Structure of the Quaternary Complex of Interleukin-2 with Its α, β, and γ_c Receptors

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Interleukin-2 (IL-2) is an immunoregulatory cytokine that acts through a quaternary receptor signaling complex containing alpha (IL-2Rα), beta (IL-2Rβ), and common gamma chain (γ_c) receptors. In the structure of the quaternary ectodomain complex as visualized at a resolution of 2.3 angstroms, the binding of IL-2Rα to IL-2 stabilizes a secondary binding site for presentation to IL-2Rβ. γ_c is then recruited to the composite surface formed by the IL-2/IL-2Rβ complex. Consistent with its role as a shared receptor for IL-4, IL-7, IL-9, IL-15, and IL-21, γ_c forms degenerate contacts with IL-2. The structure of γ_c provides a rationale for loss-of-function mutations found in patients with X-linked severe combined immunodeficiency diseases (X-SCID). This complex structure provides a framework for other γ_c-dependent cytokine-receptor interactions and for the engineering of improved IL-2 therapeutics.

The cytokine IL-2 is mainly produced by antigen-activated T cells and promotes the proliferation, differentiation, and survival of mature T and B cells as well as the cytolytic activity of natural killer (NK) cells in the innate immune defense (*1*, *2*). IL-2 is used therapeutically as an immune adjuvant in certain types of lymphoproliferative diseases and cancers, and IL-2 antagonists can prevent organ transplant rejection (*3*, *4*). However, severe dose-limiting toxicity has limited its effectiveness in the clinic. These deleterious side effects are mediated through different combinations of IL-2 receptors, which suggests that structure-based engineering of receptor-selective variants could have clinical benefit (*5*).

IL-2 exerts its pleiotropic activities through binding to different receptor complexes, depending on which of the components are expressed on the cell surface: the alpha chain (IL-2Rα), beta chain (IL-2Rβ), and common cytokine receptor gamma chain (γ_c) (*6*–*10*). Isolated IL-2Rα has been termed the "low-affinity" IL-2 receptor (binding affinity $K_d \approx$ 10 nM) and is not involved in signal transduction (*11*). A complex of IL-2Rβ and γ_c binds with intermediate affinity ($K_d \approx 1$ nM) and is the receptor form on NK cells, macrophages, and resting T cells (*2*), 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 (*12*). The association of IL-2Rβ and γ_c in the presence of IL-2 is necessary and sufficient for effective signal transduction through the heterodimerization of their cytoplasmic domains and subsequent kinase activation of multiple signaling pathways (*13*, *14*). A complex with three subunits—IL-2Rα, IL-2β, and γ_c—binds with high affinity ($K_d \approx$ 10 pM) and is the receptor form on activated T cells (*10*). The high-affinity receptor complex mediates most biological effects of IL-2 in vivo (*2*).

Whereas IL-2Rα is a specific receptor for IL-2, IL-2Rβ is also a component of the IL-15 receptor and γ_c is shared by cytokines IL-4, IL-7, IL-9, IL-15, and IL-21 (*15*). Mutations in γ_c can abolish the activity of all γ_c-dependent cytokines and result in X-linked severe combined immunodeficiency diseases (X-SCID), in which the T and NK cells are absent or profoundly reduced in number (*16*). Because the six γ_c-dependent cytokines have low sequence homology, structural information will be helpful to delineate shared versus ligand-specific binding determinants that could be exploited therapeutically. Previously, we reported the structure of the binary complex of IL-2 with IL-2Rα (*17*). We now present the crystal structure, at 2.3 Å resolution, of the quaternary complex of IL-2 with the extracellular domains of receptors IL-2Rα, IL-2Rβ, and γ_c.

Overall structure. Because of the heterogeneity of the fully glycosylated proteins expressed from insect cells, we crystallized a glycan-minimized quaternary complex, which had five potential Asn-linked glycosylation sites mutated (*18*). This material behaved identically to the fully glycosylated proteins and yielded crystals that diffracted to 2.3 Å resolution (*18*).

The quaternary complex is composed of one copy each of IL-2, IL-2Rα, IL-2Rβ, and γ_c (Fig. 1A). The orientation of IL-2Rα explains the necessity for the long connecting peptide, disordered in the structure, between the IL-2Rα globular head and the transmembrane segment. This allows the IL-2Rα binding domain to extend away from the cell surface and reach the dorsally located binding site on IL-2 (Fig. 1B). The bases of the receptors IL-2Rβ and γ_c, both class I–type cytokine receptors, converge to form a Y shape and IL-2 binds in the fork (Fig. 1, A and B). Formation of the quaternary complex is mediated by four binding sites—IL-2/IL-2Rα, IL-2/IL-2Rβ, IL-2/γ_c, and IL-2Rβ/γ_c—burying a total of 5700 Å^2 of surface area (fig. S1). The IL-2/IL-2Rα and IL-2/IL-2Rβ contacts are independent, whereas IL-2 and IL-2Rβ form a composite interface with γ_c, reflecting the cooperative nature of complex assembly.

IL-2Rα has been shown to deviate from typical cytokine receptor structure and mode of interaction with IL-2 (*17*). It is composed of two domain-swapped "sushi" modules, essentially miniature β-sheet sandwich domains. IL-2Rβ and γ_c are prototypical members of the class I cytokine receptor superfamily (*19*). Both are composed of N- and C-terminal fibronectin-III domains (D1 and D2, respectively), which are characterized by a β-sandwich sheet consisting of seven antiparallel strands arranged in a three-on-four topology. In IL-2Rβ, the D1 and D2 domains are connected by a helical linker and are bent at ~90°, whereas in γ_c the D1 and D2 domains are bent at ~120° (Fig. 1A). Both IL-2Rβ and γ_c contain the two disulfide bonds in the N-terminal domain (D1) and a "WSXWS" motif (*20*) in the C-terminal domain (D2) that are characteristic of class I cytokine receptors (*19*). However, a third disulfide bond in the γ_c D2 domain is unusual because of its central position in the interface with IL-2 and its role in enabling degenerate cytokine recognition (Cys^{160} to Cys^{209}) (discussed below).

IL-2/IL-2Rα. In the "low-affinity" complex, the atomic interactions between IL-2 and IL-2Rα, now visualized at 2.3 Å, are unchanged from the binary complex at 2.8 Å (*17*). The binding interface between IL-2 and IL-2Rα in the quaternary complex is composed of helices A′ and B′ and part of the AB loop in IL-2 and strands G, C, and D in the D1 domain and strand A in the D2 domain in IL-2Rα (table S2A). The two prominent hydrophobic ridges around residues Phe^{42} and Tyr^{45} of IL-2 insert into grooves between the IL-2Rα beta strands. Superposition of the two IL-2Rα structures in the binary and quaternary complexes shows a significant shift in the D2 domain of IL-2Rα (~2 Å), which is most likely a result of crystal packing and reflects some flexibility in the D1-D2 junction.

IL-15 is the only other cytokine that uses an atypical sushi-domain alpha receptor (IL-

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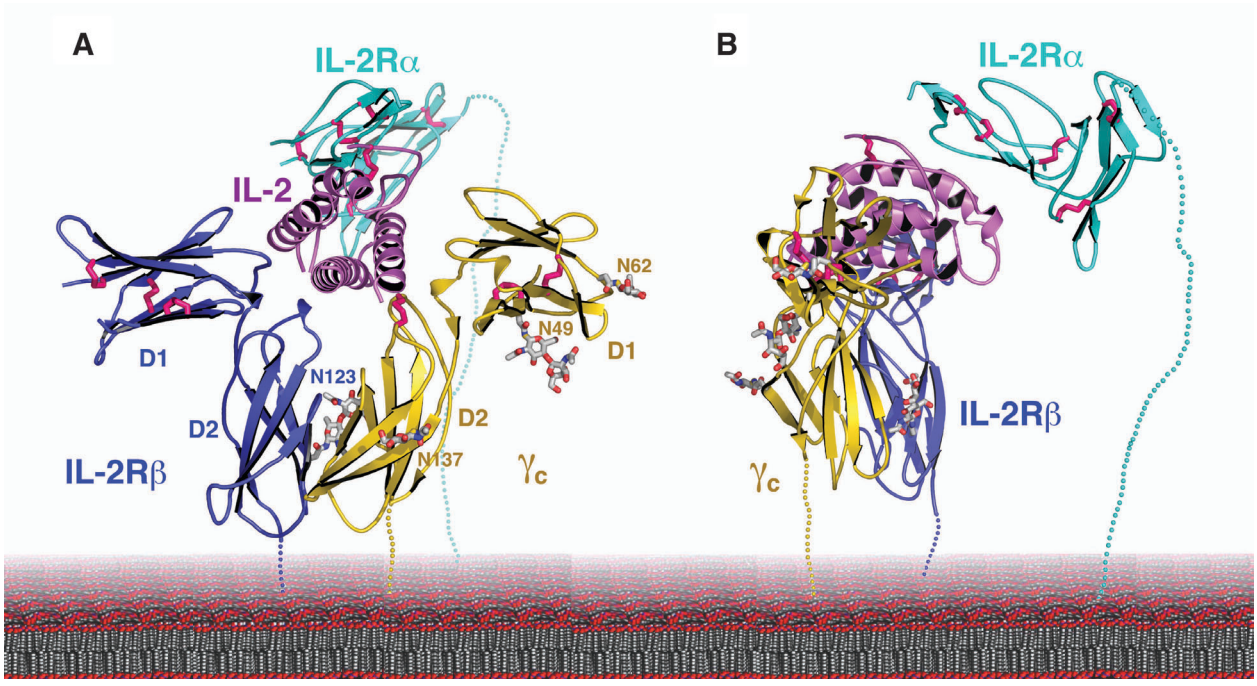

Fig. 1. Structure of the human IL-2/Rαβγ quaternary signaling complex. (**A** and **B**) Ribbon diagram of the complex structure shown in two views related by a ~90° rotation about the vertical axis. IL-2 is shown in violet, and the receptors are shown in cyan (IL-2Rα), blue (IL-2Rβ), and gold (γ_c). The observed N-linked carbohydrates at Asn^{123} of IL-2Rβ and at Asn^{49}, Asn^{62}, and Asn^{137} of γ_c are shown in gray (*20*). Disulfide bonds are shown in red. The disordered peptides connecting the C terminus of the receptors to the cell membrane are shown as dotted lines in their respective colors. The program PyMol (*43*) was used to make all figures.

15Rα), which is expressed primarily on NK cells (*21*). However, IL-15Rα is only a single sushi domain analogous to the IL-2Rα D1 domain (*22*, *23*). By analogy with IL-2, the IL-15/IL-15Rα complex likely forms first, followed by binding to IL-2Rβ and γ_c to form the quaternary signaling complex.

IL-2Rα does not appear to make any contact with either IL-2Rβ or γ_c. This is rather surprising, given that the IL-2/IL-2Rα complex binds with much higher affinity to IL-2Rβ ($K_d \approx 30$ pM) than does IL-2 binding to IL-2Rβ alone ($K_d \approx 100$ nM) (*12*, *24*) and the on-rate of IL-2 for IL-2Rβ is faster in the presence of IL-2Rα by a factor of 3 to 20 (*11*, *25*). We see no evidence of a composite receptor binding surface for IL-2, so what is the basis of the cooperativity? One possibility would be simple entropy reduction, wherein IL-2Rα captures and concentrates free IL-2 at the cell surface for presentation to IL-2Rβ and γ_c. Another possibility would be an IL-2Rα–induced conformational change in IL-2 that stabilizes the formation of the ternary complex.

To address the latter mechanism, we compared IL-2 structures in the quaternary complex, binary complex, and unbound states. The root mean square deviations for Cα atoms in the helical core between the different IL-2 molecules indicate nearly identical structures, ranging from 0.29 Å to 0.57 Å. One notable exception is at the beginning of helix C of IL-2, where, in the binary and quaternary complexes, several turns of helix C are slightly unwound and translated forward by ~1.0 Å toward IL-2Rβ (Fig. 2A). This local conformational adjustment moves IL-2 residue Asn^{88} into hydrogen-bonding distance to IL-2Rβ residue Arg^{42} (Fig. 2B). This movement possibly "primes" the next step in complex assembly by forming a more complementary IL-2Rβ binding site. Consistent with this, mutation of Asn^{88} in IL-2 ablates binding to IL-2Rβ (*5*). IL-2Rα may stabilize a favorable IL-2Rβ–binding conformation of IL-2 helix C, reducing a conformational entropy penalty that would be incurred during binding to IL-2Rβ. This priming of a "quiescent" IL-2Rβ binding site in IL-2 by IL-2Rα could effectively increase the on-rate for the IL-2 interaction with IL-2Rβ, as has been observed.

IL-2/IL-2Rβ. The interface between IL-2 and IL-2Rβ buries ~1350 Å^2 formed by residues from helices A and C in IL-2 and residues from loops CC′1, EF1, BC2, and FG2 in IL-2Rβ (table S2B). The interface is highly polar, with eight hydrogen bonds directly between IL-2 and IL-2Rβ residues. Strikingly, there are seven water molecules buried in the interface that bridge interactions between IL-2 and IL-2Rβ by forming bonds with protein atoms (Fig. 3A) (table S2B). 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} and Asn^{88}, are involved in hydrogen bonding networks to both water molecules and side chains on IL-2Rβ (Fig. 3A). The side chains of IL-2Rβ residues His^{133} and Tyr^{134} insert into a complementary cavity in IL-2 to form hydrogen and ionic bonds with Asp^{20} of IL-2 (Fig. 3B).

IL-2Rβ is also used by IL-15 to form a quaternary complex along with IL-15Rα and γ_c (*15*). IL-15 has limited sequence identity (19%) with IL-2, so its contact with IL-2Rβ is probably through a unique set of interactions. The bridging water molecules may contribute to the ability of IL-2Rβ to cross-react by accommodating the different IL-15 residues through remodeling of the intervening solvent layer.

IL-2/γ_c. Neither IL-2 nor IL-2Rβ alone have measurable affinities for γ_c (*12*). Therefore, two very weak interactions, IL-2/γ_c and IL-2Rβ/γ_c, combine to produce an intermediate-affinity IL-2/IL-2Rβ/γ_c complex. In the quaternary complex structure, the interaction surface of the IL-2/Rαβ complex with γ_c is composed of two interfaces: a small one between IL-2 and γ_c, and a larger one between IL-2Rβ and γ_c.

The IL-2/γ_c interface buries ~970 Å^2 of surface area and is the smallest of the four protein-protein interfaces in the complex. The γ_c binding surface is striking in its absence of extended side chain–specific interactions with

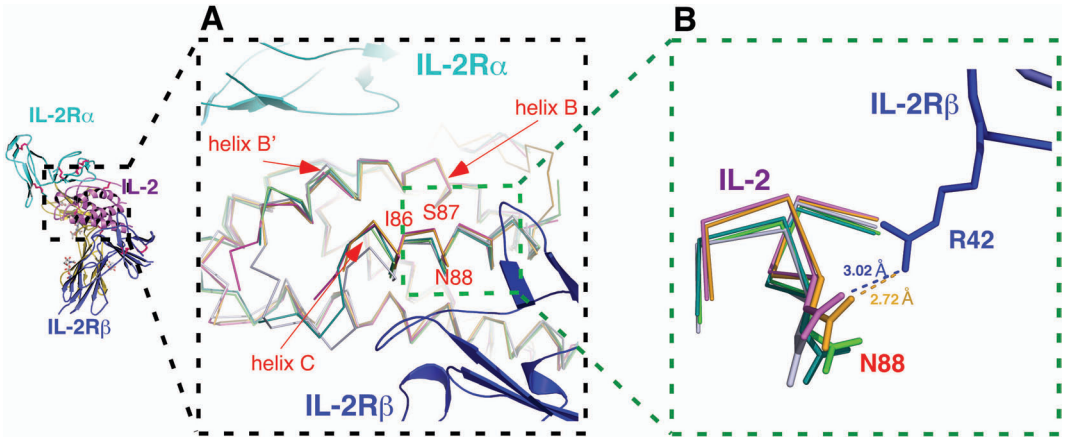

Fig. 2. IL-2Rα binding results in local conformational changes within IL-2 helix C. (**A**) Backbone superposition of IL-2 structures in quaternary complex (violet), binary complex (orange) (PDB 1Z92) (*17*), and three unbounded states: PDB 1M4C (green), 1M47 (dark green), and 3INK (gray) (*44*, *45*). (**B**) IL-2 residue Asn^{88} in helix C forms a hydrogen bond with Arg^{42} from IL-2Rβ in quaternary and binary complexes as a result of closer proximity induced by IL-2Rα binding.

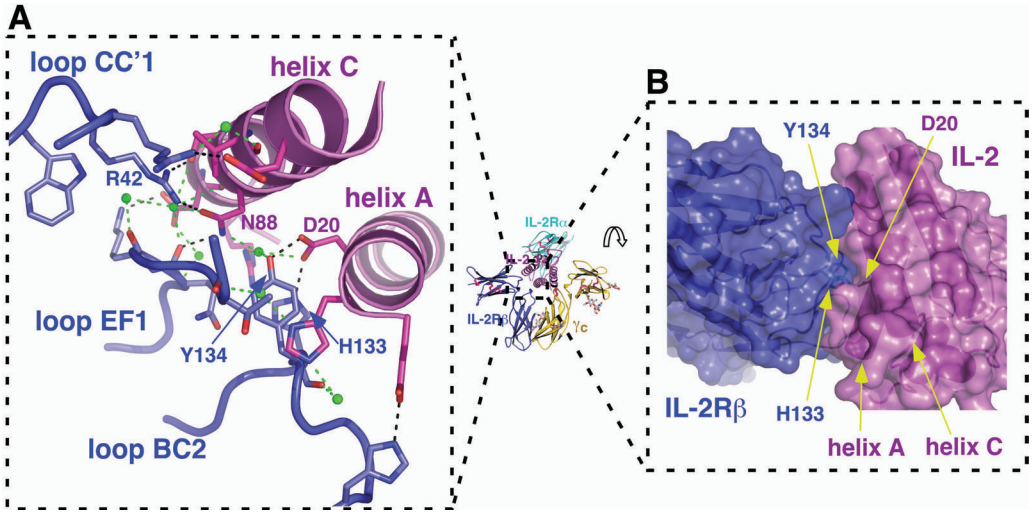

Fig. 3. A polar interface and hydration layer between IL-2 and IL-2Rβ. (**A**) All interactions between IL-2 and IL-2Rβ. The buried water molecules in the interface are shown as green spheres. The hydrogen bonds between IL-2 and IL-2Rβ are in black; those between water molecules and protein atoms are in green. (**B**) Close-up view of the shape complementarity in the interface, as viewed from above.

IL-2 and in the preponderance of main-chain contacts. Although there are several apparent ''hotspots,'' the γ_c binding surface is remarkable in its flatness and almost tangential contact with IL-2 (Fig. 4A). The γ_c structure contains an unusual disulfide bond in the heart of the interface with IL-2 that connects loops FG2 with BC2 and supports the conformations of Ser^{207} to Pro^{211} that form direct contacts with IL-2 (Fig. 4A). The disulfide also contributes to the apparent rigidity of the cytokine-binding surface, which is surprising given that one prevailing assumption for receptor cross-reactivity is structural plasticity (*26*). The γ_c binding surface does not appear to contain mobile structural elements, although we do not have a structure of the unliganded receptor for comparison.

The overall interface involves residues from helices A and D in IL-2 and residues from loops CC′1, EF1, BC2, FG2, and the linker between strands G1 and A2 in γ_c (table S2C). In contrast to the broad array of specific polar interactions between IL-2 and IL-2Rβ, small contact patches dominate the IL-2/γ_c interface. The first one is composed of residue Tyr^{103} from γ_c and residues Ser^{127} and Ser^{130} from IL-2. The Tyr^{103} aromatic ring packs flat against the side chains of Ser^{127} and Ser^{130} in IL-2 (Fig. 4, A and B). The second is around residue Gln^{126} in IL-2, which has been shown by mutagenesis to be a critical energetic hotspot. Similar to Tyr^{103}, the side chain of Gln^{126} is almost parallel to the surface formed by main-chain atoms of residues Pro^{207} to Ser^{211} in γ_c, and this orientation is further fixed by two hydrogen bonds with the receptor, to Pro^{207} O and Ser^{211} OG (Fig. 4A). A single bridged water molecule in the IL-2/γ_c interface forms hydrogen bonds with Gln^{126} of IL-2 and with Gln^{127} and Asn^{128} of γ_c, respectively (table S2C).

Previous mutagenesis studies have found that two of the flat γ_c patches we see in the structure that are involved in binding IL-2—residue Tyr^{103} and residues from Leu^{208} to Ser^{211} in γ_c—are also important for binding IL-4, IL-7, IL-15, and IL-21 (*27*–*29*). There is also evidence that γ_c binding sites for different cytokines overlap but are not identical (*30*). We propose that these two patches form the central degenerate recognition surfaces that participate in binding all cytokines in the γ_c-dependent family by using their flat surfaces, and that the peripheral polar interactions modulate specificity for individual cytokines.

IL-2Rβ/γ_c. The second part of the composite interface between IL-2/Rβ and γ_c is formed by extensive interactions between the D2 domains of IL-2Rβ and γ_c, burying more than 1750 Å^2 of surface area (Fig. 5A). The D2 domains from IL-2Rβ and γ_c are related by almost exact two-fold symmetry, and the interface is formed by 21 residues from IL-2Rβ and 19 residues from γ_c from strands C2, C′2, and E2 and loop C′E2 (table S2D). The interface is highly polar, with a peripheral ring of 17 hydrogen bonds surrounding a hydrophobic stripe in the center dominated by Trp^{166} from IL-2Rβ and Tyr^{167} from γ_c (Fig. 5B) (table S2D).

The D2-D2 interaction between IL-2Rβ and γ_c is the largest buried surface seen so far in cytokine-receptor complexes, and it underscores the role of receptor-receptor contact in the cooperative assembly of the quaternary complex. Although it is surprising that IL-2Rβ and γ_c have no measurable affinity toward one another given this extensive contact surface, a lack of interaction would prevent the receptors from heterodi-

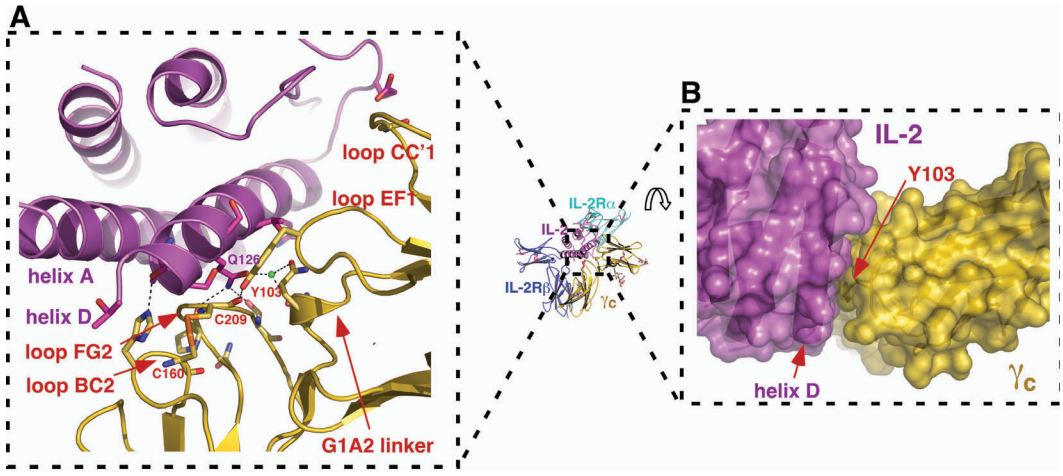

Fig. 4. Interactions between γ_c and IL-2. (**A**) Contacting residues in the IL-2/γ_c interface. (**B**) Surface representation of the relative contact patch around Tyr^{103} from γ_c, as viewed from above.

merizing and signaling in the absence of cytokine. Given the structural observations of a small IL-2/γ_c interface and a large and tightly packed IL-2Rβ/γ_c interface, we suggest that the receptor-receptor (i.e., D2-D2) contact may serve as an important energetic determinant. If so, the role of the cytokine would be to stabilize complex formation by guiding a perfect geometrical alignment of the numerous interatomic contacts (hydrogen bonds, van der Waals contacts, etc.) in the D2-D2 interface. In this respect, considering the relatively flat and chemically inert IL-2/γ_c interface, it may be that specificity is largely provided by receptor-receptor contact with IL-2Rβ rather than cytokine.

This model can in part be rationalized by the "shared" function of γ_c. γ_c is expressed on most immune cell types, but the tissue and cytokine specificity are regulated by the coordinate expression of different α receptors (or, in the case of IL-2, the β receptor). Given the lack of sequence homology between γ_c-dependent cytokines, the capacity of γ_c to discriminate among (and to cross-react with) these ligands would be more easily achieved by spreading the energetics of the interaction over the combined ~2600 $Å^2$ of surface area presented by the IL-2/IL-2Rβ composite surface, rather than focusing it all on the small portion of this surface contributed by cytokine alone (~970 $Å^2$). By comparison, in the structures of more ligand-specific cytokine receptors such as human growth hormone receptor (hGHbp) and erythropoietin receptor (epoR) complexed with their ligands, there is much less receptor-receptor contact (~900 $Å^2$ for hGHbp, no contact for epoR) (*31*, *32*).

X-SCID mutations. X-linked severe combined immunodeficiency (SCID) is a syndrome of profoundly impaired cellular and humoral immunity caused by mutations in the gene encoding the common gamma chain (*33*). The mutated gene results in faulty signaling through several cytokine receptors;

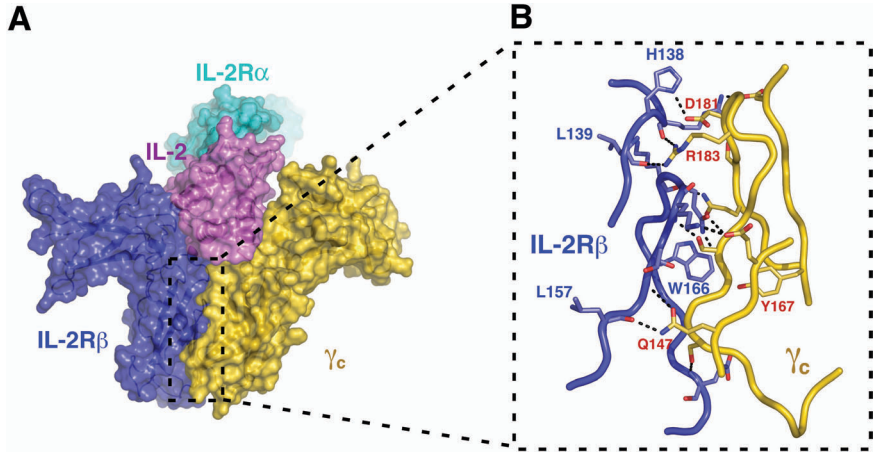

Fig. 5. Extensive receptor-receptor contact between IL-2Rβ and γ_c. (**A**) Surface representation of the quaternary complex shows the shape complementarity in the IL-2Rβ/γ_c interface. (**B**) Hydrogen-bonding interactions between IL-2Rβ and γ_c.

thus, T, B, and NK cells can be affected by a single mutation. We mapped extracellular γ_c mutations that have been found in X-SCID patients in which γ_c is expressed but is not competent for activation by any of the γ_c cytokines. Many mutations appear to concentrate near the γ_c cytokine-binding site, and several of these—Y103N, Y103C, L208P, C209R, C209Y, G210R, G210V, and C160R (*20*, *33*)—map to the γ_c binding interface with IL-2 (Fig. 6A). Mutation of Cys^{209} or Cys^{160}, which participate in the disulfide bond in the γ_c cytokine-binding surface, would be particularly destabilizing. The interface mutations would effectively ablate cytokine recognition by γ_c, but it seems likely that the receptor would still appear to be competent to signal if heterodimerized. Although the database X-SCID mutations map to all other parts of the γ_c structure, none of the X-SCID mutations map to the IL-2Rβ/γ_c interface, possibly implying a structural necessity for this area to be preserved in the expressed receptor (Fig. 6B).

Degenerate cytokine recognition by γ_c. The γ_c-dependent cytokines have, on average, 19% sequence identity to one another, with most of the homology concentrated inside the helical cores. Although we currently know the structures of only IL-2 and IL-4 in the γ_c-dependent cytokine family, we sought to identify conserved residues that might serve as a recognition code for γ_c binding throughout the family. Sequence alignment between IL-2 and other γ_c-dependent cytokines (fig. S3) indicates that residue Gln^{126}, which plays a key structural role in IL-2 interactions with γ_c, is conserved in IL-2, IL-9, IL-15, and IL-21, whereas IL-4 and IL-7 have Arg^{121} and Lys^{139} in this position, respectively. Superposition of the IL-4/IL-4Rα complex (*34*) with the IL-2 quaternary complex indicates that Arg^{121} may play a structural role similar to that of Gln^{126} in IL-2 in contacting γ_c. Although position 126 in helix D may serve as a common contact point with γ_c, there are not obvious constellations of conserved residues that allow us to dock the different cytokines with γ_c. It appears that each cytokine uses distinct structural solutions for γ_c recognition.

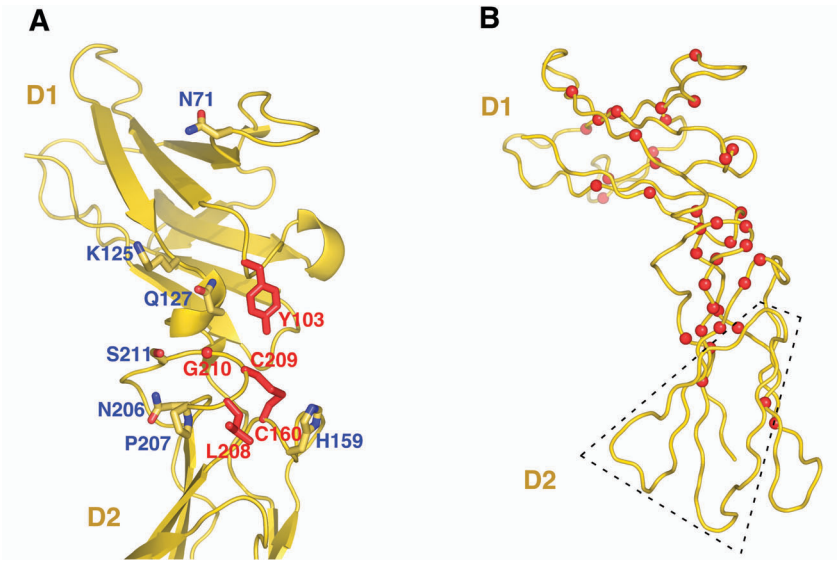

Fig. 6. Mapping known X-SCID mutations in the structure of γ_c. (**A**) Five missense mutations that are located in the γ_c cytokine-binding epitope, and make contact with IL-2 in the structure, are shown as red sticks. (**B**) Distribution of all missense mutations in the X-SCID mutation database (http://genome.nhgri.nih.gov/scid) in γ_c. The γ_c area participating in the D2-D2 interaction with IL-2Rβ is free of mutations and is indicated within the dashed line.

Cytokine recognition by shared receptors. The flat and apparently rigid surface in the common binding epitope of γ_c suggests that it uses somewhat chemically inert complementary surfaces to interact with divergent cytokine residues. Although this contrasts with notions of receptor promiscuity through binding site flexibility (*26*), it parallels structural results for gp130, the shared cytokine receptor for long-chain cytokines (*35*), in complex with three different cytokines: LIF, viral IL-6, and human IL-6 (*36*–*38*). In the gp130 system, thermodynamic compensation between rigid surfaces, rather than conformational change, enables cross-reactivity with a broad range of chemically diverse cytokine surfaces (*35*). We predict, on the basis of direct thermodynamic measurements of the quaternary complex assembly (*12*), that γ_c also uses such a mechanism for cross-reactivity. Such a large range of energetic compensation appears to be a property of binding sites found in shared receptors, which are tuned for degenerate recognition through a mechanism that bypasses the entropic penalty for conformational change (*39*).

Therapeutic implications. A recombinant human IL-2 (rIL-2) analog (Aldesleukin, Proleukin, Chiron Inc., Emeryville, CA) is currently licensed in the United States for the treatment of metastatic melanoma and renal cell carcinoma and is undergoing clinical trials for patients with HIV/AIDS (*40*, *41*). Treatment of cancer patients with rIL-2 results in robust responses but is associated with life-threatening toxicity, which limits its use (*40*). The antitumor efficacy of rIL-2 therapy has been shown to be mediated by the high-affinity quaternary complex containing IL-2Rαβγ expressed on T cells, whereas the toxic side effects are mediated through the IL-2Rβγ form of the receptor on NK cells (*42*). This hypothesis suggests that it might be possible to dissociate efficacy and toxicity by generating an IL-2 variant with selectivity for the IL-2Rαβγ receptor complex, versus the IL-2Rβγ complex of NK cells (*5*). Proof-of-concept was demonstrated with an IL-2 variant bearing an $Asn^{88} \rightarrow Arg$ mutation that conferred a factor of 3000 selectivity increase for the IL-2Rαβγ complex by crippling the interaction between IL-2 and IL-2Rβ (*5*).

In the structure we see that Asn^{88} is the side chain brought into hydrogen-bonding distance to IL-2Rβ by the structural perturbation of helix C in response to IL-2Rα binding, and is involved in an extensive hydrogen-bonding network (Fig. 3A). Such an energetically critical residue may not be the most desirable choice for generating a receptor-selective IL-2, because it may not be necessary to completely ablate binding to the IL-2Rβγ receptors. Rather, weakening the IL-2Rβ interaction, or even contact with γ_c, while maintaining near wild-type affinity for the IL-2Rαβγ complex appears tenable through structure-guided engineering. It is our hope that this quaternary complex structure can be used to design IL-2 variants that will allow its powerful clinical potential to be more fully realized.

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20. Single-letter abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
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Supporting Online Material

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References

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