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Annu Rev Immunol. Author manuscript; available in PMC 2014 April 09.

Published in final edited form as:

Annu Rev Immunol. 2009 ; 27: 29–60. doi:10.1146/annurev.immunol.24.021605.090616.

Structural Biology of Shared Cytokine Receptors

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Abstract

Recent structural information for complexes of cytokine receptor ectodomains bound to their ligands has significantly expanded our understanding of the macromolecular topology and ligand recognition mechanisms used by our three principal shared cytokine signaling receptors—gp130, γ_c, and β_c. The gp130 family receptors intricately coordinate three structurally unique cytokine-binding sites on their four-helix bundle cytokine ligands to assemble multimeric signaling complexes. These organizing principles serve as topological blueprints for the entire gp130 family of cytokines. Novel structures of γ_c and β_c complexes show us new twists, such as the use of a nonstandard sushi-type α receptors for IL-2 and IL-15 in assembling quaternary γ_c signaling complexes and an antiparallel interlocked dimer in the GM-CSF signaling complex with β_c. Unlike gp130, which appears to recognize vastly different cytokine surfaces in chemically unique fashions for each ligand, the γ_c-dependent cytokines appear to seek out some semblance of a knobs-in-holes shape recognition code in order to engage γ_c in related fashions. We discuss the structural similarities and differences between these three shared cytokine receptors, as well as the implications for transmembrane signaling.

Keywords

interleukin; signaling; structure

INTRODUCTION

The interaction of cell surface receptor extracellular domains with secreted ligands is essential to most types of cell signaling and cell-cell communication. This initial step and the subsequent activation of membrane-proximal and -distal intracellular signaling cascades lead to specific, although often redundant, cellular responses that control cell proliferation, differentiation, maturation, and survival. Cell surface receptors usually bind their ligands through highly specific molecular interactions to provide the tight regulation necessary for control of physiological responses. However, researchers increasingly appreciate that many receptor systems exhibit, to a greater or lesser extent, cross-reactivity with a spectrum of different ligands. There are many examples of polyspecific, shared receptors with central roles in signaling (1). In neurobiology, the p75 neurotrophin receptor can recognize a family of neurotrophic factors, including nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 (2). The glial cell line–derived neurotrophic factor family ligands, which include glial cell line–derived neurotrophic factor, neurturin, artemin,

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DISCLOSURE STATEMENT

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and persephin, share the RET receptor as the signaling subunit in their receptor complexes (3). In the immune system, shared receptors exist in both adaptive (T cell receptors, costimulatory molecules B7/CD28) (4) and innate immunity (NKG2D natural killer receptor, scavenger, and pattern-recognition receptors such as RAGE and Toll) (5). However, the most widespread roles for shared receptors are found for cytokines (6–9), which are secreted growth factors that control cell growth and proliferation primarily in the immune and hematopoietic systems. There are three major shared receptors in the class I cytokine receptor family: the common gamma chain (γ_c), gp130, and the common beta chain (β_c), which participate in the formation of receptor complexes for nearly 20 different cytokines (Figure 1).

Important insights into the mechanisms for cross-reactivity of shared cytokine receptors have emerged from structural studies of complexes between cytokines and extracellular domains of their receptors (10). During the past several years, crystal structures have been determined for gp130, γ_c, and β_c complexes with cytokines, including gp130 bound to human herpes virus (HHV)-8 IL-6 (11), IL-6 (12), and leukemia inhibitory factor (LIF) (13); LIF complexed with LIF receptor (14); γ_c bound to IL-2 (15, 16) and IL-4 (17); and more recently β_c bound to GM-CSF (18). Gp130, γ_c, and β_c share a rudimentary core structural blueprint for the assembly of the extracellular cytokine-receptor signaling complexes; however, there are many important deviations between these three systems that result in substantially different signaling complex topologies. Collectively, these structures allow us to delineate common and unique structural features for both ligand recognition and assembly of signaling complexes by these three major shared cytokine receptors in the class I family, which is the focus of this review.

Owing to space constraints, we could not cite all contributors to this field. We refer the reader to excellent treatises on various aspects of cytokine structure, receptor interaction, and signaling; see References 10, 19–35.

THE CLASS I CYTOKINE RECEPTORS

Cytokines represent a diverse group of small soluble proteins that when secreted by one cell can act on the same cell, in an autocrine fashion, or on another cell, in a paracrine fashion (36). Through binding to specific cell surface receptors, they initiate signals that are critical to a diverse spectrum of functions, including induction of immune responses, cell proliferation, differentiation, and apoptosis. Structural analysis has allowed the grouping of cytokines into different structural classes, including the helical cytokines (37), the trimeric tumor necrosis factor (TNF) family (38), the cysteine knot growth factors (39), and the β-trefoil growth factors (40). Cytokines can also be classified according to the type of receptor that they engage. On the basis of common structural features, the cytokine receptors are grouped into six major families: class I cytokine receptors, class II cytokine receptors, TNF receptors, IL-1 receptors, tyrosine kinase receptors, and chemokine receptors (36, 41, 42).

The class I cytokine receptors, also known as the hematopoietin receptors, constitute the largest group among the cytokine receptor family (41, 43, 44). These are type I membrane proteins with an N-terminal extracellular and C-terminal intracellular orientation. The extracellular segments of the class I cytokine receptors show a modular architecture, which is characterized by a ~200 residue–long cytokine-binding homology region (CHR) (45) possessing the classical binding motif for cytokines, as structurally delineated in the human growth hormone (hGH) receptor complex (46). The CHR module consists of two fibronectin type-III (FNIII) domains connected by a linker, and it represents the signature recognition module for helical cytokines that is present on every type I cytokine receptor (Figure 1). The upper, N-terminal domain contains four (47) conserved cysteine residues that form

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interstrand disulfide bonds. The lower, C-terminal domain has a conserved Trp-Ser-X-Trp-Ser motif (45, 46). Mutagenesis studies have shown an essential structural role for these amino acids in maintaining the tertiary structure of the protein, but they are not involved in cytokine interaction (48). These signature sequence and structural features have been used to identify novel cytokine receptors in several genomes (49–51). The cytokine-binding site for most CHR modules is at the apex of the elbow region, consisting mainly of the interstrand loops connecting the β-strands from both N- and C-terminal domains (46). The basic CHR module is present in every class I cytokine receptor, and for some, such as the receptors for hGH (46, 52) and erythropoietin (EPO), a single CHR is sufficient to mediate ligand binding and receptor homodimerization. However, many other class I cytokine receptors require additional domains, such as the Ig-like domain and additional membrane-proximal fibronectin domains, found in the gp130 family, to function and respond to cytokines (11, 12). The α receptors of IL-2 and IL-15 of the γ_c family are atypical cytokine receptors in that they do not contain a CHR, but rather sushi domains (discussed below) (53, 54).

The defining structural feature of the cytokines recognized by the class I cytokine receptors is a four-helix bundle motif (43, 55), which is composed of four amphipathic helices having solvent-facing hydrophilic sides and hydrophobic sides that form the core of the helical bundle. These four helices are oriented into a unique up-up-down-down topology that is only found in the helical cytokines (43, 55). Structural predictions, later confirmed by several crystal and NMR structures, indicated that these cytokines could be further subclassified on the basis of the length of the helices (19, 37). Short-chain cytokines, represented by IL-2 and IL-4, have helices of 8–10 residues. The long-chain cytokines, such as gp130 family cytokines, hGH, and EPO, have helices of 10–20 residues. Finally, some cytokines, such as IL-5 and interferon (IFN)-γ, have two four-helix bundles forming an eight-helix architecture (55–57).

Cytokine binding induces receptor oligomerization that leads to the juxtaposition of the intracellular domains of the signaling subunits. Unlike receptors for many growth factors (e.g., insulin, epidermal growth factor) that have intracellular domains possessing tyrosine kinase activity on the same polypeptide chain, the class I cytokine receptors have no intrinsic enzymatic activity. Rather, the intracellular domains of the class I cytokine receptors are constitutively associated with tyrosine kinases of the Janus kinase (JAK) family, and to a more restricted degree the TYK kinase (26, 31, 32, 34). After the JAK/TYK kinases are activated by ligand-induced receptor oligomerization, they phosphorylate themselves and the intracellular domains of the receptors. The phosphorylated tyrosine residues in the receptors then serve as the docking sites for a second family of proteins, the signal transducer and activator of transcription (STAT) proteins. Binding of STATs to the intracellular domains of the receptors leads to their tyrosine phosphorylation and subsequent dissociation from the receptors. The phosphorylated STATs form dimers and translocate into the nucleus, where they bind to DNA recognition sequences and act as transcription factors (33, 34, 58). Besides the major JAK-STAT signaling pathway, the class I cytokine receptors also use other signaling mechanisms such as the RAS-RAF-MAP kinase pathway (59), PI3 kinase (60), and insulin receptor substrate (61). The structural aspects of JAK-STAT communication during receptor signaling are poorly understood and represent a major future frontier in cytokine receptor structural biology.

SHARED RECEPTORS IN THE CLASS I CYTOKINE RECEPTOR FAMILY

Although the original structural paradigm for cytokine receptor complexes was derived from the homodimeric hGH system (21, 46), most of the class I cytokine receptors do not signal through homodimerization (Figure 2). In fact, most form heterodimers (e.g., IL-4, IL-7, etc.) (17), and some even form heterotrimers (IL-2 and IL-15 receptors) (15, 16), tetramers (viral

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IL-6/gp130, G-CSF/G-CSFR) (11, 62), hexamers (human IL-6/IL-6Rα/gp130) (12), and even dodecamers (GM-CSF/GM-CSFRα/β_c) (18). A significant feature of these hetero-oligomeric receptor complexes is the use of a common, shared receptor subunit as a signal-transducing chain together with a cytokine-specific chain. When subgrouped by shared receptors, there are three major classes of heteroreceptor complexes in the class I cytokine receptor family: those that use β_c, those that use gp130, and those that use γ_c (Figure 2). In most cases, the shared receptors do not show appreciable affinity for cytokines, but in the presence of cytokine-specific α receptors they can form high-affinity cytokine receptor complexes that are capable of initiating intracellular signaling cascades. Such a characteristic affinity-conversion effect is used by shared receptors as a means of imposing tissue specificity (8). Shared receptors also must recognize different cytokines that have relatively low sequence identities, requiring that they be poly-specific for different ligand surface structures and chemistries, yet specific enough not to cross-react with inappropriate cytokines. In this respect, the shared receptors may teach us much about the basic structural and chemical mechanisms of protein-protein cross-reactivity.

GP130

Gp130 is the founding member of the tall cytokine receptors and is the common signal-transducing receptor component for the gp130, or IL-6/IL-12, family of cytokines (Figure 1*b* and Figure 2*b*) that exhibit highly pleiotropic biological activities (23, 63). There are currently ten members in the gp130 family of cytokines: IL-6, IL-11, LIF, cillary neurotrophic factor (CNTF), oncostatin M (OSM), cardiotrophin 1 (CT-1), NNT-1/BSF3 [also known as cardiotrophin-like cytokine (CLC)], and IL-27. There are two viral homologs of IL-6, one from HHV-8 IL-6 and another from the Rhesus macaque rhadinovirus (Rm IL-6) (Figure 1*b*). Except for the viral IL-6 homologs that bind directly to gp130 alone (11, 64), the signaling functions of gp130 cytokines are mediated through a set of receptor complexes that are formed by combining gp130 with other receptors (Figure 2*b*) (63). The association of gp130 with cytokine-specific, nonsignaling receptor IL-6Rα (65) or IL-11Rα executes the activities of IL-6 or IL-11, respectively. Other signaling receptors such as LIF receptor (LIFR) and OSM receptor (OSMR) can also participate in signaling complexes with gp130. The nonsignaling CNTF α receptor (CNTFRα) can recognize the cytokines CNTF, CT-1, and CLC as part of a quaternary signaling complex with gp130 and LIFR (66–68). Thus, CNTFRα exhibits the ability to bind three different cytokines, which is a rare example of degeneracy by an α receptor. Gp130 can also engage the recently identified heterodimeric cytokine IL-27 (p28/EBI3) in conjunction with the signaling receptor TCCR (T cell cytokine receptor, also known as WSX-1) (69). A major distinguishing feature of gp130 cytokines is that they possess the unique site III receptor-binding site at the tip of the cytokine that is necessary for gp130 activation (12, 70, 71).

The extracellular part of gp130 is composed of six contiguous β-sandwich domains with a single Ig domain at the top (D1), followed by one CHR module (D2 and D3), and three fibronectin III-like domains (D4–D6) leading to the cell membrane (Figure 1*b*). Both the CHR and Ig domains are necessary for full activation. There are three known crystal structures of complexes involving gp130: the IL-6/IL-6Rα/gp130-D1D2D3 hexamer (12), the HHV-8 IL-6/gp130-D1D2D3 tetramer (11), and LIF/gp130-D2D3 (13). In addition, there are two low-resolution, electron microscopic (EM) three-dimensional reconstructions of the entire extracellular complexes of IL-6 (72) and IL-11 (73). The crystal structures established that gp130 cytokines use the canonical sites I and II to engage the elbow regions of the α receptor and gp130 CHR, respectively. Site III in the cytokine engages the Ig domain of gp130 so that each gp130 contacts two different cytokines in an antiparallel fashion (Figure 3*a*). This basic assembly template is used by all gp130 cytokine receptor family members, including the nonshared members of the tall receptor family such as G-

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CSF, leptin, and OSMR. Originally, G-CSF was crystallized with only the CHR of its receptor (74). Subsequently, a mutational study of G-CSF based on the viral IL-6/gp130 complex structure (which revealed the first site III) determined that G-CSF contains a site III (75). Recently, the full complex of the G-CSFR Ig domain plus CHR has been solved with G-CSF (62), and this complex is almost identical in structure to that of the viral IL-6/gp130 complex and other gp130 complexes in the use of site III (11, 12).

Several recent advances have established the site III paradigm for heterodimeric gp130/LIFR signaling complexes. The 4.0 Å structure of the D1–D5 domains of LIFR in complex with LIF confirmed that LIFR uses a high-affinity site III interaction in which the interhelical loops at one tip of LIF engage the D3 Ig domain of LIFR in an almost orthogonal orientation (Figure 3*b*) (14). Binding measurements have also confirmed that LIFR and CNTF alone interact via a high-affinity site III that is presumably analogous to that of the LIF/LIFR interaction. A recent single-particle EM analysis of full-length gp130 and LIFR in complex with CNTFRα and CNTF (76) has confirmed the architecture of the quaternary gp130/LIFR/CNTFRα/CNTF complex proposed in Boulanger et al. (13) and for the trimeric LIF/LIFR/gp130 complex modeled in Huyton et al. (14) (Figure 3*c*). The structure of an intact gp130/LIFR heterodimeric complex is an important milestone for research on this class of receptors, as there has been some controversy about the functionally active domains of LIFR involved in cytokine-mediated complex formation with gp130.

With the basic site II/III architecture of the heterodimeric gp130/LIFR signaling complex predicted from a variety of structural and biochemical data, it is now clear that other gp130-related heterodimeric cytokines such as IL-12 (p35/p40) (77), IL-23 (p19/p40) (78), and IL-27 (p28/EBI3) (79) also engage their cognate receptors in some variation of this basic organizing principle (69, 80, 81). Interestingly, in the case of these heterodimeric cytokines (which consist of a four-helix bundle cytokine in complex with a soluble α receptor), the gp130-like receptors IL-12Rβ1 (IL-12 and -23) and TCCR/WSX-1 (IL-27) lack an N-terminal Ig-like domain and hence engage site II via their CHR domains. Site III is then free to interact with the second signaling receptor IL-12Rβ2, IL-23R, or gp130, respectively, all of which contain the top-mounted Ig domain required for site III interaction. In this fashion, the presence of the Ig domain serves as a structural beacon for the receptor that engages in site III interaction. IL-12 and IL-23 also represent unique examples of two cytokines sharing both an α receptor (p40) and a signaling receptor (IL-12Rβ1), while gaining specificity by using different site III receptors (IL-12Rβ2 and IL-23R).

An additional characteristic feature of the gp130 family receptors is that that they are taller than other cytokine receptors, by virtue of three additional membrane-proximal domains. A three-dimensional reconstruction of negatively stained six-domain gp130 complexed with IL-6 and the IL-6Rα receptor indicated that the gp130 membrane-proximal legs are bent back toward one another through the flexing of a hinge between the D3 and D4 domains (73). This leg closure has also been observed in a cryoelectron microscopic analysis of the hexameric gp130/IL-11/IL-11Rα complex (74). Thus, although the FNIII leg domains have retained conformational flexibility to allow for the close apposition of the intracellular domains required for intracellular signaling, it remains unclear whether the unliganded gp130 exists in this bent conformation or if the engagement of the shorter cytokine/Rα binary complex forces gp130 to bend in order to accommodate the height differences.

THE COMMON BETA CHAIN: β_c

β_c is a type I transmembrane protein that serves as a shared signaling subunit for the receptors of IL-3, IL-5, and GM-CSF (Figure 1*c* and Figure 2*a*), which are related cytokines involved in the regulation of hematopoiesis and inflammation (82–84). Although β_c does not

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measurably bind any of the ligands alone, its coexpression with cytokine-specific α receptors enhances the affinity of cytokine binding. The activated receptor complex, consisting of the cytokine ligand plus the α and β_c receptors, initiates the intracellular signaling pathway mainly through JAK2 associated with the cytoplasmic domain of β_c receptors (85). The extracellular part of β_c has four fibronectin domains, forming two contiguous CHR modules (Figure 1*c*), with features conserved among the class I cytokine receptors. The crystal structure of the unliganded extracellular domain of β_c shows it to exist as an unusual, intertwined, strand-swapped, antiparallel homodimer (86). This structure, together with mutagenesis studies (87), led to the proposition that the possible cytokine-binding site is composed of D1 of one chain and D4 of another chain in the β_c homodimer that join in an antiparallel fashion. Excitingly, the recent crystal structure of the β_c in complex with the GM-CSFα receptor (GMRα) and GM-CSF confirms that β_c engages the cytokine via a composite D1/D4 interface similar to site II in gp130 and γ_c, while the accessory receptor GMRα engages the cytokine via a site I–like interface (Figure 4) (18). It has been reported that GMRα does not engage a JAK kinase (85), leaving the JAK2-bound β_c as the sole carrier of the signal-transducing kinase. The asymmetric unit of the β_c/GMRα/GM-CSF complex consists of a 2:2:2 hexamer with the C termini of β_c ~ 140 Å apart, therefore making it hard to reconcile how JAK kinases bound to β_c subunits of a single β_c dimer could be activated. Importantly, crystallographic contacts between β_c D4 domains of two separate β_c/GMRα/GM-CSF hexamers suggested β_c signaling may be mediated by two hexamers dimerized into a dodecameric structure (18). Site-directed mutagenesis of this interface abrogated GM-CSF-induced signaling; thus, it appears that a second β_c dimer in complex with GMRα and GM-CSF is necessary to complete the active signaling unit. These studies clarify what turns out to be a highly interesting deviation from the typical cytokine receptor signaling paradigm and can likely be extrapolated to explain the activation mechanisms of the other β_c-family cytokines IL-3 and IL-5.

THE COMMON GAMMA CHAIN: γ_c

γ_c serves as a shared signaling receptor for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (Figure 1*a* and Figure 2*c*) (8). The biological importance of γ_c is illustrated by the fact that mutations in either γ_c or the associated JAK3 kinase can abolish the function of all γ_c-dependent cytokines and cause X-linked severe combined immunodeficiency diseases (X-SCID) (88, 89). One interesting twist in the γ_c family is that the IL-2 receptor (IL-2R) and IL-15R signaling complexes are heterotrimers composed of structurally unique α subunits and shared IL-2Rβ and γ_c subunits (Figure 2*c*). This is in contrast to type I IL-4R, IL-7R, IL-9R, and IL-21R, which heterodimerize cytokine-specific α subunits and γ_c (Figure 2*c*). Another interesting twist in the γ_c family is limited sharing of several α receptors including IL-2Rβ, IL-4Rα, and IL-7Rα to recognize different cytokines as well as different receptors (Figure 2*c*). IL-2Rβ serves as a receptor for both IL-2 and IL-15. IL-4Rα heterodimerizes with γ_c to form type I IL-4R, and with IL-13Rα1 to form type II IL-4R. Type II IL-4R is also the functional receptor of IL-13 (discussed below) (25, 90, 91). IL-7Rα can also form a receptor heterodimer with TSLPR (thymic stromal-derived lymphopoietin receptor) to recognize TSLP (92, 93).

STRUCTURAL STUDIES OF CYTOKINE-RECEPTOR COMPLEXES IN IL-2 AND IL-4/IL-13 SYSTEMS

The cytokine IL-2 is a prototypical short-chain cytokine and has pleiotropic actions in the immune system (28, 94, 95). Produced mainly by activated T cells, IL-2 promotes the proliferation, differentiation, and survival of mature T and B cells and the cytolytic activity of natural killer (NK) cells (95). There are three receptor chains for IL-2: IL-2Rα, IL-2Rβ, and γ_c, which form three different receptor complexes on different target cells. Isolated

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IL-2Rα has been termed the low-affinity IL-2 receptor ($K_d \approx 10$ nM) and is not currently believed to be involved in signal transduction (96). IL-2Rβ and γ_c form the intermediate-affinity complex ($K_d \approx 1$ nM) expressed on NK cells, macrophages, and resting T cells (95), although IL-2Rβ alone has very low affinity ($K_d \approx 100$ nM) and γ_c alone has no detectable binding affinity for IL-2 (97, 98). The heterodimerization of IL-2Rβ and γ_c in the presence of IL-2 is necessary and sufficient for effective signaling through the activation of JAK1 and JAK3 kinases associated with the intracellular domains of the IL-2Rβ and γ_c, respectively (99, 100). A complex with three subunits, IL-2Rα, IL-2Rβ, and γ_c, is the high-affinity complex ($K_d \approx 10$ pM) for IL-2 and is the receptor form found on activated T cells (101). The high-affinity receptor complex mediates most biological effects of IL-2 in vivo (102). Prior to structural analysis, the Ciardelli group published a series of elegant papers measuring the various rate constants and affinities for soluble forms of the different compositions of complexes (103–107). A similar analysis of the soluble complex assembly has also been conducted using thermodynamic measurements instead of kinetic studies (97). Therefore, the IL-2 receptor system has been one of the most rigorously characterized receptor systems using both cellular and biochemical approaches.

IL-4 is another principal regulatory cytokine during the immune response and is crucially important in allergy and asthma (90). Once resting T cells are antigen activated and expand in response to IL-2, the fate decision of Th1 versus Th2 is influenced by IL-4. Th2 cells secrete IL-4, which both stimulates Th2 in an autocrine fashion and acts as a potent B cell growth factor to promote humoral immunity (90). There are two types of receptor complexes for IL-4 (Figure 2*c*) (61, 91). Type I IL-4R is predominantly expressed on the surface of hematopoietic cells and consists of IL-4Rα and γ_c. Type II IL-4R consists of IL-4Rα and IL-13Rα1 and is predominantly expressed on the surface of non-hematopoietic cells, and this receptor complex is also the functional receptor of IL-13 (108–110).

The crystal structures of the high-affinity IL-2/IL-2Rα/IL-2Rβ/γ_c quaternary complex and IL-4/IL-4Rα/γ_c, IL-4/IL-4Rα/IL-13Rα1, and IL-13/IL-4Rα/IL-13Rα1 ternary complexes have all been determined (Figure 5) (15, 17). The structural comparison between IL-2/IL-2Rα/IL-2Rβ/γ_c and IL-4/IL-4Rα/γ_c complexes allows us to probe the basis by which γ_c can recognize six distinct cytokines. For the convenience of comparison between these two structures, and to eliminate redundancy in the chapter, we describe them in parallel throughout the following sections.

OVERALL STRUCTURE

The first complex structure of γ_c to be solved was the IL-2/IL-2Rα/IL-2Rβ/γ_c quaternary complex (15, 16) (Figure 5*a*), which is composed of one copy each of IL-2, IL-2Rα, IL-2R β, and γ_c. Viewed from the perspective of the cell membrane, IL-2Rα sits on top of IL-2, and receptors IL-2Rβ and γ_c form a Y shape in which IL-2 sits in the fork (Figure 5*a*). The overall structural organization of the IL-4/IL-4Rα/γ_c ternary complex is very similar to that of the IL-2 quaternary complex with a 1:1:1 stoichiometry (Figure 5*b*) (17), except for the absence of a top-mounted IL-2Rα. In the IL-4/IL-4Rα/γ_c ternary complex, receptor IL-4Rα and γ_c form a Y-shape heterodimer that binds to IL-4 in the classical site I/site II paradigm (Figure 5*b*).

BINARY COMPLEX OF IL-2Rα/IL-2

After resting T cells are activated by antigen, expression of IL-2Rα is upregulated in order to sensitize the T cells to low concentrations of IL-2, which is required for clonal expansion. The human IL-2Rα receptor chain, also known as Tac antigen or CD25, is a ~55-kDa polypeptide consisting of an extracellular domain of 219 residues, a transmembrane domain

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of 19 residues, and a short cytoplasmic tail containing 19 residues (111–113). The short cytoplasmic tail does not participate in the signal transduction, although it is highly conserved between mice and humans, suggesting some important functional roles that remain unknown (100). From sequence analysis, IL-2Rα also clearly lacks the signature features of class I cytokine receptors such as IL-2Rβ and γ_c. With the goal of targeting IL-2Rα with therapeutics for immunosuppression, the crystallization of the IL-2/IL-2Rα binary complex was an important benchmark in the field (114). In fact, the monoclonal antibody referred to as anti-Tac targets IL-2Rα and blocks the interaction of IL-2 with IL-2Rα (115, 116). The humanized form of anti-Tac (daclizumab, or Zenapax®) has been approved by the FDA for use in preventing renal transplant rejection (117). In several other clinical trials, daclizumab also provided a reduction of rejection in patients receiving liver, cardiac, and pancreatic islet transplants (118–120).

In 2005, the structure of a recombinant soluble form of IL-2Rα complexed with IL-2 was solved to 2.8 Å (53) and revealed a very unusual structure for both the IL-2Rα as well as its mode of interaction with IL-2. The globular part of the IL-2Rα extracellular region is composed of two domain-swapped sushi modules (D1 and D2). Strands A and B from one sushi domain (A–B on C–D–E) are exchanged with strands F and G from another sushi domain (F–G on H–I–J), so the two domain-swapped sushi modules in IL-2Rα now become F on G-C–D–E (D1 domain) and A on B-H–I–J (D2 domain) (Figure 6*a*). As a result of this domain swap, IL-2Rα uses a composite surface to dock into a groove on IL-2 between the A′ and B′ helices, the same surface that serves as a binding site for antagonist drugs (121). This can be considered the dorsal surface of the cytokine with respect to the membrane, poised to present IL-2 to the side-oncoming IL-2Rβ and γ_c (Figures 5*a*, 6*c*). The IL-2/IL-2Rα binding interface (site a) has an extensive buried surface of 1670 Å^2 and is dominated by two hydrophobic clusters with a surrounding polar periphery, which may contribute to IL-2Rα's high-affinity binding and exquisite specificity. A recent mutational study identified IL-2 hot-spot residues Glu-62, Tyr-45, and Phe-42 as the most energetically critical residues in the receptor/IL-2 interface (122). Interestingly, these residues also serve as the hot-spots for several small molecule drugs that antagonize IL-2 binding to this receptor suggesting that the small molecule and receptor use the same energetic mechanism for binding. The implications are that residue contact footprints in a protein/protein interface may, in some cases, serve as a useful surrogate scaffold for design of small molecules.

The IL-2/IL-2Rα complex represents the initiating step for formation of the quaternary signaling complex, so many anticipated that IL-2Rα's role was simply to capture free IL-2 and present it to IL-2Rβ and γ_c (Figure 7*a* and Table 1). Moreover, the expression level of IL-2Rα is 10- to 20-fold higher than that of IL-2Rβ on activated T cells (123). Thus, the excess of IL-2Rα molecules and relatively high-affinity binding to IL-2 would facilitate efficient capture of free IL-2 and its delivery to IL-2Rβ through a restricted two-dimensional handoff on the same cell membrane. That IL-2R α did not appear to make any contact with either IL-2Rβ or γ_c in the quaternary signaling complex was a surprise (Figure 5*a*, 6*c*) (15). The linker connecting the globular domains of IL-2Rα to the cell membrane, which is disordered in the structure, does not appear capable of forming receptor-receptor contact with IL-2Rβ even if fully extended (Figure 6*c*). This is a rather surprising finding given the longstanding observation that the coexpression of IL-2Rα and IL-2Rβ forms the pseudo-high-affinity complex that can bind to IL-2 with a K_d of ~30 pM, much higher than IL-2 binding to IL-2Rβ alone ($K_d \approx 100$ nM) (124). Isothermal titration calorimetry (ITC) experiments with the soluble receptor ectodomains also showed a twofold affinity increase between IL-2 and IL-2Rβ in the presence of IL-2Rα (97). Kinetic studies of both membrane-bound and soluble ectodomains have shown that the on rate of IL-2 for IL-2Rβ is 3 to 20 times faster in the presence of IL-2Rα (96, 98). A simple mechanistic explanation for these affinity data would be the presence of a composite binding surface for IL-2 contributed by

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both IL-2Rα and IL-2Rβ, which is not seen in the structure (Figure 5*a*). So what is the basis of the cooperativity? One explanation is that the affinity-enhancing role of IL-2Rα is independent of structural effects and is achieved by simply capturing and concentrating free IL-2 at the cell surface, as mentioned above. The other possibility is an IL-2Rα-induced conformational change in IL-2 that favors the binding to IL-2Rβ. After comparison of the IL-2 structures in the quaternary complex, binary complex, and unbound states, one local conformational adjustment on IL-2 upon IL-2Rα binding was found at the beginning of the helix C, where several turns of the helix are slightly unwound and translated forward on the helical axis by approximately 1.0 Å toward IL-2Rβ. This local conformational change enables the movement of IL-2 residue Asn-88 into hydrogen-bonding proximity to IL-2Rβ residue Arg-42 (Figure 8*a*). Consistent with this, mutation of Asn-88 in IL-2 ablates binding to IL-2Rβ (125). We take this to suggest that IL-2Rα may induce and stabilize a favorable IL-2Rβ-binding conformation of the IL-2 C helix in IL-2, in addition to its roles in capture and delivery.

IL-15 is the only other cytokine that uses a specific sushi-domain α receptor (IL-15Rα) (126, 127). In addition, IL-15 uses IL-2Rβ and γ_c as its signaling components in the receptor heterotrimer (128, 129). Because IL-15Rα has only one sushi domain in the extracellular part, there is no possibility for a strand exchange as observed in IL-2Rα, which has been confirmed by a NMR structure of the IL-15Rα sushi domain and two complex crystal structures of IL-15Rα sushi domain with IL-15 from human and mouse (54, 130, 131). In these structures, the IL-15Rα sushi domain shows a canonical sushi fold topology (Figure 6*b*). The IL-15/IL-15Rα and IL-2/IL-2Rα binary complexes have similar cytokine-receptor docking modes: The α receptor sushi domain binds to the dorsal surface of the cytokine (Figure 6*b*), and the cytokine-receptor interaction footprints of IL-15Rα on IL-15 and IL-2Rα on IL-2 also have substantial overlap (130–132), but IL-15Rα has an approximately 1000-fold higher binding affinity to IL-15 than that of IL-2Rα to IL-2 (126). Compared to the dominant hydrophobic patches, with a surrounding polar periphery seen in the IL-2/IL-2Rα binding interface, the interaction area between IL-15 and IL-15Rα is dominated by salt bridges and hydrogen bonds with superior shape complementarity (130, 131). The charge-charge interactions may cause the low K_{off} value between IL-15 and IL-15Rα that is responsible for the high affinity. Similar to IL-2/IL-2Rα, the presence of the IL-15Rα endows IL-15 with a much higher affinity for IL-2Rβ and γ_c (30, 133). One possible explanation for this is the fact that IL-15Rα is known to present IL-15 in *trans*, from cell to cell, to the IL-2Rβ/γ_c complex (134) (Figure 6*c*). The disconnection of IL-15Rα from residing in the same membrane as the IL-2Rβ and γ_c components may require a more profound allostery for IL-15Rα to serve effectively as an affinity converter for IL-15. In other words, the *trans*-signaling role of IL-15Rα requires that IL-15 binding to IL-2Rβ is enhanced solely through conformational change rather than simply through surface capture on the same cell membrane. Although *trans*-presentation is currently believed to be the major mechanism by which IL-15 exerts its biological effects in vivo, the assembly of a *cis* IL-15/IL-15Rα/IL-2Rβ/γ_c quaternary complex on the surface of the same cell is also used (131, 135) (Figure 6*c*).

IL-2Rβ/IL-2 AND IL-4Rα/IL-4 INTERFACES

IL-2Rβ and IL-4Rα are functional and structural counterparts in their respective signaling complexes, and both form one of the two major signaling subunits in their γ_c receptor complexes. The ~75-kDa human IL-2Rβ chain is composed of an extracellular domain of 214 residues, a transmembrane domain of 25 residues, and an intracellular domain of 286 residues (136). The ~140-kDa human IL-4Rα chain has 207 residues in the extracellular domain, 24 in the transmembrane region, and 569 in the intracellular domain (137). Although the nomenclature is confusing, IL-2Rβ is analogous to the α receptors for other γ_c

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cytokines, but because IL-2 has the additional nonstandard initiating receptor, IL-2Rα, IL-2Rβ is then referred to as the β receptor on the basis of the sequence of interactions with IL-2. The intracellular domains of IL-2Rβ and IL-4Rα possess the box 1 and box 2 motifs at the membrane-proximal region that constitute the binding sites for JAK1 (34). The cytokine binding–induced association of IL-2Rβ or IL-4R α with γ_c will bring their intracellular domains into close proximity, inducing the activation of the JAK kinases (Figure 5*a*,*b*).

After the capture of IL-2 by IL-2Rα, the delivery of IL-2 to IL-2Rβ in *cis* represents the second step in the formation of the quaternary IL-2 receptor complex (Figure 7*a* and Table 1). The binding interface between IL-2 and IL-2Rβ (site I) buries ~1350 $Å^2$ and is formed by residues from helices A and C in IL-2 and loops CC′1, EF1, BC2, and FG2 in IL-2Rβ (Figure 8*a*). The interface is highly polar, with eight hydrogen bonds directly between IL-2 and IL-2Rβ residues and seven buried water molecules mediating the interactions between IL-2 and IL-2Rβ by forming hydrogen bonds with protein atoms (Figure 8*a*). Solvent exchange with the layer of water molecules between IL-2Rβ and IL-2 could explain the fast on and off rates and the weak affinity of the IL-2/IL-2Rβ binary complex. Two residues of IL-2 that have been shown by mutagenesis to be critical for IL-2Rβ binding, Asp-20 (138) and Asn-88 (125), are involved in hydrogen bonding networks to both water molecules and side chains on IL-2Rβ. There is excellent knob-in-hole shape complementarity between IL-2Rβ and IL-2 (Figure 8*a*).

As mentioned, IL-2Rβ is also used by IL-15 to form a quaternary complex along with the IL-15Rα and γ_c (30, 133) (Figure 6*c*). IL-15 has limited sequence homology (19%) with IL-2, so its contacts with IL-2Rβ are almost certainly through a unique set of interactions. The apparently central role that water molecules play in bridging hydrogen bonds between IL-2Rβ and IL-2 would contribute to the ability of IL-2Rβ to cross-react through remodeling of this hydration layer to accommodate the IL-15 residues.

The first step in the formation of the IL-4/IL-4Rα/γ_c complex is the binding of IL-4 with IL-4Rα receptor (139, 140) (Figure 7*b* and Table 1). These interactions were first elucidated in the IL-4/IL-4Rα binary complex (141). The comparison of IL-4/IL-4Rα binary and IL-4/IL-4Rα/γ_c ternary complex structures reveals that the engagement of γ_c does not cause substantial conformational changes in the mode of interaction with IL-4Rα. Minor differences in peripheral interface contacts between the IL-4 type I binary and ternary complexes are likely due to the differing resolutions of the structures and crystal packing forces. The interacting residues from helices A and C in IL-4 and loops CC′1, EF1, AB1, BC2, and FG2 in IL-4Rα bury a total surface area of ~1520 $Å^2$ (site I) (Figure 8*a*). The chemical nature of the IL-4/IL-4Rα interface is also highly polar, similar to that of the IL-2/IL-2Rβ interface. The interacting residues in the IL-4/IL-4Rα interface can be grouped into two major clusters centered at Glu-9 (IL-4) to Tyr-134 (IL-4Rα) and Arg-88 (IL-4) to Asp-72 (IL-4Rα), respectively, each containing an inner polar core surrounded by outer hydrophobic residues (Figure 8*a*). Considering there are only two bridging water molecules in the IL-4/IL-4Rα interface, the buried polar and charged interactions contribute to the rapid on rate ($K_{on} \approx 1.3 \times 10^6 M^{-1}s^{-1}$) and slow off rate ($K_{off} \approx 2.1 \times 10^{-3}\ s^{-1}$) that result in high-affinity binding between IL-4 and IL-4Rα (142). An exhaustive double-mutant analysis of the IL-4-IL-4Rα interaction has been carried out by the Sebald group (143), and we refer the reader to this paper for a rigorous, energetic deconvolution of the cytokine-receptor interface.

In summary, both the IL-2 and IL-4 interactions with their respective α chains are characterized by polar and charged contacts. This is consistent with the high degree of specificity these receptors show for their cytokine, in contrast to γ_c's degeneracy. Although not completely generalizable, polar and charged contacts primarily mediate specificity in

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protein-protein interactions because their energetic content is greatly affected by the precise structural context within an interface (144). Van der Waals and hydrophobic interactions are more suitable to a promiscuous binding surface because these are less structure-selective interactions that are a result more of water exclusion than of specific structural context and pairwise atomic contacts (144).

RECRUITMENT OF γ_c BY IL-2/IL-2Rαβ AND IL-4/IL-4Rα

The interaction of the IL-2/IL-2Rα/IL-2Rβ ternary complex with γ_c is the last step in formation of the IL-2 signaling complex on activated T cells (Figure 7*a* and Table 1). IL-2 has a very low affinity for γ_c alone ($K_d \approx 700$ μM) (97, 98), requiring precomplexation with IL-2Rα/IL-2Rβ (in the high-affinity complex) or IL-2Rβ alone (in the intermediate-affinity complex) to bind γ_c with K_d in the low nM range. IL-2Rβ and γ_c do not have a measurable affinity for one another (97). The two weak interactions, IL-2 with γ_c and IL-2Rβ with γ_c, combine to produce a high affinity for γ_c. In the complex structure, two contact surfaces, a small one between IL-2 and γ_c and a larger one between IL-2Rβ and γ_c, form the interaction surface between IL-2/IL-2Rαβ and γ_c (Figure 5*a*).

The IL-2/γ_c binding surface (site IIa) reflects its degenerate recognition capabilities, showing a remarkable flatness and almost tangential contact with IL-2 (Figure 8*b*). The IL-2/γ_c interface is the smallest of the four protein/protein interfaces in the complex, burying ~970 Å^2 of surface area. In contrast to the IL-2Rβ interface, the γ_c binding surface is rather devoid of extended side chain–specific interactions with IL-2 and exhibits primarily main chain contact (Figure 8*b*).

Unlike the IL-2/IL-2Rβ interface that has a broad array of specific polar interactions, the IL-2/γ_c interface is composed of small contact patches (Figure 8*b*). The first one is composed of residue Tyr-103 from γ_c and residues Ser-127 and Ser-130 from IL-2. In γ_c, the Tyr-103 side chain does not protrude outward toward IL-2 but is instead pinned back via a hydrogen bond with the Cys-209 main chain and is therefore positioned so that its aromatic ring packs flat against the side chains of Ser-127 and Ser-130 in IL-2 (Figure 8*b*). The second contact patch is around residue Gln-126 in IL-2, whose side chain is flattened and almost parallel to the surface formed by main chain atoms of residues Pro-207 to Ser-211 in the FG2 loop of γ_c (Figure 8*b*).

The second part of the composite interface between IL-2/IL-2Rβ and γ_c is formed by extensive interactions between the D2 domains of IL-2Rβ and γ_c (site IIb) (Figure 5*a*). The residues in the IL-2Rβ/γ_c interface bury over 1750 Å^2 of surface area, which is the most extensive seen so far between receptor domains in cytokine-receptor complexes. The IL-2Rβ/γ_c interface is predominantly composed of polar interactions, with a total of 17 hydrogen bonds surrounding a small hydrophobic stripe.

After the initial binding of IL-4 with the IL-4Rα receptor, the association of γ_c is also mediated through a composite interface: IL-4/γ_c and IL-4Rα/γ_c (Figure 7*b* and Table 1). The IL-4/γ_c interface buries a total surface of ~ 1020 Å^2, which is slightly larger than that of the IL-2/γ_c interface but still the smallest in the IL-4/IL-4Rα/γ_c complex (site IIa) (Figure 8*b*). The Tyr-103 residue and the FG2 loop fixed by the Cys-160 to Cys-209 disulfide bond in γ_c interact with IL-4 in a binding mode similar to that of γ_c within the IL-2 receptor complex (Figure 8*b*). There is low sequence identity between IL-2 and IL-4, and so the contacting residues in IL-4 with Tyr-103 are Glu-122 and Ser-125, which still contact the aromatic ring of Tyr-103 through van der Waals contacts instead of forming specific polar interactions (Figure 8*b*). In the IL-4/γ_c binding interface, the Arg-121 residue from IL-4, which replaces the critical Asn-126 position in IL-2, contacts with main chain atoms of residues Pro-207 to

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Ser-211 in γ_c through its long methylene side chain (Figure 8*b*). While maintaining these two binding epitopes that are also observed in the IL-2/γ_c interface, the IL-4/γ_c interface has an additional hydrophobic patch consisting of Tyr-124 in IL-4 and the interacting Leu-208 and Tyr-182 in γ_c (Figure 8*b*).

Also contributing to the IL-4Rα/γ_c interface are extensive receptor-receptor contacts between the D2 domains of the respective CHR (site IIb) (Figure 5*b*). However, the packing between IL-4Rα/γ_c is less intimate than that of the IL-2Rβ/γ_c interface, as evidenced by its much smaller buried surface (~1200 Å^2 versus ~1750 Å^2). The IL-4Rα/γ_c interface also has extensive polar interactions surrounding a small hydrophobic stripe in the center, dominated by Tyr-154 from IL-4Rα and Phe-186 from γ_c. The IL-4Rα-γ_c interaction is much weaker (K_d ~ uM) (17) in the IL-4 ternary complex than that between IL-2Rβ and γ_c in the IL-2 quaternary complex (Table 1). The fact that receptor-receptor contacts seen in the respective complexes are less extensive explains much of this affinity difference.

DEGENERATE CYTOKINE RECOGNITION BY γ_c

Protein-protein interactions and, in fact, most receptor-ligand interactions are usually characterized by specificity for one ligand. Therefore, the molecular basis for γ_c recognition of six different cytokines has been not only a fundamental question in understanding cytokine recognition, but also a basic problem in physical chemistry. As a shared signaling subunit, the engagement of γ_c is the last step in the formation of functional signaling complexes (Figure 7*a*,*b*). On the basis of the structural information from the IL-2 quaternary complex and the IL-4 ternary complex, we can conclude that the preformed complexes of cytokines with the α receptors provide a composite binding site for γ_c that is composed of two interaction interfaces: cytokine/γ_c and α receptor/γ_c. The small buried surface area (~970 Å^2 between IL-2 and γ_c and ~1020 Å^2 between IL-4 and γ_c) and the formation of relatively nonspecific atomic interactions characterize the cytokine/γ_c binding interface. In contrast, the α receptor/γ_c binding interface has a large buried surface area (~1750 Å^2 between IL-2Rβ and γ_c and ~1200 Å^2 between IL-4Rα and γ_c) composed predominantly of specific polar interactions.

Extensive mutagenesis work has identified Tyr-103, Cys-160, and Cys-209 in γ_c as critical residues for the engagement of all γ_c-dependent cytokines (145–147). Because certain residues around these hot-spot positions were exclusively implicated in the binding of different cytokines, such as Ile-100 and Leu-102 for IL-4 binding and Asn-44, Leu-161, and Glu-162 for IL-21 binding, the γ_c-binding sites for different cytokines overlap but are not identical (146, 147). The side chain of Tyr-103 does not form specific side chain interactions with residues in IL-2 and IL-4, but it presents its aromatic ring for contact with residues Ser-127 and Ser-130 in IL-2 and Glu-122 and Ser-125 in IL-4 (Figure 8*b*). Residues Cys-160 and Cys-209 form a unique disulfide bond that connects loops FG2 and BC2 in the D2 domain of γ_c (Figure 8*b*). This disulfide bond fixes the bent conformation of loop FG2, where the main chain atoms from residues Ser-207 to Pro-211 directly contact the methylene side chain of residue Gln-126 in IL-2 or Arg-121 in IL-4 (Figure 8*b*). The critical role of these hot-spot residues in γ_c is also illustrated by the fact that some of their mutations have been found in the human γ_c gene of X-SCID patients (88).

Although the cytokines recognized by γ_c have an average 19% sequence identity among them, helix D is the most conserved region, which is also the major contact area used by γ_c-dependent cytokines to bind γ_c. Phe-114 and Ile-115 are two strictly conserved positions at the N terminus of helix D in IL-2, but they are not involved in γ_c binding, and their hydrophobic side chains point into the helical core. Residue 126 in IL-2 helix D is a conserved Asn in the γ_c-dependent cytokines IL-9, IL-15, and IL-21, while IL-4 and IL-7

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contain Arg-121 and Lys-139 at this position. The Gln-126 in IL-2 and Arg-121 at this position in IL-4 both contribute to binding with γ_c in their respective complexes (Figure 8*b*). Although this position serves as a common contact point for γ_c, its importance in binding with γ_c can vary with different cytokines. In IL-2, Gln-126 is the major γ_c-binding determinant, and mutation of this site greatly reduces binding affinity (148). In IL-4, Arg-121 is only one of the minor γ_c-binding determinants, and the nearby residues Ile-11, Asn-15, and Tyr-124 serve as the major determinants (146).

In the absence of obvious conserved hot-spot residues in γ_c-dependent cytokines, shape complementarity appears to play a dominant role. This assertion is supported by the observation that both IL-2 and IL-4 provide a shallow groove that accommodates the protruding hot-spot binding residues in γ_c. In IL-2, the bulky side chains from residues Thr-123, Gln-126, Ser-127, Ile-129, and Ser-130 form the walls of a canyon, which receives a protruding ridge on γ_c composed of Tyr-103 and the Cys-160 to Cys-209 disulfide bond (Figure 8*b*). The groove in IL-4 is surrounded by residues Thr-118, Arg-121, Glu-122, Tyr-124, and Ser-125, which are in the same positions with those of IL-2 (Figure 8*b*). The formation of the groove for the placement of hot-spot residues in γ_c would only require that residues have side chains with similar volume and shape, which can be achieved without the need for strictly conserved residues. We expect that other γ_c-dependent cytokines, IL-7, IL-9, IL-15, and IL-21, will also form a similar shallow groove on helix D that serves as the docking site for the protruding binding site on γ_c. The functional role of this groove appears to facilitate complex formation with γ_c by guiding a perfect geometrical alignment of the D2 domains of the cytokine-specific α receptor and γ_c, resulting in the numerous interatomic contacts (H-bonds, van der Waals, etc.) in the D2/D2 interface between α receptor and γ_c. In other words, the knob-in-hole shape complementarity between the groove on the cytokine and the protruding γ_c-binding site acts as a guide to align the receptor D2 domains for the intimate interaction that they exhibit.

RECRUITMENT OF IL-13Rα1 IN TYPE II IL-4 AND IL-13 RECEPTOR COMPLEXES

As previously mentioned, IL-4Rα represents an important subfamily of γ_c-cytokine receptors in that it serves as a shared signaling receptor within three different cell surface complexes. IL-4Rα and IL-13Rα1 form receptor heterodimers on cells of nonhematopoietic stem cell origin, functioning as the type II receptor complex for both IL-4 and IL-13. IL-13Rα1 is derived from the same ancestral subgroup as γ_c (149), and its divergence derives from its extra N-terminal Ig-like domain that has contact with the dorsal surfaces of both IL-4 and IL-13 (site III) in IL-4/IL-4Rα/IL-13Rα1 and IL-13/IL-4Rα/IL-13Rα1 complexes (Figure 5*c*,*d*). The IL-13Rα1 D1 domain–binding sites on IL-4 and IL-13 have extensive overlap with the respective α receptor–binding sites on IL-2 and IL-15. In site III of both complexes, the C′ strand of IL-13Rα1 interacts with the C-D strand of IL-4 and IL-13, forming an antiparallel beta sheet. The residues Trp-65 and Ile-78 on the C′ strand of IL-13Rα1 form a hydrophobic patch that opposes complementary hydrophobic residues in IL-13—these interactions are missing between IL-4 and IL-13Rα1 (Figure 5*c*,*d*). Consistent with this structural data, mutational studies have shown that after deleting the D1 domain, the IL-13Rα1 D2D3 CHR module does not detectably bind to IL-13, but it can still form a ternary complex with IL-4 and IL-4Rα (17). This difference in the energetics of site III interactions between the respective cytokines likely explains the requirement of IL-13Rα1 D1 domain for signaling in the IL-13 type II complex, in contrast to the IL-4 type II complex (150).

Although IL-4 and IL-13 use the same IL-4Rα/IL-13Rα1 receptor heterodimer for signaling, the type II IL-4 and IL-13 complexes assemble in the reverse cooperative sequences (Figure

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7*c*,*d*). Similar to the type I IL-4 complex, type II IL-4 complex is formed by the initial high-affinity binding of IL-4 with IL-4R α (site I) with a subnanomolar K_d, followed by the recruitment of IL-13Rα1 (site II and site III) with a much lower affinity of 487 nM (Figure 7*c* and Table 1). In contrast, for the type II IL-13 complex, IL-13 first binds to IL-13Rα1 with an affinity of 30 nM, and the IL-13/IL-13Rα1 binary complex then recruits IL-4Rα with an affinity of 20 nM (Figure 7*d* and Table 1).

The driver (receptor for initial cytokine interaction) and trigger (receptor recruited for signaling) terminology was previously proposed for the assembly of γ_c heterodimeric complexes (151). The type II IL-4 and IL-13 complexes have switched the driver and trigger in their respective assembly pathways. The recruitment of the trigger IL-4Rα in the type II IL-13 complex is more energetically favorable ($K_d \approx 20$ nM) than the recruitment of the trigger IL-13Rα1 by the type II IL-4 complex ($K_d \approx 487$ nM). Measurement of STAT6 phosphorylation induced by IL-4 and IL-13 in the human epithelial carcinoma cell line A549 (expressing IL-4Rα and IL-13Rα1, but not γ_c) revealed that IL-4 is more potent (17). In this A549 cell line, the expression level of IL-13Rα1 is higher than that of IL-4Rα. In other cell lines where IL-13Rα1 expression level is limiting, IL-13 can become more potent than IL-4 in stimulating signaling (152). It was therefore proposed that when IL-13Rα1 is abundant, the high-affinity binding of IL-4 with its driver IL-4Rα would determine the signaling potency. When IL-13Rα1 is limiting, the IL-13 and IL-13Rα1 binding affinity ($K_d \approx 30$ nM) would still allow the efficient formation of IL-13/IL-13Rα1 binary complex, and the subsequent relatively high-affinity binding ($K_d \approx 20$ nM) with trigger IL-4Rα could favor the formation of the type II IL-13 complex, resulting in more potent IL-13-induced signaling. These studies showed that membrane-proximal signaling events induced by a cytokine could be collectively influenced by many factors: the structural aspects of extracellular cytokine receptor interactions (e.g., receptor orientation and conformation), the concentration of cytokine, receptor expression level, the sequence of receptor assembly, and cytokine receptor binding affinity. Each of these factors could potentially be manipulated to effect a therapeutic endpoint in this system that has obvious clinical importance for asthma.

COMPARISON OF γ_c WITH gp130

With a raft of structures of both γ_c and gp130 complexes with cytokines, we can now assess the similarities and differences between the structural mechanisms by which these shared receptors cross-react (Figure 9). The ectodomain of γ_c is composed of one CHR domain, whose elbow region at the interdomain boundary is the contact area for six different short-chain cytokines. While the ectodomain of gp130 is taller than that of γ_c and consists of one top-mounted Ig domain, one CHR module, and three extra membrane-proximal fibronectin domains (Figure 3), the main gateway entry point for all long-chain gp130 family cytokines is also the elbow of the CHR module, analogous to γ_c (12). The structural analogies between the CHR modules on γ_c and gp130 are, then, very clear. Their cytokine-binding surfaces are similar in the distribution of hydrophobic and hydrophilic residues, with a largely hydrophobic core shared region and discontinuous peripheral polar patches, but gp130 has more buried surface area within the cytokine-receptor interface compared with γ_c, consistent with gp130 engaging the larger long-chain cytokines (Figure 9*a*). The buried surface area on γ_c contributing to IL-2 and IL-4 binding is ~500 Å^2, whereas the buried surface area on gp130 upon binding human IL-6, human LIF, and viral IL-6 is 710, 700, and 610 Å^2, respectively. Gp130 also has a larger core hydrophobic region of its interfaces. This can possibly be understood from the standpoint that gp130 is a shared receptor capable of binding to some cytokines, such as LIF and OSM, in the absence of an α receptor, therefore not requiring the receptor-receptor contact necessary in the γ_c complexes. γ_c, in contrast, requires an α receptor for all cytokine interactions and therefore has a smaller binding interface with cytokines, as the energetics are distributed across both α receptor and

cytokine. In the hydrophobic core of the interfaces of both γ_c complexes, Tyr-103 is the hot-spot residue that contributes about 20% of the buried surface area upon binding with IL-2 and IL-4 (Figure 9*c*). Gp130 has Phe-169, an analog of Tyr-103 in γ_c, in the center of the hydrophobic core of its binding site, that contributes the largest fraction of buried surface area and is critical for ligand engagement of all cytokines (71, 153–155) (Figure 9*c*). Another noteworthy observation is the rigidity of the binding surfaces on γ_c and gp130. A comparison between the unliganded (153) and liganded forms of gp130 shows almost no rotameric flexibility in the side chains of interacting residues. Although we do not have the structure of unliganded γ_c, the superimposition of γ_c structures onto the complex of IL-2 and IL-4 shows a similar rigidity of the side chains of interacting residues. Thus, conformational plasticity is most likely not used by either shared receptor as a means of cross-reactivity. More generally, the idea that conformational plasticity will be a mechanism to enable cross-reactivity in protein-ligand interactions has largely been supplanted by the observation that degeneracy can be provided simply through enthalpy-entropy compensation of rigid interacting surfaces (13, 156).

The binding epitopes on γ_c-dependent cytokines for γ_c are on helices A and D, whereas the binding epitopes for gp130 on the gp130 family cytokines are on helices A and C. The binding surfaces on IL-2 and IL-4 for γ_c are quite similar in the distribution of hydrophobic and hydrophilic residues (Figure 8*b*). Structurally nonidentical but positionally analogous residues on the respective γ_c cytokines form the grooves similar in topology and chemical nature for the docking of γ_c residue Tyr-103 and the loop formed by the Cys-160 to Cys-209 disulfide bond (Figure 8*b*). In contrast, the surfaces contributing to the binding to gp130 in HHV-8 IL-6, LIF, and IL-6 are very different in the distribution of hydrophilic and hydrophobic residues (Figure 8*b*). The gp130-binding surface on HHV-8 IL-6 has the largest hydrophobic area, and the binding surface on IL-6 is significantly more polar. The contact surface on LIF is the most polar, consistent with four well-defined water molecules that participate in an intermolecular hydrogen bond network observed in the crystal structure of LIF in complex with gp130 (13). The topology and chemical nature of the observed grooves in gp130 family cytokines for the docking of Phe-169 from gp130 vary greatly between different cytokines (Figure 8*c*). The groove on HHV-8 IL-6 represents one extreme with a deep pocket. The surface grooves on human IL-6 and LIF that accommodate Phe-169 are more similar in overall topology, but are not as deep as observed in HHV-8 IL-6. In human IL-6, the Phe-169 does not sit deeply in the pocket, but instead packs directly against the side chains from IL-6. The gp130-binding groove on LIF is the most polar of all three cytokines. The polar head groups of hydrophilic residues from LIF form the walls of the pocket and direct the aromatic ring of Phe-169 to pack against LIF in a similar fashion to the packing of Phe-169 against human IL-6.

Analysis of the binding surfaces on γ_c and gp130 indicates that they use chemically inert complementary surfaces to bind to different cytokines, as opposed to adjusting their main chain and/or side chain conformations to interact specifically with divergent cytokine residues. This observation contrasts with notions of receptor promiscuity through binding site flexibility (157). Because structural adaptation is not used by γ_c or gp130 as a means of cross-reactivity, the basis for degenerate recognition lies in the unique chemistry of the CHR epitope. Extensive thermodynamic studies of the binding between gp130 and different cytokines by ITC have revealed that their interactions are all primarily entropy driven, presumably because of desolvation of the interacting surfaces (LIF: 5 cal/molK; human IL-6: 45 cal/molK; OSM: 30 cal/molK; and CNTF: 62 cal/molK), albeit to varying extents commensurate with the surface polarity of the cytokine (10). We interpret these data to mean that gp130 uses desolvation as a structurally insensitive means of cross-reacting with structurally unique surfaces. The multiple solvent-exposed aromatic residues are likely covered with immobilized water clathrates in the unbound state, so that expulsion of the

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water into bulk solvent would be extremely entropically favorable. ITC analysis of γ_c interactions with the preformed IL-2/IL-2Rβ and IL-2/IL-2Rα/IL-2Rβ complexes shows that the entropic contribution is smaller than that of gp130 (IL-2/IL-2Rβ: 4.85 cal/molK; IL-2/IL-2Rα/IL-2Rβ: 0.72 cal/molK) (97). We still lack comparative data for the binding of γ_c with other γ_c-dependent cytokines, but we expect that γ_c uses similar thermodynamic solutions for the recognition of different cytokines. The entropy contribution may be smaller than that of gp130 because γ_c has a smaller contact area. Also, compared with gp130, the entropy contribution by the elbow region (site II) of γ_c to binding likely plays less of a role in cytokine recognition because of the extensive D2-D2 interactions between γ_c and the α receptors. The D2-D2 contacts are more polar in nature than are the cytokine-elbow region contacts and would presumably be more enthalpically driven, which would offset the entropy-driven recognition of the cytokine. Thus, as for gp130, γ_c uses enthalpy-entropy compensation to modulate its binding affinity for diverse surface chemistries.

CONCLUSIONS AND FUTURE PERSPECTIVES

Gp130, β_c, and γ_c—our principal shared cytokine receptors—appear to use a variety of both distinct and similar mechanisms to recognize diverse cytokines and ultimately to assemble into productive signaling complexes. The complexes exhibit the basic core template in which the CHR of the cytokine-specific α receptors and the shared receptors engage the sides of the four-helix bundle in the typical site I/site II manner seen in homodimeric receptor complexes. The presence of an additional site III interacting Ig domain distinguishes gp130 from γ_c, and the antiparallel β_c dimer demarks the most obvious signature of the β_c complexes. In gp130, the heterodimers between gp130 and α receptors are nonproductive because in most cases the α receptors do not contain intracellular signaling motifs. Thus, site III is necessary to dimerize gp130, each of which is bound to a JAK, and signal. This stands in contrast to the γ_c family, in which the α receptors do contain intracellular signaling domains, and so the γ_c/α receptor heterodimers are productive: γ_c does not need to be dimerized. Another major difference is the extensive receptor-receptor contact seen between γ_c and the α receptors. The relatively energetic role of this contact versus the cytokine/γ_c contact remains to be determined, but it may be that the receptor-receptor contact between the D2 domains of the CHRs is the primary driving force for heterodimerization and that the role of the cytokines is to tip the energetic balance toward heterodimer formation. Finally, the chemical basis of degeneracy is also nicely paralleled in both receptors. Gp130 and γ_c are structurally rigid and present relatively flat, hydrophobic binding sites for cytokine engagement that are rather devoid of highly charged and specific polar contacts. There are several important future questions that remain regarding the extracellular structures of these shared receptors. For gp130, several cytokines such as CNTF, OSM, and LIF heterodimerize gp130 with LIFR. Now that the basic template for dimerization of gp130 and LIFR has been elucidated by EM and structural studies, a high-resolution structure of the intact heterodimer remains an exciting puzzle to solve. In the γ_c family, with only two complex structures so far, there is some indication of the presence of a possible recognition code between cytokines and γ_c. This would be very exciting and different from gp130 cytokines, which do not appear to share any sequence or structural motifs necessary for gp130 engagement. The mechanism by which β_c cross-reacts with IL-3 and IL-5 awaits additional complex structures for comparison with GM-CSF.

However, the major structural frontier for cytokine receptors remains to obtain a better picture of the intracellular machinery. So far, we have no idea about the tertiary structure of any cytokine receptor intracellular domain. These regions may be unstructured unless bound to adaptors JAK and STAT. For STAT molecules, there are several structures now bound to DNA (158, 159) and also recently bound to phosphorylated peptides from cytokine receptors (160). However, we do not have any full-length JAK structures. The crystal structures of

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kinase domains of JAKs are a step forward (161, 162), but ultimately we need to know how the N- and C-terminal ends of JAK communicate, as well as how the cytokine receptors box1 and box2 bind to JAK proteins. To fulfill these goals, higher-order imaging techniques such as EM and tomography will likely be used to obtain snapshots of entire cytokine receptors in lipid environments to preserve the integrity of the transmembrane regions.

Acknowledgments

The authors acknowledge the contribution of Figure 4 from Michael Parker and Jack King-Scott prior to publication. We also acknowledge helpful discussions with Warren Leonard, Nicole Hanick, Mathias Rickert, Fernando Bazan, Rob Kastelein, and Marty Boulanger. This work was supported by NIH (RO1-AI51321), Keck Foundation, Sandler Foundation, Damon Runyon Cancer Research Foundation, and the Howard Hughes Medical Institute.

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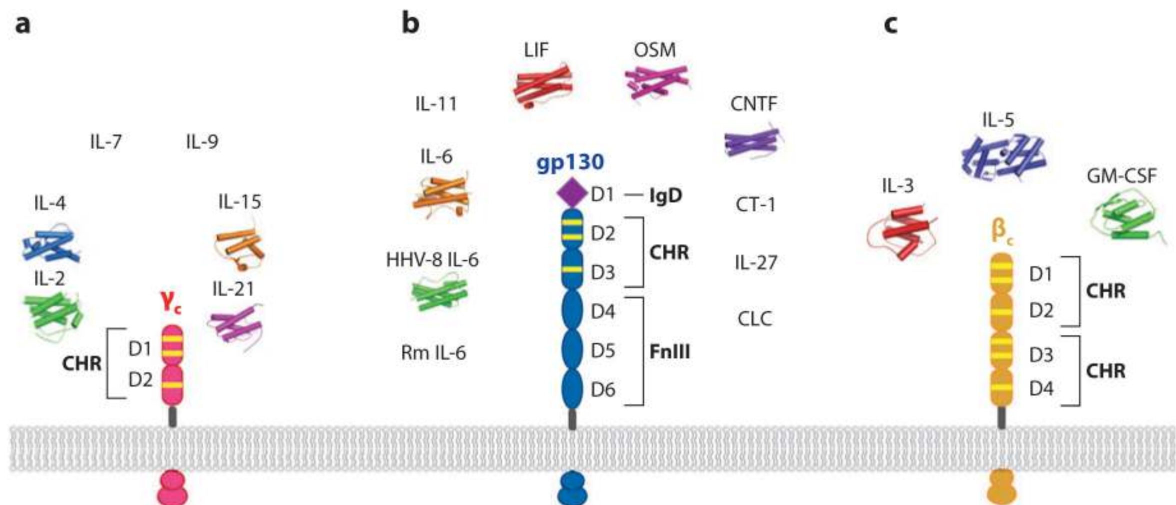

Figure 1.

Diversity of shared cytokine-receptor interactions. Shared cytokine receptors γ_c (*a*), gp130 (*b*), and β_c (*c*) are represented schematically on a cell membrane. The respective interacting cytokines with known three-dimensional structures are shown with cylinder representations of the four-helix bundles. (Abbreviations: LIF, leukemia inhibitory factor; OSM, oncostatin-M; CNTF, cillary neurotrophic factor; CLC, cardiotrophin-like cytokine; CHR, cytokine-binding homology region; HHV-8, human herpes virus; GM-CSF, granulocyte-macrophage colony-stimulating factor.)

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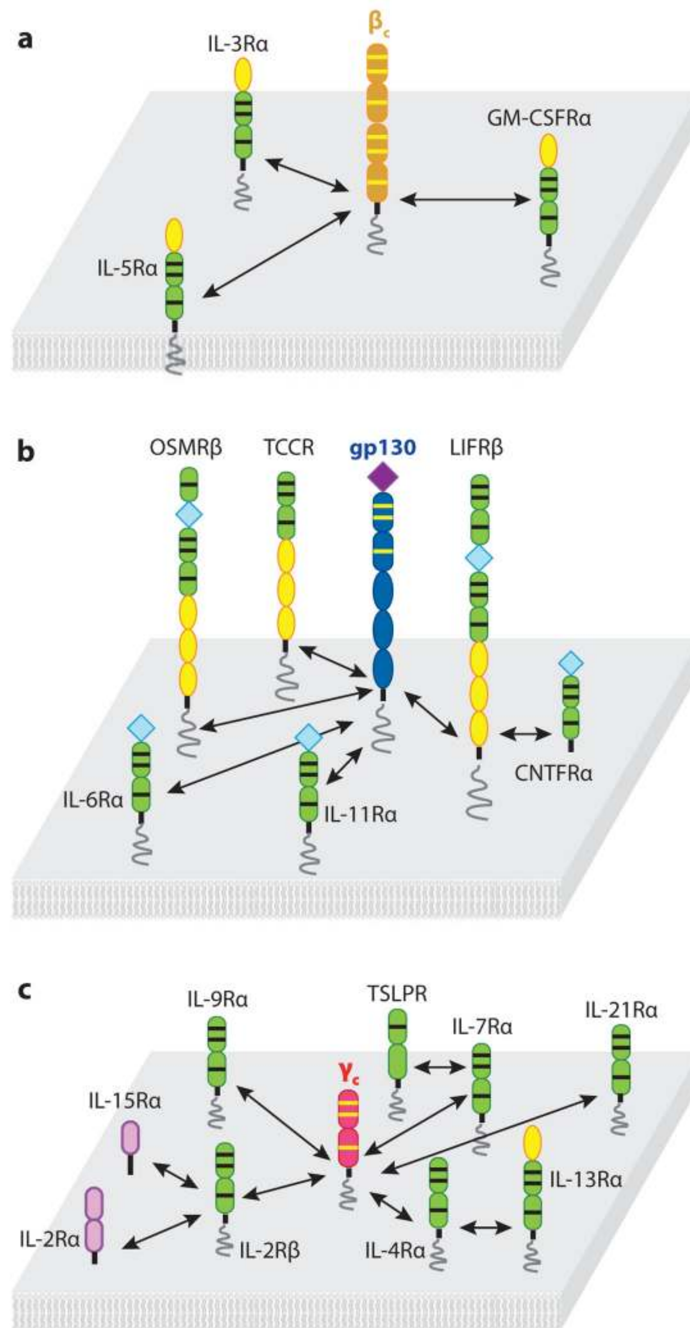

Figure 2.

Diversity of shared receptor-receptor interactions. Shared cytokine receptors β_c (*a*), gp130 (*b*), and γ_c (*c*) and their various receptor partners are depicted. These complexes are formed by the combination of ligand-specific α and/or β receptors with shared cytokine receptors. (Abbreviations: TCCR, T cell cytokine receptor; TSLPR, thymic stromal-derived lymphopoietin receptor.)

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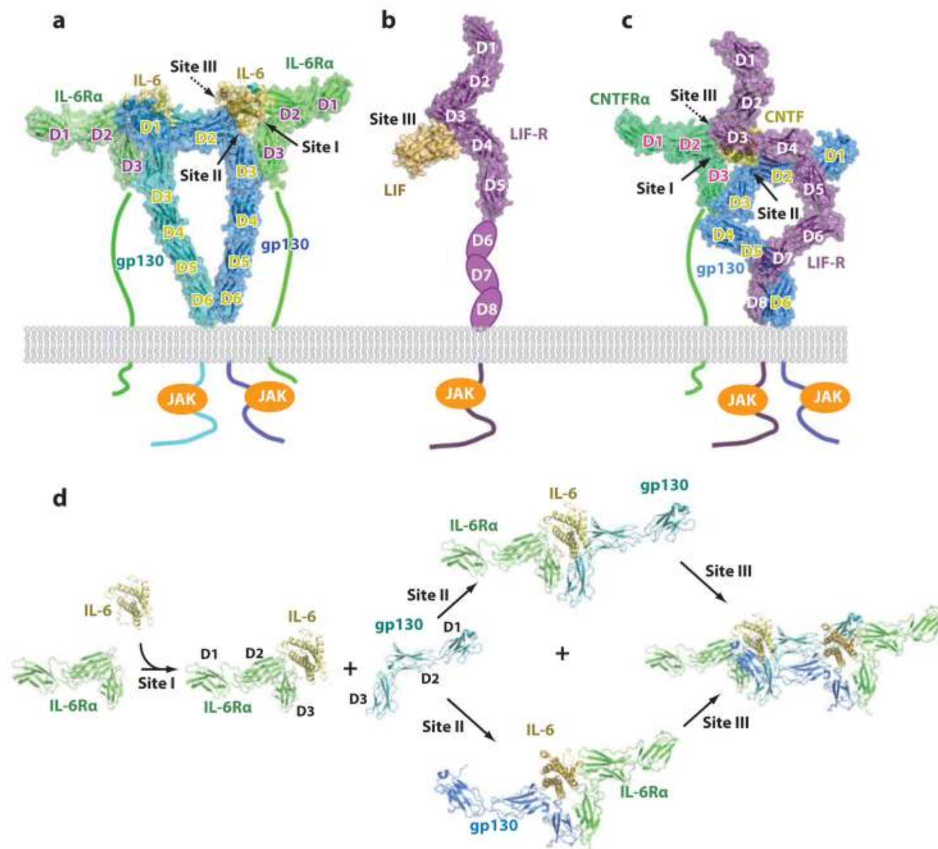

Figure 3.

Structures of human IL-6/IL-6Rα/gp130 hexameric complex (*a*), mouse LIFR in complex with human LIF (*b*), and human CNTF/CNTFRα/LIFR/gp130 (*c*) assembled from known crystallographic, biochemical, and electron microscopic data. In (*a*) the model shown was derived from the crystal structure of the IL-6 hexamer headpiece (12) together with the single-particle reconstruction of the entire extracellular complex (72). IL-6 signaling is mediated through homodimerization of gp130 in a symmetric hexameric arrangement with a nonsignaling IL-6Rα receptor. In (*b*) the model shown derives from the 4 Å crystal structure of the LIF/LIFR complex (14) missing the membrane-proximal domains that are depicted as cartoons. In (*c*), the quaternary LIFR/gp130/CNTF/CNTFRα complex is derived from a combination of the crystal structures of LIF/gp130 (13), CNTF (163), and a single-particle reconstruction of the entire quaternary complex (76). CNTF signals through the asymmetric heterodimerization of gp130 and LIFR and the nonsignaling CNTFα receptor. In panel (*d*), the assembly pathway for IL-6 signaling is depicted as elucidated from References 12, 164. IL-6 first engages IL-6Rα through a site I interaction to form a composite interface (site II) that recruits gp130. This trimeric structure can then engage a second trimer through two site III interfaces to form a productive signaling complex.

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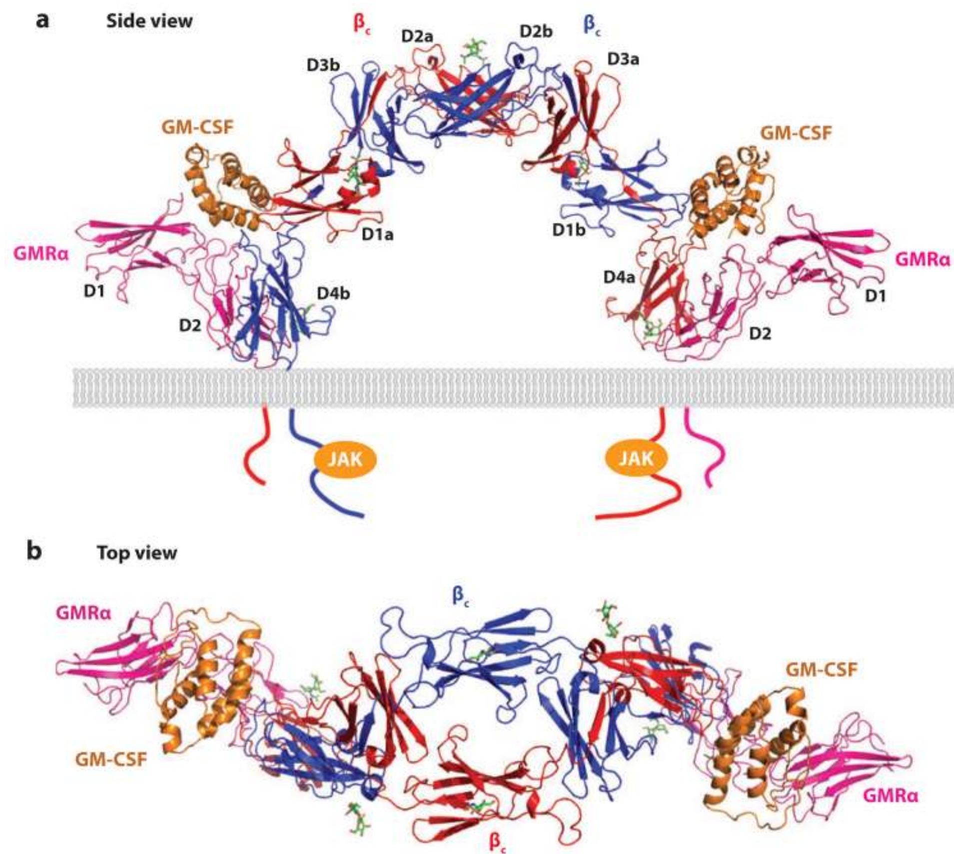

Figure 4.

The 2:2:2 GM-CSF/GMRα/β_c complex viewed from the side (*a*) and from the top (*b*) (18). GMRα engages the cytokine GM-CSF via a canonical site I interaction, whereas the β_c receptor engages site II on GM-CSF by using a composite cytokine-binding homology region (CHR) interface generated by domain 1 (D1) of one β_c subunit and domain 4 (D4) of the second β_c.

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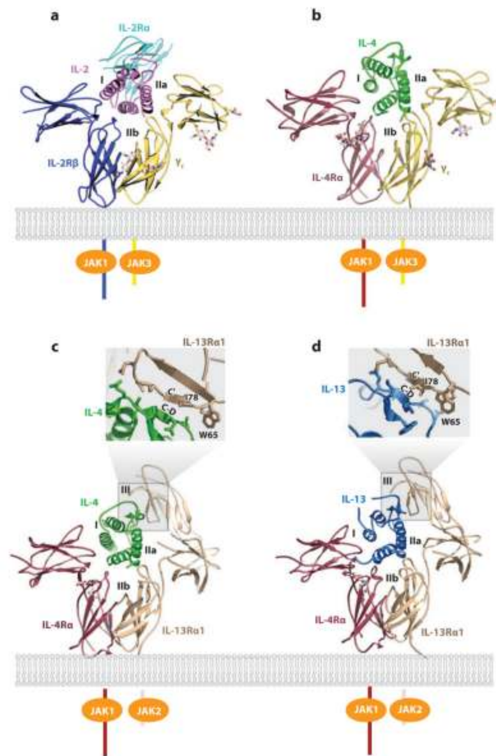

Figure 5.

Extracellular complex structures of IL-2/IL-2Rα/IL-2Rβ/γ_c (*a*) (15), IL-4/IL-4R α/γ_c (*b*), IL-4/IL-4Rα/IL-13Rα1 (*c*), and IL-13/IL-4Rα/IL-13Rα1 (*d*) (17) extracellular signaling complexes depicted on a cell membrane.

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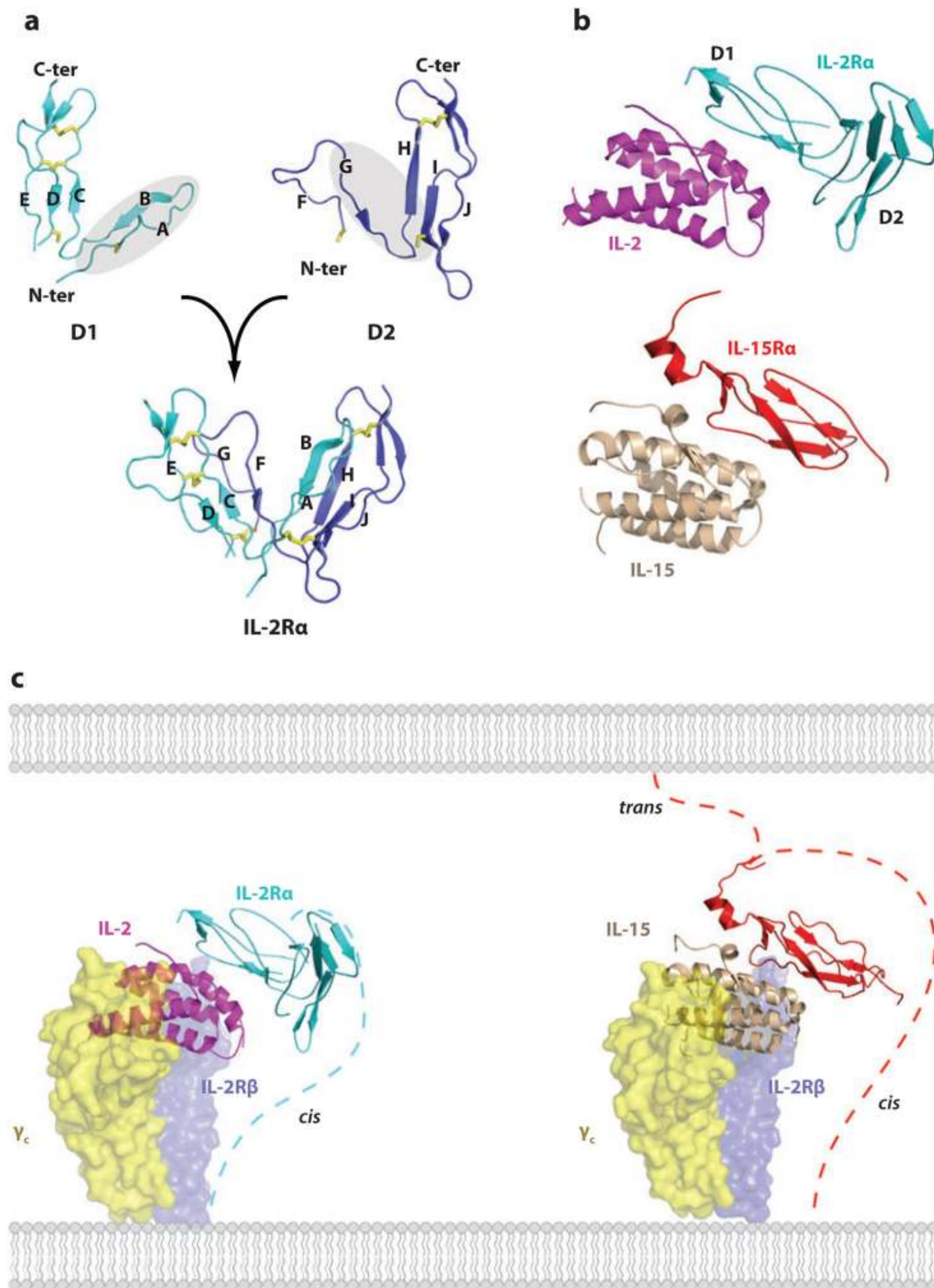

Figure 6.

IL-2/IL-2Rα and IL-15/IL-15Rα binary complexes. (*a*) IL-2Rα is composed of two sushi modules (D1 and D2) that swap opposing β-strands, forming a noncanonical sushi fold topology (53). (*b*) IL-2Rα and IL-15Rα contact the dorsal surface of IL-2 and IL-15, respectively, with respect to the membrane (130, 131). (*c*) IL-2 quaternary complex and modeled IL-15 quaternary complexes. IL-2Rα presents IL-2 in *cis*, whereas IL-15Rα presents IL-15 in *cis* or *trans*.

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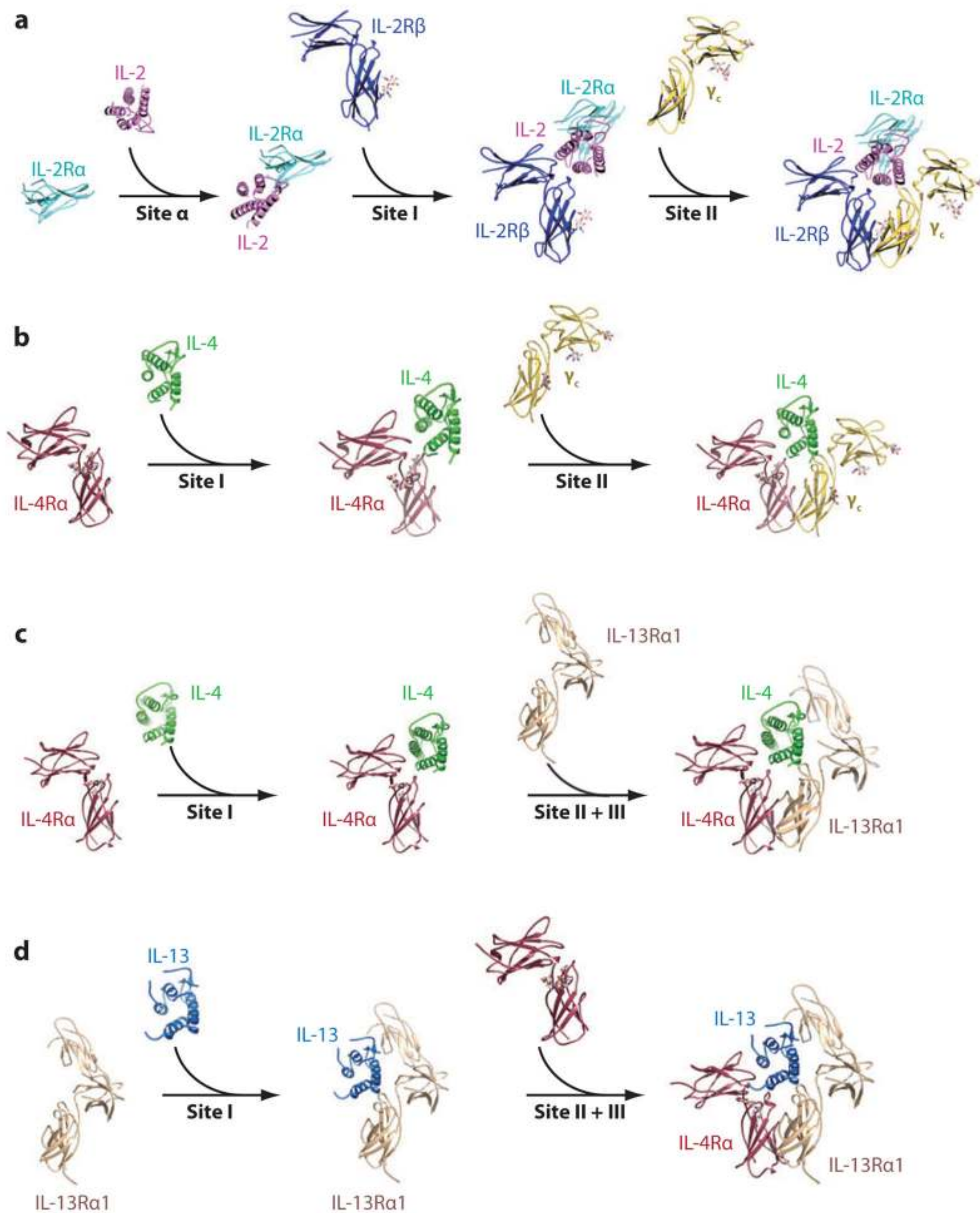

Figure 7.

The sequential assembly pathways of the IL-2/IL-2Rα/IL-2Rβ/γ_c quaternary (*a*), IL-4/IL-4Rα/γ_c (*b*), IL-4/IL-4Rα/IL-13Rα1 (*c*), and IL-13/IL-4Rα/IL-13Rα1 (*d*) ternary complexes. See Table 1 for the interaction affinity and thermodynamic parameters of each binding site.

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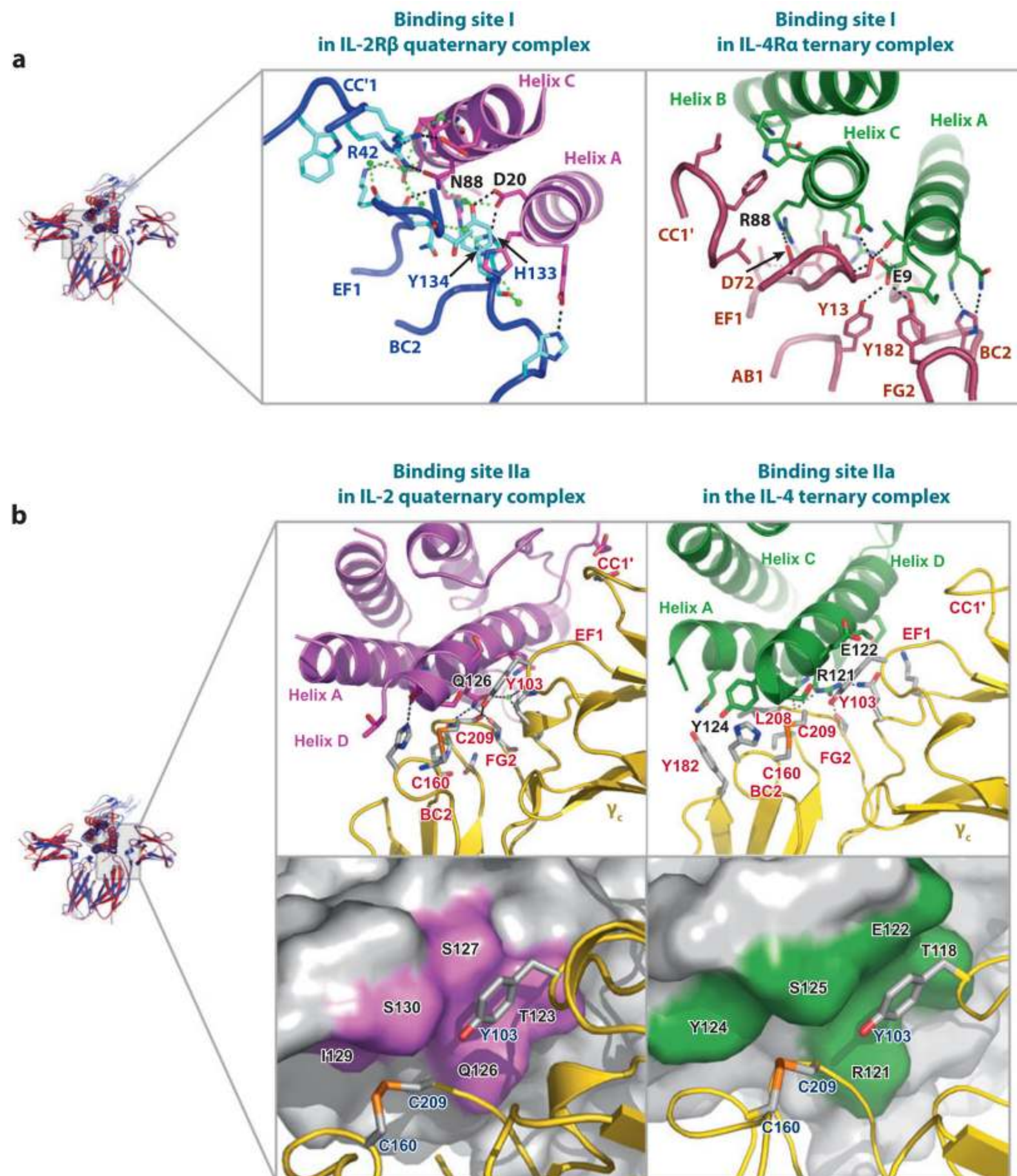

Figure 8.

Specific versus degenerate binding sites I and IIa in the IL-2/IL-2Rα/IL-2Rβ/γ_c and IL-4/IL-4Rα/γ_c complexes. (*a*) Left panel shows the binding site I between IL-2 helices A and C and IL-2Rβ loops in the elbow region. The corresponding binding site I in the IL-4 ternary complex is shown in right panel. (*b*) Binding site IIa in the IL-2 quaternary complex (γ_c/IL-2) and in the IL-4 ternary complex (γ_c/IL-4) are shown in left and right panels, respectively.

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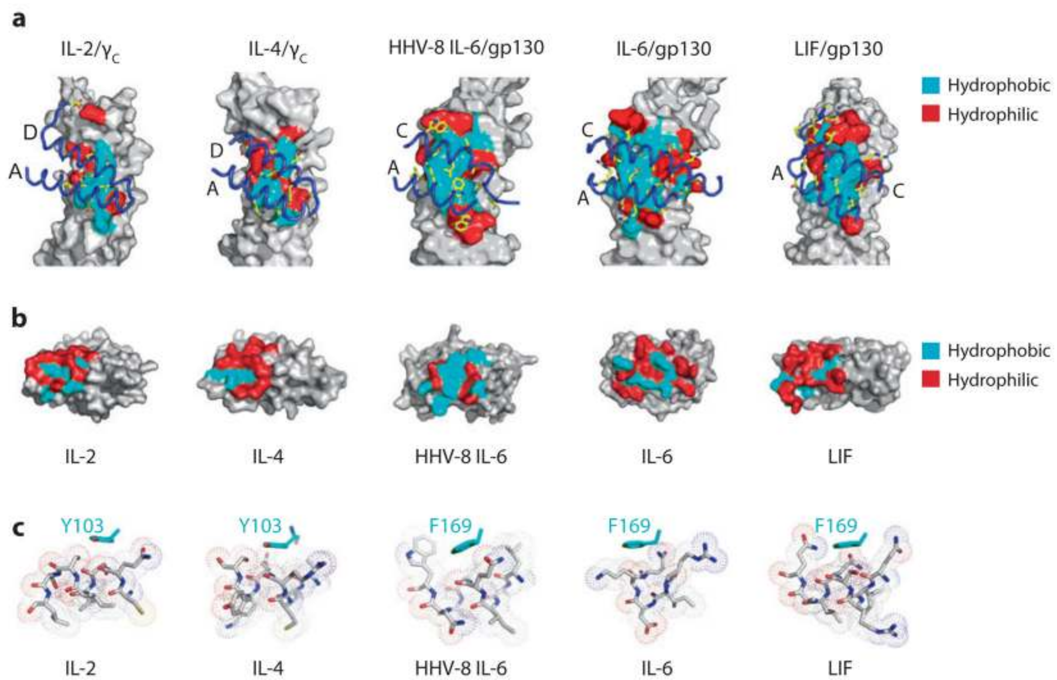

Figure 9.

Cross-reactivity of the cytokine-binding homology region (CHR) of γ_c and gp130. (*a*) Contact surface of γ_c and gp130 when bound to IL-2, IL-4, HHV-8 IL-6, IL-6, and LIF. Hydrophobic residues are colored as blue surface area and hydrophilic residues as red surface area. The helices and the contacting residues for each of the cytokines are shown docked onto the surface of the receptor. (*b*) Surface representations showing the contact surface of each cytokine. Note that IL-2 and IL-4 have a similar distribution of hydrophobic and hydrophilic residues in the contact area. For gp130 family cytokines, HHV-8 IL-6 has primarily hydrophobic contact surface area. The contact area on IL-6 is more polar, and LIF has the most significant hydrophilic contact surface. (*c*) The packing environment of the common binding epitope residues Tyr-103 (γ_c) and Phe-169 (gp130) against the cytokines.

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Table 1

Interaction affinity and thermodynamic parameters of binding sites in IL-2/IL-2Rα/IL-2Rβ/γ_c quaternary complex, IL-4/IL-4Rα/γ_c, IL-4/IL-4Rα/IL-13Rα1, and IL-4/IL-4Rα/IL-13Rα1 ternary complexes[a]

Complex	Binding site	K_d (nM)	ΔH (Kcal/mol)	ΔS (cal/molK)	ΔG (Kcal/mol)
IL-2/IL-2Rα/Rβ/γ	Site a	10	−5.2	18.4	−10.5
	Site I	63	−6.9	8.9	−9.5
	Site II	12	−10.3	0.72	−10.4
IL-4/IL-4Rα/γ_c	Site I	1	−11.2	3.5	−12.2
	Site II	559	−11.7	−10.5	−8.6
IL-4/IL-4Rα/IL-13Rα1	Site I	1	−11.2	3.5	−12.2
	Site II+III	487	−4.8	13.0	−8.7
IL-13/IL-4Rα/IL-13Rα1	Site I	30	−15.0	−15.6	−10.3
	Site II+III	20	−5.8	16.0	−10.6

[a]Data compiled from References 17 and 97.