Structural Basis for the Blockage of IL-2 Signaling by Therapeutic Antibody Basiliximab

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Structural Basis for the Blockage of IL-2 Signaling by Therapeutic Antibody Basiliximab

Jiamu Du,* Hui Yang,*,† Dapeng Zhang,‡ Jianchuan Wang,*† Huaizu Guo,‡ Baozhen Peng,* Yajun Guo,‡ and Jianping Ding*

IL-2 signaling plays a central role in the initiation and activation of immune responses. Correspondingly, blockage of this pathway leads to inhibition of the immune system and would provide some therapeutic benefits. Basiliximab (Simulect), a therapeutic mAb drug with specificity against IL-2Rα of T cells, was approved by U.S. Food and Drug Administration in 1998. It has been proven to be effective in the suppression of the IL-2 pathway and hence has been widely used to prevent allograft rejection in organ transplantation, especially in kidney transplants. In this study, we report the crystal structure of the basiliximab Fab in complex with the ectodomain of IL-2Rα at 2.9 Å resolution. In the complex structure, the Fab interacts with IL-2Rα with extensive hydrophobic and hydrophilic interactions, accounting for a high binding affinity of 0.14 nM. The Ag binding site of basiliximab consists of all six CDR loops that form a large binding interface with a central shallow hydrophobic groove surrounded by four hydrophilic patches. The discontinuous epitope is composed of several segments from the D1 domain and a minor segment from the D2 domain that overlap with most of the regions responsible for the interactions with IL-2. Thus, basiliximab binding can completely block the interactions of IL-2 with IL-2Rα and hence inhibit the activation of the IL-2 signal pathway. The structural results also provide important implications for the development of improved and new IL-2Rα-targeted mAb drugs. The Journal of Immunology, 2010, 184: 000–000.

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The coordinates and structure factors presented in this article have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank under accession number 3IU3.

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Abbreviations used in this paper: γc, common γ chain; PDB, Protein Data Bank.

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The Journal of Immunology

Interleukin-2 is the first cytokine to be identified, characterized, purified, and cloned (1–5). It plays a pivotal role in immune responses against pathogenic infection (6–8). During the defense against pathogens, recognition and binding of the foreign Ags by the TCRs stimulate both the secretion of IL-2 and changes the conformation of IL-2 to a favorable receptor, IL-2Rβ (p75 or CD122) is shared with IL-15 (15–18), and the γc (p55 or CD132) is a common receptor shared by many cytokines including IL-2, IL-4, IL-7, IL-9, and IL-15 (19, 20). IL-2Rα and IL-2Rβ contribute to the rapid association and slow dissociation of IL-2, respectively (16), whereas receptors β and γ mediate the transmembrane signal transduction (21, 22). Structural studies have shown that IL-2 has a four-helix bundle structure (23), IL-2Rα is composed of two β-strand–swapped “sushi-like” domains, and both IL-2Rβ and γc are composed of two fibronectin type III domains (24–26). In the structures of the IL-2/IL-2Rαγc quaternary complexes, IL-2Rα binds to a surface groove of IL-2 and makes extensive interactions with IL-2 via mainly the D1 domain and a few residues of the D2 domain, and IL-2Rβ and γc bind to IL-2 on the opposite side of IL-2Rα and have minor interactions with each other but no contact with IL-2Rα (24–26). The γc receptor alone has no detectable affinity to IL-2, and binding of γc to IL-2 needs the presence of IL-2Rβ (27). It is inferred that binding of IL-2Rα to IL-2 stabilizes the binding site for IL-2Rβ and that IL-2 and IL-2Rβ together form a composite binding site for γc. The structural, biochemical, and computational data together suggest a sequential binding scenario of IL-2 by its receptors: first IL-2Rα, which is abundantly expressed on the T cell surface, captures and enriches the secreted IL-2 and changes the conformation of IL-2 to a favorable IL-2Rβ binding state, then the formed IL-2/IL-2Rα complex approaches IL-2Rβ through two-dimensional cell surface diffusion to form the IL-2/IL-2Rβγc complex, and finally γc is recruited to form the biologically active IL-2/IL-2Rβγc complex to transduce the signaling cascade (25–28).

Because of the central role of the IL-2 signaling in the activation of immune defense, blocking of this signal pathway could suppress the immune system (7). The activation of T cells through the IL-2 signaling is initiated by the binding of IL-2Rα to IL-2. IL-2Rα is a specific receptor for IL-2, whereas IL-2Rβ and γc are shared by other cytokines. Intriguingly, IL-2α is not expressed on resting T and B cells but abundantly expressed on activated T cells, especially by
the T cells participating in some pathological conditions such as organ allograft rejection (29, 30), some autoimmune diseases (31, 32), and T cell leukemia (31, 33). The critical role of IL-2Rs in the IL-2 signal pathway and its specific expression pattern make it a good clinical target. It has been shown that blocking of IL-2Rs can interfere with the interactions between IL-2 and IL-2R and hence inhibit the IL-2 signal pathway, resulting in suppression of the immune system, which provides clinical benefits to organ transplantation patients. Two mAb drugs against IL-2Rα, basiliximab (Simultene; Novartis Pharmaceuticals, East Hanover, NJ) and daclizumab (Zenapax; Roche, Basel, Switzerland), have been approved by the U.S. Food and Drug Administration for the prevention of allograft rejection in organ transplantation, especially in kidney transplants. Basiliximab is a mouse-human chimera mAb with the variable domain of murine anti–IL-2Rα mAb RTF5 and the constant domains of human IgG1 (34) that has had great success in the prevention of renal allograft rejection (35). This mAb binds specifically to the ectodomain of IL-2Rα. With the phage display method, the epitope recognized by basiliximab was mapped to residues 116–122 of the D2 domain of IL-2Rα, which is part of the region interacting with IL-2 and thus explains in part why the binding of basiliximab with IL-2Rs can block IL-2 signaling (36). However, the detailed molecular mechanism of the inhibition of IL-2 signaling by basiliximab remains unclear.

In this study, we report the crystal structure of the basiliximab Fab in complex with the IL-2Rα ectodomain. Structural analysis of this complex and its comparison with the crystal structures of IL-2 in complex with IL-2Rsβγ reveal the molecular basis for the high specificity and high affinity of basiliximab with IL-2Rα and the molecular mechanism for the blockage of the IL-2 signaling by basiliximab. The structural results also have important implications for the design and development of improved and new mAb drugs against IL-2Rα.

Materials and Methods

Protein preparation and purification

The mAb basiliximab was purchased from Novartis Pharmaceuticals. The mAb was diluted to a concentration of 1 mg/ml with a buffer of 1 mM EDTA and 100 mM sodium acetate (pH 5.5), followed by digestion with 10 μg/ml papain (Sigma-Aldrich, St. Louis, MO) at 37°C for 5 h. The reaction was quenched with 20 mM iodoacetamide. The Fab fragment was separated by ion exchange chromatography using a Q Sepharose Fast Flow column (GE Healthcare, Uppsala, Sweden). The pooled Fab fragment was further purified by gel filtration chromatography using a Superdex G-200 16/60 column (GE Healthcare). The Fab sample was concentrated to 10 mg/ml and then exchanged to a buffer of 10 mM HEPES (pH 7.0) and 50 mM NaCl.

The cDNA encoding the ectodomain of human IL-2Rα (residues 1–217) was cloned into a modified pFastBac vector (Invitrogen, Carlsbad, CA) that fuses a gsp67A signal sequence and a hexahistidine tag at the N and C termini of the target protein, respectively. The recombinant protein was expressed and secreted into the medium using a Bac-to-Bac baculovirus expression system (Invitrogen). The harvested medium was centrifuged twice to remove the remaining cells and concentrated to a suitable volume followed by dialysis against a buffer of 10 mM Tris (pH 8.0) and 500 mM NaCl. The recombinant IL-2Rα was purified by affinity chromatography using a Ni-NTA Superflow column (Qiagen, Valencia, CA). Because the wild-type IL-2Rα would form a disulfide-linked dimer with the free cysteine at position 192 (37), the purified disulfide-linked protein was reduced with 10 mM cysteine and further alkylated with 20 mM iodoacetamide as previously described (27). The resultant protein was purified further by gel filtration chromatography using a Superdex G-200 16/60 column, and the fraction corresponding to the monomeric IL-2Rα was collected for further structural and biochemical studies. The recombinant IL-2Rα is heavily glycosylated, and its apparent molecular weight is ~43 kDa as monitored by reduced SDS-PAGE, whereas the calculated molecular weight is ~25 kDa.

The protein samples of the basiliximab Fab and IL-2Rα ectodomain were mixed at a molar ratio of 1:5:1 at 4°C for 12 h and then loaded onto a Superdex G-200 16/60 column. The protein complex was eluted with a buffer of 10 mM HEPES (pH 7.0) and 50 mM NaCl and then concentrated to 3 mg/ml for crystallization. The purity and homogeneity of the complex were confirmed by SDS-PAGE and dynamic light scattering analysis.

Crystallization and diffraction data collection

Crystallization was performed using the hanging drop vapor diffusion method at 20°C. In a drop containing 0.5 μl of the protein complex sample and 0.5 μl of the reservoir solution (0.2 M KCl, 0.05 M HEPES (pH 7.5), and 45% pentane-tetrol propoxylate (5/4 PO/OH) (38) equilibrated against the reservoir solution with the use of a nylon loop and flash-cooled into the liquid N2 stream (−170°C). Diffraction data were collected at Shanghai Synchrotron Radiation Facility beamline BL-17U1 and processed with the program HKL2000 (39). The statistics of the diffraction data are summarized in Table I.

Structure determination and refinement

The structure of the basiliximab Fab in complex with the IL-2Rα ectodomain was determined by the molecular replacement method implemented in the program Phaser (40) with the structure of the basiliximab Fab as the search model, followed by manual fitting of the IL-2Rα ectodomain. The refinement function search and the subsequent translational function search with the structure of the free-form basiliximab Fab (Protein Data Bank [PDB] code 1MIM) (41) used as the search model yielded an outstanding solution in the asymmetric unit. Further locate the position of the IL-2Rα ectodomain, we used all of the three available structures of IL-2Rα (PDB codes 2BS1, 1Z92, and 2ERJ) (24–26) as templates with Phaser and other commonly used programs implemented with the molecular replacement method. However, these attempts were unsuccessful to find a solution for IL-2Rα. After several cycles of structure refinement using the program CNS (42), the electron density for the D1 domain of IL-2Rα was developed gradually. The structure of the D1 domain of IL-2Rα (PDB code 2BS1) (25) was manually placed into the electron density. After several rounds of structure refinement using the program Phenix (43) and model building using the program O (44), the complete D1 and D2 domains of IL-2Rα were modeled and fit well into the electron density. However, as in all of the other IL-2Rα structures (24–26), several flexible regions of IL-2Rs have no defined electron density and thus could not be modeled, including the linker region between domains D1 and D2 (residues 62–100) and the C-terminal region (residues 159–217). The final model contains 119 of 217 residues of the IL-2Rα ectodomain. There was a long stretch of electron density near residue Asn69 of IL-2Rα that could be modeled as an N-linked core trisaccharide (MANβ1-4GlcNAcβ1-4GlcNAcβ1-Asn) without ambiguity. All of the diffraction data were used in the structure refinement except 5% of randomly chosen diffraction data were set aside for free R factor cross-validation. The stereochemical geometry of the final structure model was analyzed with the program Procheck (45). The statistics of the refinement and structure model also are listed in Table I. Structural analysis was performed using the programs in the CCP4 suite (46) and the PISA server (47). Figures were prepared using the program PyMol (www.pymol.org).

Surface plasmon resonance analysis

The kinetic studies of the interaction between basiliximab and the IL-2Rα ectodomain were performed by the surface plasmon resonance method using a Biacore 3000 instrument (GE Healthcare) at 25°C. The mAb basiliximab was immobilized on a CMS sensor chip (GE Healthcare) using an amine coupling kit (GE Healthcare). The purified IL-2Rα ectodomain was dialyzed against the HBS (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20) buffer (GE Healthcare) and used as the analyte in the binding assay. The association was monitored for a 240 s period, and the dissociation was monitored by flowing the HBS buffer for 600 s subsequently. An irrelevant chimeric mAb, the anti-CD20 mAb Rituximab (Roche), was used as a reference. The experimental data were analyzed with a 1:1 Langmuir model using the program BLAevaluation (GE Healthcare).

Accession code

The coordinates and structure factors of the basiliximab Fab in complex with the IL-2Rα ectodomain have been deposited in the Research Collaboratory for Structural Bioinformatics PDB (www.rcsb.org/pdb/) with the accession code 3HJ3.

Results

Overall structure of the basiliximab Fab in complex with the IL-2Rα ectodomain

The crystal structure of the basiliximab Fab in complex with the IL-2Rα ectodomain was determined by the molecular replacement method implemented with the structure of the free-form basiliximab.
Fab as the search model, followed by manual fitting of the IL-2Rα ectodomain. This structure was refined to a resolution of 2.9 Å, yielding an R factor of 21.1% and a free R factor of 26.2% (Table I). There are three Fab/IL-2Rα complexes in an asymmetric unit. The structure model has good stereochemical geometry with only Thr50 of each L chain located in the disallowed regions of the Ramachandran plot, which resides in a classic γ-turn (48). Thr50 is located in the generously allowed region of the Ramachandran plot in the free-form basiliximab Fab and has slightly differed ϕ and ψ angles (41). Although there are some variations in the numbers of disordered residues, the three complexes are very similar (superposition of all of the Cα atoms yields a root-mean-square deviation of 1.3–1.4 Å for IL-2Rα, 0.2–0.3 Å for the Fab, and 0.5–0.6 Å for the complex, respectively), and the one with the most detectable residues and best electron density has been selected for structural analysis.

The basiliximab Fab in the complex consists of the H chain residues 1–215 that fold into the VH and CH1 domains and the L chain residues 1–208 that fold into the VL and CL domains (Fig. 1A). The overall structures of the variable domains and constant domains of the Fab in the complex are similar to those in the free-form Fab reported previously (41) (superposition of all of the Cα domains of the Fab in the complex are similar to those in the free-form Fab, indicating that binding of IL-2Rα does not induce a significant conformational change of the Fab.

As in the other IL-2Rα structures reported previously (PDB codes 1Z92, 2ERJ, and 2BSI) (24–26), the IL-2Rα ectodomain in the Fab/IL-2Rα complex is composed of two typical β-strand-swapped “sushi-like” domains D1 (residues 1–61) and D2 (residues 101–158) that assemble like a bent arm with an elbow angle of ~90°. The electron density for both D1 and D2 domains was well defined, especially in the regions participating in interactions with the Fab (Fig. 1B). However, again similar to that seen in the other IL-2Rα structures (24–26), the linker region between the D1 and D2 domains (residues 62–100) and the C-terminal region (residues 159–217) connecting the D2 domain to the transmembrane domain of IL-2Rα could not be observed in this complex. Thus, only 119 out of a total of 217 residues of the IL-2Rα ectodomain were modeled, reflecting the flexible nature of IL-2Rα. A detailed structure comparison indicates that the overall structure of the IL-2Rα ectodomain in this complex is similar to that in its complexes with IL-2 (24–26). Superposition of the different structures yields a root-mean-square deviation of 0.9–1.8 Å for the D1 domain, 1.6–2.0 Å for the D2 domain, and 1.4–1.7 Å for the whole IL-2Rα molecule. The major structural differences occur in two solvent-exposed regions (residues 109–117 and 132–144 of the D2 domain) that are not in contact with other protein molecules. It is noteworthy that Asn49 of IL-2Rα in this complex is glycosylated and an N-linked core

![FIGURE 1. Overall structure of the basiliximab Fab in complex with the IL-2Rα ectodomain. A, A stereoview of the overall structure of the complex. The Fab is colored with the L chain in yellow and the H chain in green, and IL-2Rα is colored with the D1 domain in cyan and the D2 domain in purple. The sugar chain of the glycosylated Asn49 of IL-2Rα is shown with a ball-and-stick model. B, A stereoview of a representative SIGMAA-weighted 2Fο − Fc electron density map (1σ contour level) in regions of the D1 domain of IL-2Rα (residues 21–30 and 53–61) that are involved in the interactions with the Fab. The atomic coordinates of the residues are shown with ball-and-stick models. The disulfide bonds between Cys28 and Cys59 and between Cys30 and Cys61 are clearly defined.]

Table I. Summary of diffraction data and structure refinement statistics

<table>
<thead>
<tr>
<th>Summary of Diffraction Data</th>
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<tbody>
<tr>
<td>Wavelength (Å)</td>
<td>0.9796</td>
</tr>
<tr>
<td>Space group</td>
<td>P6122</td>
</tr>
<tr>
<td>a = b (Å)</td>
<td>137.1</td>
</tr>
<tr>
<td>c (Å)</td>
<td>459.1</td>
</tr>
<tr>
<td>Resolution range (Å)²</td>
<td>50.0–2.90 (3.00–2.90)</td>
</tr>
<tr>
<td>Observed reflections</td>
<td>836,837</td>
</tr>
<tr>
<td>Unique reflections (I/σ(I) &gt; 0)</td>
<td>56,779</td>
</tr>
<tr>
<td>Average redundancy</td>
<td>14.8 (10.0)</td>
</tr>
<tr>
<td>Average I/σ(I)</td>
<td>17.0 (2.0)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>98.0 (94.5)</td>
</tr>
<tr>
<td>Wilson B factor (Å²)</td>
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<tr>
<td>Mosaicity</td>
<td>0.24</td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>16.5 (56.1)</td>
</tr>
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</table>

Statistics of Refinement and Model

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<tr>
<th>Number of reflections [Fo &gt; 0 or Fc]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Working set</td>
<td>53,580</td>
</tr>
<tr>
<td>Free R set</td>
<td>2,651</td>
</tr>
<tr>
<td>R factor/free R factor (%)</td>
<td>21.5/26.2</td>
</tr>
<tr>
<td>Number of protein atoms</td>
<td>12,450</td>
</tr>
<tr>
<td>Number of sugar atoms</td>
<td>117</td>
</tr>
<tr>
<td>Average B factor of all atoms (Å²)</td>
<td>87.6</td>
</tr>
<tr>
<td>Fab/IL-2Rα/sugar (Å²)</td>
<td>74.3/132.2/132.6</td>
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<tr>
<td>Root-mean-square bond lengths (Å)</td>
<td>0.006</td>
</tr>
<tr>
<td>Root-mean-square bond angles (°)</td>
<td>1.1</td>
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<tr>
<td>Ramachandran plot (%)</td>
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<tr>
<td>Most favored regions</td>
<td>86.4</td>
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<tr>
<td>Allowed regions</td>
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<tr>
<td>Generously allowed regions</td>
<td>1.4</td>
</tr>
<tr>
<td>Disallowed regions</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to the highest resolution shell.

aRmerge = Σ||Fo|−|Fc||/Σ||Fo||. 

bR factor = Σ|Fo|−|Fc|/Σ||Fo||(|Fo|+|Fc|). 

Materials and Methods

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FIGURE 1. Overall structure of the basiliximab Fab in complex with the IL-2Rα ectodomain. A, A stereoview of the overall structure of the complex. The Fab is colored with the L chain in yellow and the H chain in green, and IL-2Rα is colored with the D1 domain in cyan and the D2 domain in purple. The sugar chain of the glycosylated Asn49 of IL-2Rα is shown with a ball-and-stick model. B, A stereoview of a representative SIGMAA-weighted 2Fο − Fc electron density map (1σ contour level) in regions of the D1 domain of IL-2Rα (residues 21–30 and 53–61) that are involved in the interactions with the Fab. The atomic coordinates of the residues are shown with ball-and-stick models. The disulfide bonds between Cys28 and Cys59 and between Cys30 and Cys61 are clearly defined.
trisaccharide (MANβ-1, 4GlcNAcβ-1, 4GlcNAcβ-1-Asn⁴⁹) could be modeled without ambiguity. This is the first observation of glycosylation of this residue, because in the previously reported structures Asn⁴⁹ is either disordered or mutated to serine to prohibit the glycosylation of the protein (24–26). In our structure, although Asn⁴⁹ and the sugar chain do not have direct contact with the basiliximab Fab, the region near Asn⁴⁹ (residues 38–48) participates in the interactions with the Fab, which might contribute to the stabilization of the glycosylated Asn⁴⁹. However, the biological significance of the glycosylation is unclear.

**Interactions between the basiliximab Fab and the IL-2Rα ectodomain**

In the complex structure, the basiliximab Fab forms extensive hydrophilic and hydrophobic interactions with IL-2Rα via a large interface, including 10 hydrogen bonds, 4 salt bridges, and 138 van der Waals contacts (Fig. 2A, Tables II and III). Although the complex has a shape complementarity value of 0.57, which is slightly smaller than the average value (0.64–0.68) for Ag/Ab complexes (49), formation of the complex buries a very large solvent-accessible surface area of 2255.4 Å² (1108.6 Å² on the Fab and 1146.8 Å² on IL-2Rα), which is much higher than the common value seen in the other Ag/Ab complexes (50, 51). The Fab H chain contributes 768.9 Å² of the buried surface area, and the L chain 339.7 Å², consistent with the notion that the mAb H chain usually makes more contributions than the L chain in Ag binding (52, 53).

![FIGURE 2](image-url)

**FIGURE 2.** Interactions between the basiliximab Fab and the IL-2Rα ectodomain. A, The interaction interface between the Fab and IL-2Rα viewing down at the Fab. The basiliximab Fab is shown with an electrostatic potential surface with the locations of some of the residues involved in the interactions with IL-2Rα labeled in green. IL-2Rα is shown with a stick model with the D1 domain in cyan and the D2 domain in purple, and the residues involved in the interactions with the Fab are shown in ball-and-stick models and labeled in black. B, The interaction interface between the Fab and IL-2Rα viewing down at IL-2Rα in the same orientation as in Fig. 2A. IL-2Rα is shown with an electrostatic potential surface with the locations of some of the residues involved in the interactions with the Fab labeled in cyan. The Fab is shown with a stick model with the CDR loops L1, H2, and H3 in green and the CDR loops L1, L2, and L3 in yellow, and the residues involved in the interactions with IL-2Rα are indicated in ball-and-stick models and labeled in black. C, A stereoview showing the hydrogen bonding and salt bridge interactions between IL-2Rα (in cyan) and the L chain of the Fab (in yellow). The hydrogen bonds are indicated with gray dashes, and the salt bridges are indicated with orange dashes. D, A stereoview showing the hydrogen bonding and salt bridge interactions between IL-2Rα (D1 domain in cyan and D2 domain in purple) and the CDR loop H1 of the Fab (in green). E, A stereoview showing the hydrogen bonding and salt bridge interactions between IL-2Rα (in cyan) and the CDR loops H2 and H3 of the Fab (in green). The color coding of the structural elements is the same as that in Fig. 1A.
Surrounding the hydrophobic groove there are three positively charged surface patches and one negatively charged surface patch at the Ag binding site. One positively charged patch is composed of several residues of CDRs L1 and L3, including Arg<sup>L29</sup>, Arg<sup>L30</sup>, and Ser<sup>L31</sup> that form both hydrophilic and hydrophobic interactions with residues Asp<sup>199</sup> and Asn<sup>198</sup> of IL-2R<sub>a</sub> (Fig. 2A, 2B). In particular, Asp<sup>199</sup> stretches its side chain into a shallow cavity on the surface of the Fab and forms a hydrogen bond with Asp<sup>199</sup> of IL-2R<sub>a</sub> (Fig. 2C). The second positively charged patch is composed of the residues Arg<sup>L29</sup> and Arg<sup>L30</sup>, and Asn<sup>L31</sup> forms a hydrogen bond with Arg<sup>L29</sup> and Ser<sup>L31</sup> (Fig. 2C, Table II). The third positively charged patch is composed of mainly CDR H1 residues Arg<sup>H29</sup> and Tyr<sup>H30</sup> that interact with a negatively charged surface patch of IL-2R<sub>a</sub> formed by two short segments (residues 1–6 and residues 116–120 (Fig. 2A, 2B). In particular, Arg<sup>H29</sup> forms a salt bridge with Asp<sup>199</sup> and a hydrogen bond with His<sup>L120</sup> and Tyr<sup>H30</sup> forms a hydrogen bond with Asp<sup>14</sup> (Fig. 2D, Table II). The fourth surface patch is negatively charged and composed of mainly CDR H2 residues Asn<sup>H53</sup>, Asp<sup>H55</sup>, and Glu<sup>H63</sup> that interact with a positively charged surface patch of IL-2R<sub>a</sub> formed by the residues Arg<sup>H36</sup>, Arg<sup>H38</sup>, and Lys<sup>H38</sup>, each of which makes two hydrogen bonds or salt bridges with the Fab, and Leu<sup>H44</sup> and Tyr<sup>H41</sup>, each of which makes many hydrophobic interactions with the Fab.

### Discussion

**The epitope of basiliximab**

Previously, the basiliximab epitope was mapped to residues 116–122 of the D2 domain of IL-2R<sub>a</sub> using the phage display method (36). In the basiliximab Fab/IL-2R<sub>a</sub> complex structure, only two residues of this segment are involved in the interactions with the Fab. Specifically, Ile<sup>118</sup> makes two van der Waals contacts with the Fab, and His<sup>L120</sup> forms one hydrogen bond with Arg<sup>H29</sup> and five van der Waals contacts with the Fab (Tables II and III). These interactions constitute only a small portion of the interactions at the third hydrophilic patch described above and play a less significant role in the recognition and binding of basiliximab. These results indicate that the potential epitope identified by the phage display method is incomplete and the functional role of the identified region in the recognition is undefined. A similar situation also was seen in the structural studies of the Rituximab Fab in complex with a peptide corresponding to its epitope on CD20. With the phase display method, the Rituximab binding epitope was mapped to two segments 170ANPS<sup>173</sup> and 182YCYSI<sup>186</sup> on the CD20 extracellular loop. The structural studies of the Ag/Ab complex indicate that the 182YCYSI<sup>186</sup> motif is not directly involved in the interaction with the Ab; instead it plays a critical role in the formation of a disulfide bond to define the proper geometry of the 170ANPS<sup>173</sup> motif so that the latter can be recognized by Rituximab (57, 58).

### Molecular mechanism of the inhibition of the IL-2 signal pathway by basiliximab

Structural analysis of the basiliximab Fab/IL-2R<sub>a</sub> complex and its comparison with the crystal structures of the IL-2/IL-2R<sub>a</sub> and IL-2/IL-2R<sub>b</sub> complexes provides insights into the molecular
mechanism of the inhibition of the IL-2 signal pathway by basiliximab. In the crystal structures of the IL-2/IL-2Rα and IL-2/IL-2Rαβγ complexes, IL-2Rα makes interactions with IL-2 mainly via the D1 domain (24–26). Specifically, Met225, Leu242, and Tyr243 of IL-2Rα form a hydrophobic surface patch to interact with residues Phe42 and Leu72 of IL-2 (24). Surrounding the hydrophobic patch, several hydrophilic residues of IL-2Rα interact with IL-2 by forming numerous hydrogen bonds, salt bridges, and van der Waals contacts. In total, there are 21 residues of IL-2Rα participating in the interactions with IL-2, forming 8 hydrogen bonds, 2 salt bridges, and 100 van der Waals contacts (25). The interaction interface buries 971.2 Å² of the solvent-accessible surface area on IL-2-Rα. Structural comparison of these complexes with the basiliximab Fab/IL-2Rα complex indicates that the residues of IL-2Rα responsible for the interactions with IL-2 overlap largely with the epitope of basiliximab. Fifteen out of the 21 residues (71.4%) are involved in the interactions with the basiliximab Fab, and ~641.6 Å² (66.1%) of the buried solvent-accessible surface area on IL-2Rα is covered by the Ab (Fig. 3A). Especially, the hydrophobic patch formed by residues Met225, Leu242, and Tyr243 of IL-2Rα plays a key role in the binding of both basiliximab and IL-2. Furthermore, the basiliximab binding epitope comprises several other residues besides those involved in the interactions with IL-2, and the basiliximab Fab/IL-2Rα interface comprises more hydrophobic and hydrophilic interactions than the IL-2/IL-2Rα interface. These results may explain in part the biochemical data that the binding affinity of basiliximab to IL-2Rα (0.14 nM) is ~71-fold higher than that of IL-2 to IL-2Rα (10 nM) (27). Therefore, the binding of basiliximab to IL-2Rα would compete for IL-2 binding to the receptor. In the presence of a sufficient amount of basiliximab, the IL-2 binding sites of IL-2Rα would be blocked, and thus IL-2 signaling cannot be initiated and executed due to the lack of binding of IL-2 to IL-2Rα and the formation of the functional IL-2/IL-2Rαβγ complex (Fig. 3A, 3B). This provides the molecular mechanism of the inhibition of IL-2 signaling by basiliximab.

Implications for drug development
IL-2 signaling plays an important role in the activation of immune responses against foreign intrusion. Blockage of this signal pathway could provide therapeutic benefits to reduce or eliminate allograft rejection in organ transplantation. The biological and structural data have shown that activation of IL-2 is initiated by the binding of IL-2Rα and further facilitated by the binding of IL-2Rβ and γc (24–27). Among the three receptors, only IL-2Rα is IL-2-specific, whereas IL-2Rβ and γc are shared with other cytokines and are less specific. Therefore, IL-2Rα is a more suitable drug target for blocking the IL-2 signal pathway. Basiliximab and daclizumab are two IL-2Rα–specific mAb drugs that have been used in clinical applications for the prevention of allograft rejection in organ transplantation.

Although basiliximab binds to IL-2Rα with a high affinity (Kd = 0.14 nM), the Fab/IL-2Rα complex has a relatively lower shape complementarity value of 0.57, suggesting that the paratope of basiliximab could be modified to have higher shape and chemical complementarities with IL-2Rα and thus achieve a tighter binding and better specificity. In our previous structural studies of the Rituximab Fab in complex with a CD20 peptide, we have proposed some mutations on the CDR loops of the Ab that might be able to improve its binding affinity (57). Recently, these suggestions have been validated, and the results have shown that some of the mutations could substantially improve the affinity (59). Structural analysis of the basiliximab Fab/IL-2Rα complex also provides some hints for improving the mAb drug. For instance, Met225 intrudes its side chain into the hydrophobic groove at the paratope of basiliximab but forms only one van der Waals contact with Gly499. Modeling studies show that a substitute for Gly499 with a slightly larger hydrophobic residue, such as alanine, valine, leucine, or isoleucine, could form more hydrophobic interactions with Met225 and the surrounding residues Leu23, Leu242, and Tyr243 without steric conflict, thus making the interaction interface gain better shape and chemical complementarities. Moreover, because Lys452 forms a hydrogen bond with the main-chain carbonyl of Gly499 and a weak salt bridge with Glu422 (4.3 Å), mutation of Lys452 to arginine would make it form a more favorable salt bridge with Glu422. In addition, Ser455 is located near the interaction interface but has no direct contact with IL-2Rα; however, substitution of this residue with a large positively charged residue such as arginine would make it form a favorable salt bridge with Glu42 (within 3.5 Å). In other words, mutations of the aforementioned residues at the paratope of basiliximab could introduce additional favorable interactions between the mAb and IL-2α and thus improve the binding affinity and specificity of the mAb.
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Disclosures

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References


