Crystal structure of the IL-2 signaling complex: Paradigm for a heterotrimeric cytokine receptor

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IL-2 is a cytokine that functions as a growth factor and central regulator in the immune system and mediates its effects through ligand-induced hetero-trimerization of the receptor subunits IL-2R α , IL-2R β , and γ_c . Here, we describe the crystal structure of the trimeric assembly of the human IL-2 receptor ectodomains in complex with IL-2 at 3.0 Å resolution. The quaternary structure is consistent with a stepwise assembly from IL-2/IL-2R α to IL-2/IL-2R α /IL-2R β to IL-2/IL-2R α /IL-2R β / γ_c . The IL-2R α subunit forms the largest of the three IL-2/IL-2R interfaces, which, together with the high abundance of charge-charge interactions, correlates well with the rapid association rate and high-affinity interaction of IL-2R α with IL-2 at the cell surface. Surprisingly, IL-2R α makes no contacts with IL-2R β or γ_c , and only minor changes are observed in the IL-2 structure in response to receptor binding. These findings support the principal role of IL-2R α to deliver IL-2 to the signaling complex and act as regulator of signal transduction. Cooperativity in assembly of the final quaternary complex is easily explained by the extraordinarily extensive set of interfaces found within the fully assembled IL-2 signaling complex, which nearly span the entire length of the IL-2R β and γ_c subunits. Helix A of IL-2 wedges tightly between IL-2R β and γ_c to form a three-way junction that coalesces into a composite binding site for the final γ_c recruitment. The IL-2/ γ_c interface itself exhibits the smallest buried surface and the fewest hydrogen bonds in the complex, which is consistent with its promiscuous use in other cytokine receptor complexes.

common γ chain | cooperativity | IL-2 receptor | receptor assembly | structure-activity relationship

In 1976, lymphocyte-conditioned medium was found to support the long-term growth of T lymphocytes (T cells) (1), but the active component was not characterized as a variably glycosylated 15.5-kDa protein until 1981 (2). Subsequently, IL-2 was purified to homogeneity (3), and its cDNA was cloned in 1983 (4). The discovery of IL-2 permitted the creation of monoclonal T cells (5), which were instrumental in the characterization of many aspects of T cell biology. IL-2 is the primary cytokine responsible for the rapid expansion, differentiation, and survival of antigen-selected T cell clones during an immune response, but is also important for B cell, natural killer (NK) cell (6), and regulatory T cell (T_{reg}) (7) function.

IL-2 was the first cytokine found to mediate its effects via a cell surface binding site with all of the characteristics of classic hormone receptors, including high affinity, stereospecificity, and saturability (8). Kinetic binding studies revealed that the high affinity binding ($K_d \approx 10$ pM) to the IL-2 receptor (IL-2R) is due to a rapid rate of association ($k_{on} \approx 10^7$ M⁻¹s⁻¹), but relatively slow dissociation rate ($k_{off} \approx 10^{-4}$ s⁻¹) (9). The high-affinity IL-2R comprises three separate, noncovalently linked chains, termed α (IL-2R α , p55, CD25) (10), β (IL-2R β , p75, CD122) (11, 12), and γ (γ_c , IL-2R γ , p65, CD132) (13), the latter being a common receptor component for many cytokines, including IL-4, IL-7, IL-9, IL-15, and IL-2 (14). IL-2R α contributes the rapid association rate of IL-2 binding, whereas IL-2R β is responsible for its slow dissociation rate (9).

A critical number of IL-2Rs must be triggered before an individual T cell will make the irrevocable, quantal (all-or-none) decision to pass through the G₁ restriction point so as to undergo DNA replication and subsequent mitosis (15, 16). However, exactly how the cell senses this threshold of triggered IL-2Rs has remained obscure. A clue to this issue may then reside in the structure-activity relationship (SAR) of the IL-2/IL-2R interaction.

Here, we present the crystal structure of the fully assembled, human IL-2 receptor ectodomains in complex with its ligand at 3.0 Å resolution. The molecular architecture of this heterotrimeric receptor can now be analyzed to decipher the assembly, signaling, and disassembly mechanisms of this enigmatic cytokine receptor complex, and provide insights into the cooperativity of high-affinity binding, as well as the promiscuous use of γ_c in other cytokine receptor complexes.

Results

Assembly of the Quaternary IL-2 Signaling Complex. The high-affinity complex of IL-2 with its extracellular IL-2R α , IL-2R β , and γ_c receptor domains was assembled from the individual receptor ectodomains expressed in insect cells. Throughout purification of γ_c , this receptor subunit existed as a stable homotrimer, as determined by gel filtration. When incubated at 37°C in the presence of IL-2R α , IL-2R β , and IL-2, the γ_c trimer could dissociate and incorporate into the stable quaternary IL-2 signaling complex. However, upon chemical deglycosylation, γ_c aggregated and could not be dissociated for engagement in the high-affinity complex. Thus, self interaction through trimerization and surface carbohydrate sites on γ_c may serve an important solubility role, masking epitopes for IL-2 and IL-2R β recognition until formation of a productive signaling complex.

Structure Determination of the Quaternary IL-2 Signaling Complex.

The 3.0-Å crystal structure was determined by molecular replacement by using the coordinates from an independently assembled IL-2/IL-2R complex (17), in which mutations at Asn-17^BGln and Asn-45^BGln eliminated two glycosylation sites and which crystallized in a different space group (C2). A brief comparison of the IL-2/IL-2R complexes in the triclinic (here) and the monoclinic crystal form (17) is presented in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site. The x-ray structure was refined to R_{free} and R_{cryst} of 26% and 22%, respectively (Table 2, which is published as supporting information on the PNAS web site). Two independent complexes in the asymmetric unit offered a valu-

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Abbreviations: IL-2R, IL-2 receptor; Dn, domain n.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 2ERJ).

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Interface Between IL-2R β and IL-2. IL-2 forms the second largest interface with IL-2R β (1,150 Å², Table 1) with good shape complementarity (0.68) and high specificity via 10 hydrogen bonds (Table 3). Recognition of IL-2 by IL-2R β is dominated by the central, protruding Tyr-134 β , which contributes 17% of the buried surface area on IL-2R β (Fig. 3). For IL-2, Asp-20^{IL2} and His-16^{IL2} seem to be the most critical residues; Asp-20^{IL2} hydrogen bonds to His-133 β and Tyr-134 β , whereas His-16^{IL2} is tucked into a slot created by Tyr-134 β , Gln-188 β , and the methyl groups of Thr-74 β and Thr-73 β . Major van der Waals contacts in

Fig. 3. Interface of IL-2R β with IL-2 and γ_c . The interface of IL-2R β (cyan/red) and IL-2 (yellow/red) is intermediate in buried surface area among the three IL-2/IL-2R interfaces. Asp-20 and His-16 of IL-2 are located at the center of the binding region and are involved in many van der Waals contacts and hydrogen bonds. The interface features the highest number of hydrogen bonds, consistent with its high specificity and low k_{off} rate of IL-2 dissociation. The large contact area with γ_c is colored brown and includes two residues (red) that also interact with IL-2 (red residues).

the interface are also made with Arg-41^β, Val-75^β, His-133^β, Leu-19^{IL2}, Asp-84^{IL2}, Asn-88^{IL2}, and Val-91^{IL2}.

Interface Between γ_c and IL-2. The γ_c /IL-2 interaction (970 Å²) is the smallest of the three IL-2/IL-2R interfaces, although with good surface complementarity (0.72). Furthermore, this contact surface features only two hydrogen bonds (Table 3) and weak electrostatic interactions (Fig. 4), indicating low specificity. The contribution of main-chain atoms to the buried surface area is intermediate (17% on average) between those of IL-2R α (21%) and IL-2R β (12%) (Table 1). Tyr-103 γ , His-159 γ , and Leu-208 γ together with the disulfide (Cys-160 γ -Cys-209 γ) contribute 53% of the buried surface on γ_c and represent the major binding determinant. The importance of the disulfide bridge was previously appreciated from mutagenesis data (25, 26), but its direct involvement in ligand binding was unexpected. With a buried surface area of 72 Å² and one hydrogen bond, Gln-126^{IL2} is the most critical IL-2 residue that contacts γ_c in accordance with biochemical data (27). Glu-15^{IL2}, Thr-123^{IL2}, and Ile-129^{IL2} contribute other significant interactions to the IL-2/ γ_c interface.

Interface Between IL-2R β and γ_c . In stark contrast to the relatively small IL-2/ γ_c interface, the IL-2R structure reveals a surprisingly large and specific interaction between the D2 receptor domains of IL-2R β and γ_c (1,640 Å²). The predominantly polar contacts are evenly distributed among the 22 residues of IL-2R β and 31 residues of γ_c without any obvious hot-spot residues. The low percentage of aromatic residues among the side-chain contributions (12%), in combination with 14 hydrogen bonds, seems to enforce much higher specificity of γ_c with IL-2R β than of γ_c to IL-2. Glu-136 β and

Fig. 4. Interface of γ_c with IL-2 and IL-2R β . This γ_c interface (green/red) with IL-2 (yellow/red) is characterized by the smallest total buried surface area and formation of only two hydrogen bonds. Strikingly, the elbow region of γ_c engages only partially with IL-2. Furthermore, γ_c exhibits a large elbow angle, which is atypical of class-I cytokine receptors. These structural properties are likely to contribute to promiscuity of this receptor subunit. The large contact area with IL-2R β is colored brown and includes three residues (red) which also interact with IL-2 (red residues).

His-138^β form the hinge of the D1/D2 IL-2R^β elbow and significantly interact with both IL-2 and γ_c . Similarly, Tyr-182^γ, Pro-207^γ, and Leu-208^γ form the hinge of the D1/D2 γ_c elbow and interact with both IL-2 and IL-2R^β.

Interface Between IL-2R α and γ_c : Crystal Artifact or Physiologically Relevant. A crystal contact of considerable size and specificity is formed between γ_c and a symmetry-related IL-2R α molecule in the neighboring unit cell. Remarkably, this interaction is observed in both copies of the asymmetric unit, as well as in another crystal form (17). IL-2R α D2 strands H, I, and J interact with the surface of the γ_c elbow region that is not involved in IL-2 binding. Structural features include high shape complementarity (0.68 and 0.78, respectively), a large buried surface area (1,150 Å²) and nine interdomain hydrogen bonds. In this arrangement, the D2 domains of IL-2R β / γ_c are parallel to each other, with their C termini toward the membrane (Fig. 7, which is published as supporting information on the PNAS web site). Such lateral interactions would lead to one-dimensional lattices of IL-2R complexes on the cell surface that could promote higher-order signaling assemblies, although no measurable affinity between soluble IL-2R α and γ_c has been detected (28). However, this same set of experiments also did not detect any binding between IL-2R β and γ_c , which in fact form an extensive interface in the fully assembled IL-2R.

Discussion

Despite only 10–20% sequence identity to other cytokine receptors, the IL-2R β and γ_c ectodomains of the IL-2 receptor

have long been recognized to consist of two tandem fibronectin type-III domains that contain sequence signature motifs as in other cytokine receptors (13, 29). As expected, a DALI search (30) identified other cytokine receptors, such as IL-4R (Z-score of 17.3, rms deviation (rmsd) of 2.4 Å over 176 residues) and erythropoietin receptor (16.5, 3.4 Å over 182 residues) as the closest structural homologs of IL-2R β and γ_c . However, the crystal structure of the complete IL-2/IL-2R complex now enables analysis of the organization of the quaternary assembly and the nature and extent of each of the IL-2/IL-2R interfaces and receptor subunit interactions, as well as the role of the individual receptor subunits in the high-affinity complex assembly and signal transduction.

The precise role of IL-2R α has so far remained elusive. This receptor subunit, together with IL-2R β , can form a pseudo-high-affinity site for IL-2 on the cell surface ($K_d \approx 300$ pM), yet IL-2R β itself binds IL-2 only with modest affinity ($K_d \approx 450$ nM) (31). In contrast, a soluble form of IL-2R β comprising solely its ectodomain exhibits comparable IL-2 affinity ($K_d \approx 144$ nM) to its membrane-bound form, but only ≈ 2 -fold increased affinity ($K_d \approx 64$ nM) upon addition of soluble IL-2R α (28). Minor changes observed in the IL-2 structure in response to IL-2R α binding are probably sufficient to explain the 2-fold increase in IL-2R β affinity for IL-2 in solution. However, because IL-2R α does not make any interactions with IL-2R β , the massive difference in the K_d of IL-2 with membrane-anchored IL-2R β in the presence or absence of IL-2R α suggests that this subunit primarily serves as a ligand carrier. For potent cell signaling, secreted IL-2 must be captured at the cell surface to minimize its loss by diffusion away from the cell. The high affinity of IL-2R α for IL-2 and the large excess of IL-2R α over IL-2R β and IL-2R γ on activated T cells facilitate this capture and delivery to IL-2R β or to any IL-2R β / γ_c complex through two-dimensional cell surface diffusion. Computational modeling suggests that a stepwise assembly mechanism leads to a greater number of high-affinity signaling complexes than signaling via a preformed, fully assembled IL-2R, considering the thermodynamics, kinetics, receptor density, and cell density of the IL-2/T cell system (32). Alternatively, cooperativity in formation of the ternary IL-2/IL-2R α /IL-2R β complex may result from a preformed IL-2R α /IL-2R β complex on the cell surface, which would then undergo large-scale conformational rearrangements upon assembly to higher-order IL-2 signaling complexes. Isothermal titration calorimetry (ITC) experiments (28) have, in fact, measured significant affinity for this heterodimer ($K_d \approx 280$ nM), but the structure of the quaternary IL-2 complex is difficult to reconcile with this finding.

The preponderance of charge-charge interactions at the IL-2/IL-2R α interface may serve to dominate the kinetics of IL-2 recruitment by its receptor. Long-range electrostatic interactions are known to accelerate the association rate k_{on} , whereas short range interactions govern the dissociation rate k_{off} (33). The rapid rate of IL-2 association with IL-2R α (9) is then consistent with strong electrostatic interactions and high electrostatic complementarity, whereas the slow dissociation rate ascribed to IL-2R β (9) correlates well with the properties of the IL-2/IL-2R β interface (Table 1).

Thus, the IL-2R α /IL-2 complex is likely to be preferentially delivered to IL-2R β , forming the pseudo-high-affinity complex, in agreement with isothermal titration calorimetry experiments (28). This complex then recruits γ_c , possibly from γ_c trimers, burying a large surface area between the receptor subunits (Table 1) and forming a tight three-way junction comprising IL-2R β , γ_c , and IL-2. Consistent with the lowest K_d value measured for a single step in IL-2R assembly (28), association of γ_c with IL-2/IL-2 β constitutes the ultimate thermodynamic driving force toward the assembly of the IL-2 signaling complex. On activated T cells, IL-2/IL-2 β would be part of the ternary IL-2/IL-2R α /IL-2 β complex whereas, on

Fig. 5. Three-way junction between IL-2, IL-2R β , and γ_c . IL-2 (yellow ribbon representation), IL-2R β , and γ_c (the surfaces are colored as in Figs. 3 and 4) form a three-way junction at the heart of the high-affinity IL-2 signaling complex. The network of residues that mediate these contacts (colored red) provides a compelling structural basis for cooperativity in the IL-2/IL-2R complex assembly.

certain natural killer cells and monocytes, IL-2/IL-2 β would not be associated with IL-2R α due to the lack of this receptor subunit on these cells (9). Intriguingly, the buried surface areas of IL-2R β and of γ_c that are involved in receptor/receptor and receptor/IL-2 contacts nearly span the entire length of IL-2R β and of γ_c . Because the buried surface areas of IL-2R β and of γ_c that are involved in receptor/receptor and receptor/IL-2 contacts overlap somewhat (Figs. 3 and 4), we propose that IL-2 binding to IL-2R β may induce conformational changes in the contiguous γ_c contact area of IL-2R β , which primes the IL-2/IL-2R α /IL-2R β and the IL-2/IL-2R β complex, respectively, for γ_c recruitment. The IL-2-dependent IL-2R β / γ_c association is further enhanced by three γ_c residues (Tyr-182 γ , Pro-207 γ , and L208 γ), which interface with both IL-2 and IL-2R β , two IL-2R β residues (Glu-136 β and His-138 β) which are correspondingly buried in IL-2/ γ_c interface, and by three IL-2 residues (Leu-112, Glu-15^{IL2}, and Leu-19^{IL2}), which interface with both receptor subunits (Fig. 5). This cooperative mechanism could then account for the nondetectable affinities (<50 μ M) of IL-2R β to γ_c in the absence of IL-2, and IL-2 to γ_c (28), and would also provide an essential safety mechanism against premature signaling via the IL-2R β / γ_c dimer in the absence of ligand. Collectively, the three-way junction between IL-2, IL-2R β , and γ_c provides a compelling structural basis for cooperativity in IL-2/IL-2R complex assembly initiated by, and therefore dependent on, IL-2 or IL-2/IL-2R α .

The structural features of the IL-2/ γ_c interface provide a plausible mechanism of how the common γ chain γ_c is capable of binding to six different cytokines (34). First, the IL-2/ γ_c interface is characterized by the smallest buried surface area, and only a portion of the D1/D2 junction of γ_c actually contacts IL-2 (Fig. 4). Second, the relatively large elbow angle between D1 and D2 of γ_c with respect to other cytokine class I receptors increases the prospective ligand-binding surface in the interdomain region. Most strikingly, little specificity is conferred upon the IL-2/ γ_c interface due to the formation of only 2 hydrogen bonds, as opposed to 7 and 10 in the other two IL-2/IL-2R interfaces. Our structural results also agree well with extensive mutagenesis work on defining the epitopes on γ_c for various cytokines (25, 26, 35). Tyr-103 γ , Cys-160 γ , and Cys-209 γ , identified as hot-spot residues in all of the cytokine/ γ_c interfaces, form a hydrophobic patch in the center of the IL-2/ γ_c interface. Because certain residues located around this hot spot were