RII), which neutralizes the proinflammatory cytokine IL-1 (Dinarello, 2009), and the DcR family of receptors (DcR1-3), which bind to the apoptosis-inducing TNF-related molecules TRAIL and FasL (Ashkenazi and Dixit, 1999). Additionally, a number of viruses use decoy receptors to modulate the activities of the TNF, IL-1, and IFN families of cytokines (Alcami, 2003). From our affinity measurements IL-13R α 2 has at least a 5000-fold greater affinity for IL-13 than IL-13R α 1, so any expression of IL-13R α 2 on the surface of a cell would cause a substantial downregulation of IL-13 signaling. It is therefore very likely that a significant role for IL-13R α 2 in vivo is as a decoy receptor and brake on IL-13 signaling.

The high affinity of IL-13R α 2 for IL-13 would also seem to necessitate tight regulation of IL-13R α 2 expression and/or localization, due to the significant effect even small amounts of IL-13R α 2 could have on IL-13 signaling. It has been shown in U937 cells that IL-13R α 2 is only expressed at low levels on the cell surface but is rapidly upregulated in response to IFN- γ (Daines and Hershey, 2002). Additionally, strong IL-13R α 2 staining is seen in intracellular compartments (Daines and Hershey, 2002), suggesting the functional receptor may be held in intracellular stores and only trafficked to the cell surface in response to very specific cytokine-mediated signals. IL-13R α 2 is also internalized quickly upon IL-13 binding (Kawakami et al., 2001),

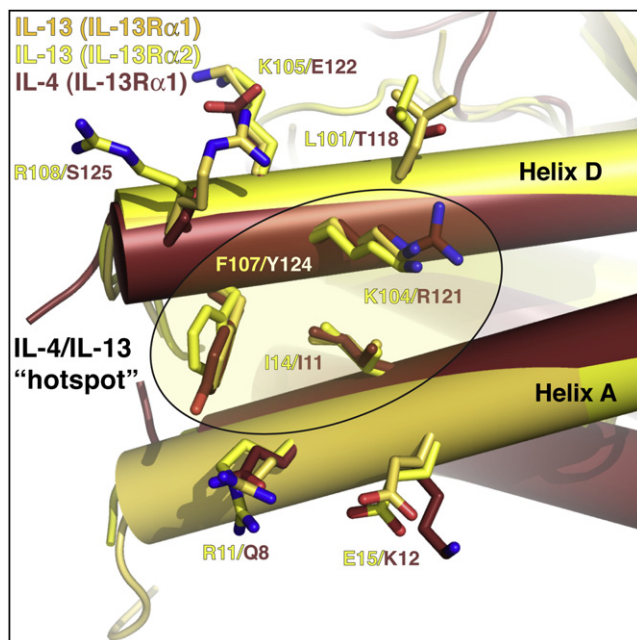


Figure 6. An IL-4/IL-13 Hotspot Mediates binding to the IL-13 Receptor Family

The A and D helices of IL-13 molecules from our IL-13/IL-13R α 2 complex and the IL-13/IL-13R α 1 complex (PDB 3BP0) were aligned along with the A and D helices of IL-4 from the IL-4/IL-13R α 1 complex (PDB 3BP1N). Side chains interacting with IL-13R α 1 or IL-13R α 2 are shown as stick models. The IL-13 receptor binding hotspot consisting of the IL-13/IL-4 residues I14/I11, K104/R121, and F107/Y124 is highlighted.

suggesting that both the surface expression level and the amount of free receptor are kept under stringent control.

With regards to signaling, it has been shown that IL-13R α 2 does not signal through a canonical Jak/STAT signaling pathway due a lack of interaction with IL-4R α as well as a short intracellular domain lacking Jak interaction motifs (Andrews et al., 2006). In the IL-13 type 2 signaling complex, the binary IL-13/IL-13R α 2 complex interacts with IL-4R α through a composite cytokine-receptor and receptor-receptor binding surface (LaPorte et al., 2008). Comparison of the receptor-receptor dimerization interface of IL-13R α 1 with the same region on IL-13R α 2 shows significant structural differences that likely preclude an IL-4R α /IL-13R α 2 interaction. IL-13R α 1 residues F297 and P300 (shown to be conserved also in common γ chain) (LaPorte et al., 2008) correspond to tyrosine (Y293) and alanine (A296) in IL-13R α 2, with Y293 in particular pushed out into the interface and likely sterically restricting an interaction with IL-4R α (Figure S7). Functional studies, SPR analysis, and native gel electrophoresis have all demonstrated no measurable affinity between IL-4R α and the IL-13/IL-13R α 2 complex (Andrews et al., 2002, 2006; data not shown). The unusually high affinity of IL-13R α 2 for IL-13 would also seem to be mutually exclusive with canonical Jak/STAT signaling, due to the need for “tuneability,” or a scaling up and down in signaling in response to differing levels of ligand. Alternate STAT-independent signaling modalities, as proposed previously (Fichtner-Feigl et al., 2006), may rely on undescribed mechanisms that do not require “tuneable” affinity.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification

Human IL-13 (amino acids 21–132) and the IL-13R α 2 ectodomain (amino acids 29–331) were cloned into the pACGP67-A vector (BD Biosciences) in frame with an N-terminal gp67 signal sequence and C-terminal hexahistidine tag and produced using the baculovirus expression system. Baculovirus stocks were prepared by transfection and amplification in *Spodoptera frugiperda* (Sf9) cells grown in SF900II media (Invitrogen), and protein expression was carried out in suspension *Trichoplusia ni* (HiFive) cells grown in InsectXpress media (Lonza).

For biophysical experiments, IL-13, IL-13R α 1 (LaPorte et al., 2008), and IL-13R α 2 were individually expressed and captured from HiFive supernatants after 48 hr by nickel agarose (QIAGEN), concentrated and purified by size exclusion chromatography on a Superdex 200 column (GE Healthcare), equilibrated in 5 mM HEPES (pH 7.0) and 150 mM NaCl (HEPES-buffered saline). For crystallization, glycan-minimized IL-13/IL-13R α 2 complex was generated by coinfection of kifunensine-treated HiFive cells with IL-13, IL-13R α 2, and Endo-H viruses. After nickel purification, the complex was treated overnight with carboxypeptidase-A, diluted to $\sim$ 1 mg/ml, and modified by reductive methylation (Walter et al., 2006). The Endo-H trimmed and methylated complex was then concentrated and purified by size exclusion in HEPES-buffered saline. Peak fractions were pooled and concentrated to $\sim$ 10–12 mg/ml for crystallization.

Crystallization and Data Collection

Initial crystallization trials yielded needle-like crystals from the PACT crystallization screen (Molecular Dimensions) using the sitting drop method at 25°C. Diffraction-quality crystals were ultimately grown in sitting drops at 25°C by mixing 0.1 µl protein [10 mg/ml in 5 mM HEPES-NaOH (pH 7.0) and 150 mM NaCl] with an equal volume of 100 mM MES (pH 6.0), 200 mM CaCl₂, 20% PEG-6000, and 4% v/v polypropylene glycol. Crystals grew to a maximum size of 100 × 100 × 100 µm in 2–3 weeks. Crystals were flash frozen in liquid nitrogen using mother liquor containing 25% glycerol as a cryoprotectant. A 3.1 Å data set was collected under cryo-cooled conditions at beamline 9-2 at the Stanford Synchrotron Radiation Laboratory. Diffraction data were processed using MOSFLM (Leslie, 1992) and SCALA (Potterton et al., 2003). Data processing statistics can be found in Table 1.

Structure Determination and Refinement

Initial phase information was obtained by molecular replacement with the program PHASER (McCoy et al., 2007) using the coordinates of IL-13, followed by placement of the individual domains of IL-13R α 1 from the IL-13/IL-4R α /IL-13R α 1 ternary complex structure (PDB ID 3BPO) (LaPorte et al., 2008). After the IL-13R α 1 domains were placed, the sequence was converted to poly-alanine and iterative rounds of simulated annealing refinement with CNS (Brunger, 2007) and model adjustment with COOT (Emsley and Cowtan, 2004) were carried out to build the IL-13R α 2 model. Once all amino acid changes and register problems were resolved, final rounds of positional, ADP, and TLS refinement were carried out using the PHENIX package (Adams et al., 2002) resulting in final R_{work} and R_{free} values of 21.9% and 26.9%, respectively. Ramachandran analysis by MolProbity (<http://molprobity.biochem.duke.edu>) (Davis et al., 2007) indicates 90.4% of residues reside in the most favorable regions, with none in the disallowed regions. Contact maps and buried surface area values were calculated using the Protein Interfaces, Surfaces, and Assemblies (PISA) server (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) (Krissinel and Henrick, 2007). Overlay of coordinates was carried out using SUPERPOSE from the CCP4 suite (Potterton et al., 2003). All structural figures were prepared using PyMOL (<http://www.pymol.org>) (DeLano, 2002).

The final model consists of two IL-13/IL-13R α 2 complexes in the asymmetric unit, with IL-13 chain A and IL-13R α 2 chain C making up one complex and IL-13 chain B and IL-13R α 2 chain D making up the second copy. Several loops in between strands of the Ig-like domains of the IL-13R α 2 molecules were modeled as alanine or unmodeled due to a lack of electron density. A number of lysines throughout the structure were modeled as dimethyl-lysine when additional electron density was found around the terminal amine, and one calcium atom bound to each IL-13R α 2 was modeled into the structure.

coordinated by Asp93 away from the IL-13/IL-13R α 2 binding interfaces. IL-13R α 2 chains C and D each have a single N-acetylglucosamine moiety attached to Asp215 in the model. The amino acid numbering scheme in the PDB file corresponds to the mature, signal peptide-cleaved chain of IL-13 and the non-cleaved sequence of IL-13R α 2 to facilitate comparison with the IL-13/IL-13R α 1 complex (PDB 3BPO). Chains B (IL-13) and D (IL-13R α 2) were used for all figures in the paper.

Surface Plasmon Resonance

SPR experiments were conducted on a Biacore T100 instrument. IL-13 and all alanine mutants were expressed fully glycosylated in HiFive cells and purified via gel filtration, and all mutants behaved identically to wild-type IL-13. IL-13R α 1 and IL-13R α 2 contained a C-terminal biotinylation peptide (LHHIL-DAQMKVWNHR) and were expressed and purified, followed by biotinylation in vitro with GST-BirA ligase according to established protocols (O'Callaghan et al., 1999). After the biotinylation reaction was complete, receptors were re-purified by size exclusion chromatography to remove excess biotin. Protein concentrations were quantified by UV spectroscopy at 280 nm using a Nano-drop2000 spectrometer (Thermo Scientific), and readings were taken three times and averaged to determine protein concentration. All data was analyzed using the Biacore T100 evaluation software version 2.0 with a 1:1 Langmuir binding model.

Experiments were either conducted using a Biacore SA sensor chip or a Biacore Biotin CAPture Kit (GE Healthcare). Biotinylated receptors were captured at a low density (200–300 RU) and kinetic runs were conducted at 50 μ l/min. An unrelated biotinylated protein was immobilized as a reference surface for the SA sensor chip with matching RU to the experimental surface. For the SA sensor chip, IL-13R α 1 was regenerated using 10 mM sodium acetate (pH 4.0) and 250 mM NaCl and IL-13R α 2 was regenerated using 10 mM glycine (pH 2.5) and 125 mM NaCl.

All measurements were made using 3-fold serial dilution of IL-13 and IL-13 mutants as indicated. IL-13/IL-13R α 1 kinetics data was determined using 160 s association and 60 s dissociation. IL-13/IL-13R α 2 kinetics data was determined using 300 s association and 10,800 s (180 min) dissociation. The initial single-concentration binding screen to identify IL-13 mutants with altered binding to IL-13R α 2 used 3 nM IL-13, with 120 s association and 360 s dissociation. Kinetic experiments for IL-13 mutants binding to IL-13R α 1 were conducted identically to wild-type IL-13R α 1 measurements. When the data could not be fit for kinetics, equilibrium data was determined at 30 μ l/min flow rate with 270 s association and 60 s dissociation. Kinetic data for IL-13 mutants binding to IL-13R α 2 was conducted with 300 s association and 1800 s (30 min) dissociation time for IL-13 I14A, K104A, and F107A, with 3600 s (60 min) dissociation for IL-13 K105A.

Isothermal Titration Calorimetry

Calorimetric titrations were carried out on a VP-ITC calorimeter (MicroCal) at 30°C. Fully glycosylated proteins were expressed in HiFive cells and gel purified in HEPES-buffered saline (5 mM HEPES-NaOH and 150 mM NaCl). Proteins were quantified by UV spectroscopy as described above. Each experiment was conducted with 30 injections of 10 μ l each of titrant. Injections were conducted over 20.5 s with 360 s between injections to allow the reaction to come to equilibrium. For IL-13/IL-13R α 1 measurements, IL-13R α 1 was the titrant at a concentration of 40 μ M and IL-13 was the titrand at 5 μ M. For the wild-type IL-13/IL-13R α 2 measurements, IL-13R α 2 was the titrant at 8 μ M and IL-13 was the titrand at 1 μ M. For measurement of the IL-13 mutants K104A and F107A with IL-13R α 2, IL-13R α 2 was the titrant in the syringe at a concentration of 40 μ M and IL-13 was the titrand at 5 μ M. For displacement ITC measurements (Sigurskjold, 2000), 5 μ M IL-13 pre-incubated for 30 min with 100 μ M IL-13R α 1 was used as the titrand and 40 mM IL-13R α 2 was used as the titrant. All data processing was performed using Origin 7.0. Stoichiometry measurements via ITC were all $n = 0.88$ – 1.0 , indicating 1:1 molecular interactions.

ACCESSION NUMBERS

The atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession number 3LB6.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and one table and can be found with this article online at doi:10.1016/j.str.2010.01.003.

ACKNOWLEDGMENTS

We thank Natalia Goriatheva for expert technical assistance and members of the Garcia laboratory for helpful discussions. P.J.L. is a Damon Runyon Fellow, supported by the Damon Runyon Cancer Research Foundation (DRG-1928-06). M.E.B. is supported by a Regina Casper Stanford Graduate Fellowship and a National Science Foundation Fellowship. This work was funded by National Institutes of Health (AI51321 to K.C.G.) and the Howard Hughes Medical Institute (K.C.G.).

Received: November 20, 2009

Revised: January 12, 2010

Accepted: January 19, 2010

Published: March 9, 2010

REFERENCES

- Adams, P.D., Grosse-Kunstleve, R.W., Hung, L.-W., Ioerger, T.R., McCoy, A.J., Moriarty, N.W., Read, R.J., Sacchettini, J.C., Sauter, N.K., and Terwilliger, T.C. (2002). PHENIX: building new software for automated crystallographic structure determination. *Acta Crystallogr. D Biol. Crystallogr.* 58, 1948–1954.
- Alcami, A. (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nat. Rev. Immunol.* 3, 36–50.
- Aman, M.J., Tayebi, N., Obiri, N.I., Puri, R.K., Modi, W.S., and Leonard, W.J. (1996). cDNA cloning and characterization of the human interleukin 13 receptor alpha chain. *J. Biol. Chem.* 271, 29265–29270.
- Andrews, A.L., Holloway, J.W., Puddicombe, S.M., Holgate, S.T., and Davies, D.E. (2002). Kinetic analysis of the interleukin-13 receptor complex. *J. Biol. Chem.* 277, 46073–46078.
- Andrews, A.L., Nasir, T., Bucchieri, F., Holloway, J.W., Holgate, S.T., and Davies, D.E. (2006). IL-13 receptor alpha 2: a regulator of IL-13 and IL-4 signal transduction in primary human fibroblasts. *J. Allergy Clin. Immunol.* 118, 858–865.
- Arima, K., Sato, K., Tanaka, G., Kanaji, S., Terada, T., Honjo, E., Kuroki, R., Matsuo, Y., and Izuahara, K. (2005). Characterization of the interaction between interleukin-13 and interleukin-13 receptors. *J. Biol. Chem.* 280, 24915–24922.
- Ashkenazi, A., and Dixit, V.M. (1999). Apoptosis control by death and decoy receptors. *Curr. Opin. Cell Biol.* 11, 255–260.
- Barnes, P.J. (2002). Cytokine modulators as novel therapies for asthma. *Annu. Rev. Pharmacol. Toxicol.* 42, 81–98.
- Boulay, J.L., O'Shea, J.J., and Paul, W.E. (2003). Molecular phylogeny within type I cytokines and their cognate receptors. *Immunity* 19, 159–163.
- Brunger, A.T. (2007). Version 1.2 of the Crystallography and NMR system. *Nat. Protoc.* 2, 2728–2733.
- Caput, D., Laurent, P., Kaghad, M., Lelias, J.M., Lefort, S., Vita, N., and Ferrara, P. (1996). Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor alpha chain. *J. Biol. Chem.* 271, 16921–16926.
- Chiaromonte, M.G., Mentink-Kane, M., Jacobson, B.A., Cheever, A.W., Whitters, M.J., Goad, M.E., Wong, A., Collins, M., Donaldson, D.D., Grusby, M.J., and Wynn, T.A. (2003). Regulation and function of the interleukin 13 receptor alpha 2 during a T helper cell type 2-dominant immune response. *J. Exp. Med.* 197, 687–701.
- Daines, M.O., and Hershey, G.K. (2002). A novel mechanism by which interferon-gamma can regulate interleukin (IL)-13 responses. Evidence for intracellular stores of IL-13 receptor alpha-2 and their rapid mobilization by interferon-gamma. *J. Biol. Chem.* 277, 10387–10393.

- Daines, M.O., Tabata, Y., Walker, B.A., Chen, W., Warriar, M.R., Basu, S., and Hershey, G.K. (2006). Level of expression of IL-13R α 2 impacts receptor distribution and IL-13 signaling. *J. Immunol.* **176**, 7495–7501.
- Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall, W.B., 3rd, Snoeyink, J., Richardson, J.S., and Richardson, D.C. (2007). MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res.* **35**, W375–W383.
- DeLano, W.L. (2002). The PyMOL Molecular Graphics System (Palo Alto, CA: DeLano Scientific).
- Dinarello, C.A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* **27**, 519–550.
- Donaldson, D.D., Whitters, M.J., Fitz, L.J., Neben, T.Y., Finnerty, H., Henderson, S.L., O'Hara, R.M., Jr., Beier, D.R., Turner, K.J., Wood, C.R., and Collins, M. (1998). The murine IL-13 receptor α 2: molecular cloning, characterization, and comparison with murine IL-13 receptor α 1. *J. Immunol.* **161**, 2317–2324.
- Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132.
- Fichtner-Feigl, S., Strober, W., Kawakami, K., Puri, R.K., and Kitani, A. (2006). IL-13 signaling through the IL-13 α 2 receptor is involved in induction of TGF- β 1 production and fibrosis. *Nat. Med.* **12**, 99–106.
- Fichtner-Feigl, S., Terabe, M., Kitani, A., Young, C.A., Fuss, I., Geissler, E.K., Schlitt, H.J., Berzofsky, J.A., and Strober, W. (2008). Restoration of tumor immunosurveillance via targeting of interleukin-13 receptor- α 2. *Cancer Res.* **68**, 3467–3475.
- Hershey, G.K. (2003). IL-13 receptors and signaling pathways: an evolving web. *J. Allergy Clin. Immunol.* **111**, 677–690, quiz 691.
- Hilton, D.J., Zhang, J.G., Metcalf, D., Alexander, W.S., Nicola, N.A., and Willson, T.A. (1996). Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc. Natl. Acad. Sci. USA* **93**, 497–501.
- Junttila, I.S., Mizukami, K., Dickensheets, H., Meier-Schellersheim, M., Yamane, H., Donnelly, R.P., and Paul, W.E. (2008). Tuning sensitivity to IL-4 and IL-13: differential expression of IL-4R α , IL-13R α 1, and γ chain regulates relative cytokine sensitivity. *J. Exp. Med.* **205**, 2595–2608.
- Kahlon, K.S., Brown, C., Cooper, L.J., Raubitschek, A., Forman, S.J., and Jensen, M.C. (2004). Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. *Cancer Res.* **64**, 9160–9166.
- Kawakami, K., Takeshita, F., and Puri, R.K. (2001). Identification of distinct roles for a dileucine and a tyrosine internalization motif in the interleukin (IL)-13 binding component IL-13 receptor α 2 chain. *J. Biol. Chem.* **276**, 25114–25120.
- Kelly-Welch, A.E., Hanson, E.M., Boothby, M.R., and Keegan, A.D. (2003). Interleukin-4 and interleukin-13 signaling connections maps. *Science* **300**, 1527–1528.
- Kioi, M., Kawakami, M., Shimamura, T., Husain, S.R., and Puri, R.K. (2006). Interleukin-13 receptor α 2 chain: a potential biomarker and molecular target for ovarian cancer therapy. *Cancer* **107**, 1407–1418.
- Krissinel, E., and Henrick, K. (2007). Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* **372**, 774–797.
- LaPorte, S.L., Juo, Z.S., Vaclavikova, J., Colf, L.A., Qi, X., Heller, N.M., Keegan, A.D., and Garcia, K.C. (2008). Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132**, 259–272.
- Leonard, W.J. (1999). Type I cytokines and interferons and their receptors. In *Fundamental Immunology*, W. Paul, ed. (Philadelphia: Lippincott-Raven), pp. 741–774.
- Leslie, A.G.W. (1992). Recent changes to the MOSFLM package for processing film and image plate data. *Joint CCP4+ESF-EAMCB Newsletter on Protein Crystallography*. **26**.
- Lupardus, P.J., and Garcia, K.C. (2008). The structure of interleukin-23 reveals the molecular basis of p40 subunit sharing with interleukin-12. *J. Mol. Biol.* **382**, 931–941.
- McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., and Read, R.J. (2007). Phaser crystallographic software. *J. Appl. Crystallogr.* **40**, 658–674.
- Mentink-Kane, M.M., Cheever, A.W., Thompson, R.W., Hari, D.M., Kabatereine, N.B., Vennervald, B.J., Ouma, J.H., Mwatha, J.K., Jones, F.M., Donaldson, D.D., et al. (2004). IL-13 receptor α 2 down-modulates granulomatous inflammation and prolongs host survival in schistosomiasis. *Proc. Natl. Acad. Sci. USA* **101**, 586–590.
- Mintz, A., Gibo, D.M., Slagle-Webb, B., Christensen, N.D., and Debinski, W. (2002). IL-13R α 2 is a glioma-restricted receptor for interleukin-13. *Neoplasia* **4**, 388–399.
- Morimoto, M., Zhao, A., Sun, R., Stiltz, J., Madden, K.B., Mentink-Kane, M., Ramalingam, T., Wynn, T.A., Urban, J.F., Jr., and Shea-Donohue, T. (2009). IL-13 receptor α 2 regulates the immune and functional response to *Nippostrongylus brasiliensis* infection. *J. Immunol.* **183**, 1934–1939.
- O'Callaghan, C.A., Byford, M.F., Wyer, J.R., Willcox, B.E., Jakobsen, B.K., McMichael, A.J., and Bell, J.I. (1999). BirA enzyme: production and application in the study of membrane receptor-ligand interactions by site-specific biotinylation. *Anal. Biochem.* **266**, 9–15.
- O'Toole, M., Legault, H., Ramsey, R., Wynn, T.A., and Kasaian, M.T. (2008). A novel and sensitive ELISA reveals that the soluble form of IL-13R- α 2 is not expressed in plasma of healthy or asthmatic subjects. *Clin. Exp. Allergy* **38**, 594–601.
- Potterton, E., Briggs, P., Turkenburg, M., and Dodson, E. (2003). A graphical user interface to the CCP4 program suite. *Acta Crystallogr. D Biol. Crystallogr.* **59**, 1131–1137.
- Sigurskjold, B.W. (2000). Exact analysis of competition ligand binding by displacement isothermal titration calorimetry. *Anal. Biochem.* **277**, 260–266.
- Walter, T.S., Meier, C., Assenberg, R., Au, K.F., Ren, J., Verma, A., Nettleship, J.E., Owens, R.J., Stuart, D.I., and Grimes, J.M. (2006). Lysine methylation as a routine rescue strategy for protein crystallization. *Structure* **14**, 1617–1622.
- Wills-Karp, M. (2004). Interleukin-13 in asthma pathogenesis. *Immunol. Rev.* **202**, 175–190.
- Wood, N., Whitters, M.J., Jacobson, B.A., Witek, J., Sypek, J.P., Kasaian, M., Eppihimer, M.J., Unger, M., Tanaka, T., Goldman, S.J., et al. (2003). Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor α 2. *J. Exp. Med.* **197**, 703–709.
- Wynn, T.A. (2003). IL-13 effector functions. *Annu. Rev. Immunol.* **21**, 425–456.