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Structural insights into the assembly and activation of the IL-27 signaling complex

Yibo Jin¹†, Paul K Fyfe²†, Scott Gardner¹, Stephan Wilmes², Doryen Bubeck¹,‡, * and Ignacio Moraga²*,‡

Abstract

Interleukin 27 (IL-27) is a heterodimeric cytokine that elicits potent immunosuppressive responses. Comprised of EBI3 and p28 subunits, IL-27 binds GP130 and IL-27Rα receptor chains to activate the JAK/STAT signaling cascade. However, how these receptors recognize IL-27 and form a complex capable of phosphorylating JAK proteins remains unclear. Here, we used cryo electron microscopy (cryoEM) and AlphaFold modeling to solve the structure of the IL-27 receptor recognition complex. Our data show how IL-27 serves as a bridge connecting IL-27Rα (domains 1–2) with GP130 (domains 1–3) to initiate signaling. While both receptors contact the p28 component of the heterodimeric cytokine, EBI3 stabilizes the complex by binding a positively charged surface of IL-27Rα and Domain 1 of GP130. We find that assembly of the IL-27 receptor recognition complex is distinct from both IL-12 and IL-6 cytokine families and provides a mechanistic blueprint for tuning IL-27 pleiotropic actions.

Keywords cytokine; IL-27; cryo electron microscopy; GP130

Introduction

IL-27 is an immunosuppressive cytokine involved in resolving T cell-mediated inflammation. IL-27 inhibits Th17 responses (Stumhofer et al., 2006; Yang et al., 2008; Diveu et al., 2009) and induces differentiation of T regulatory cells (Hall et al., 2012). T cell stimulation by IL-27 promotes secretion of the anti-inflammatory cytokine, IL-10, further contributing to a reduction in the inflammatory response (Stumhofer et al., 2007). Together, these properties make IL-27 an attractive drug target to treat T cell-mediated inflammatory disease. IL-27 also contributes to immune exhaustion by regulating expression of co-inhibitory receptors (Chihara et al., 2018; DeLong et al., 2019). Elevated levels of IL-27 gene signatures are found in cancer and are associated with poor prognoses (Jia et al., 2016). Understanding how IL-27 engages its cellular receptors, GP130 and IL-27Rα, to form a signaling complex will provide fundamental insight into immune regulation, which could facilitate the development of new therapeutics that target IL-27 responses.

IL-27 is a heterodimeric cytokine composed of two gene products, Epstein–Barr virus-induced gene 3 (EBI3) and IL-27p28 (p28; Vignali & Kuchroo, 2012). Grouped within the IL-12 family of heterocytokines, p28 and EBI3 share sequence homology with two components of IL-12: p35 and p40, respectively (Kourko et al., 2019). While p28 exhibits a classical four-α helical fold, EBI3 is structurally similar to soluble cytokine receptors (Rousseau et al., 2010). Unlike other heterodimeric cytokines (IL-12 and IL-23) which activate Signal Transducer and Activator of Transcription (STAT) STAT3 and STAT4, IL-27 primarily induces activation of STAT1 and STAT3 pathways (Tait Wojno et al., 2019). These differences can be attributed to the engagement of GP130 by IL-27, which is shared across the IL-6 cytokine family (Trinchieri et al., 2003).

IL-27 bridges two major cytokine families, raising the question of whether competition or synergism could occur in dimer formation between them. Under some conditions, p28 and EBI3 can be secreted independently and associate with other proteins to induce differential responses (Crabe et al., 2009; Stumhofer et al., 2010; Collinson et al., 2012; Wang et al., 2012; Garbers et al., 2013). Promiscuity in chain combinations is characteristic of the IL-6/IL-12 family and allows the system to produce different biologically active factors starting from relatively few precursor molecules.

GP130 binds a range of cytokines to elicit diverse cellular responses (Grotzinger et al., 1997; Hunter & Jones, 2015). The receptor has two cytokine-binding sites. The first is located within the elbow between its cytokine homology regions (CHR) and has been shown to engage site 2 of cytokines, including IL-6 (Boulanger et al., 2003b), Leukemia inhibitory factor (LIF; Boulanger et al., 2003a), and Ciliary neurotrophic factor (CNTF; Skiniotis et al., 2008). The N-terminal immunoglobulin (Ig) domain of GP130 comprises an additional cytokine-binding interface that recognizes an
responses.

To overcome challenges in cytokine stability, we engineered an IL-27 cytokine variant in which the two monomeric components (EBI3 and p28) were fused by a short linker (Wilmes et al., 2021; Fig EV1A). The fusion cytokine was expressed in insect cells and purified together with domains 1–3 (D1, D2, D3) of GP130 and the first two domains of IL-27Rα (Fig EV1B and C). We then used cryoEM to solve the structure of the complete IL-27 cytokine-recognition complex (Figs 1A and EV2A–C). To address preferred orientation issues of the sample, we collected and merged datasets recorded at 0, 30, and 35 degrees along the tilt axis of the microscope (Fig EV2D). We used the ab initio reconstruction protocols within cryoSPARC (Punjani et al., 2017) to generate an initial model of the complex. Maps were further refined using a combination of nonuniform and heterogeneous refinement procedures (Fig EV3). The final map was refined to a reported resolution of 4.0 Å using the gold standard FSC 0.143 cut-off (Fig EV2C); with local resolution ranging from 3.5 to 15.1 Å (Fig EV2E). In our map, individual helices of p28 and β-strands of both EBI3 and IL-27Rα are clearly resolved (Fig EV4A). The chirality of the helical bundle central to the p28 cytokine was used to assign the handedness of the reconstruction. Although density for GP130 was less well ordered, we observe density for all three domains including the low-density core of the CHR domains, which guided placement of GP130 as a single rigid body. Given the lack of side-chain density at this resolution, models were derived from AlphaFold predictions (Jumper et al., 2021) refined with strong adaptive distance restraints and geometric restraints imposed (Table EV1). While there may be some uncertainty in the side-chain rotation, interface residues are defined largely by the rigid-body fit of AlphaFold models.

The overall structure of the IL-27 cytokine recognition complex exhibits a classical architecture with a helical cytokine bundle central to the assembly (Fig 1B). For the IL-27 complex, the helical bundle is sandwiched by two ‘L’-shaped densities and an additional prong that contacts the back face of the heterodimeric cytokine. Our data agrees with a recently published structure of the human IL-27 signaling complex (Caveney et al., 2022), with slight differences in rotation of the receptors relative to the helical cytokine.

**Principle binding interfaces for IL-27 cytokine recognition**

Cytokines from the IL-12/IL-6 family have three highly-conserved principle binding interfaces: site 1, site 2, and site 3 (Wang et al., 2009). For the IL-27 recognition complex, site 1 is occupied by the second half of the heterodimeric cytokine, EBI3 (Fig 2A and B). EBI3 is comprised of two Fibronectin type III (FNIII) domains bent into an ‘L’-shaped arrangement. Docking and minimal refinement of the EBI3 Alphafold model into the map show that the bend of the EBI3 elbow is formed by a cluster of aromatic residues (EBI3: Y39, F96, F159, and Y209) together with a proline (EBI3: P40), which create a hydrophobic groove recognized by p28:W93 (Fig 2B). This feature of knobs and holes shape recognition is consistent with other cytokine-binding interfaces (Wang et al., 2009). Our p28:EBI3 interface is supported by mutagenesis studies showing that equivalent residues in the human cytokine (EBI3:F39; p28:W97) are essential for cytokine complex formation (Rousseau et al., 2010). The same study identified an aspartic acid on EBI3 that also influences stability of the heterodimer (Rousseau et al., 2010). In our model, the equivalent murine residue (EBI3:D205) is nearby an arginine on p28 (p28:R217) and may be important for maintaining the interface (Fig 2B).

For the IL-6:GP130 cytokine recognition complex, site 2 of IL-6 is occupied by the two CHR domains of GP130 (D2 and D3; Boulanger et al., 2003b). To understand how GP130 recognizes chemically unique cytokines, we next investigated the IL-27 site 2 interface. In contrast to the IL-6 recognition complex, we find that site 2 of p28 is formed by the apex of the elbow region between the two CHR domains of IL-27Rα (Fig 2A and C). Here, we find that the knob and hole pattern is encoded by cluster of proline residues (IL-27Rα: P153, P152) and an aromatic residue (W151) of IL-27Rα, which form a pocket to bind a tyrosine extending from p28 (p28:Y48; Fig 2C). In the IL-6 complex, the equivalent tyrosine on IL-6 slots into a hydrophobic groove formed by the interface between D2 and D3 of GP130 (Boulanger et al., 2003b).

The position of EBI3 at site 1 and IL-27Rα at site 2 is coordinated by a second interaction interface. EBI3 directly contacts the second CHR domain of IL-27Rα. By contrast to the hydrophobic knob and hole recognition motifs observed for p28, this secondary interface is dominated by electrostatic interactions (Figs 2A and D, and EV5A–E). Positively charged residues of EBI3 (EBI3: R169, R176, K192)
face a negatively charged surface of IL-27Rα (IL-27Rα: D133, E137, E141, E187). These residues could be involved in a series of salt bridges that further contribute to the stability of the assembly.

The three N-terminal domains of GP130 are essential for cytokine recognition and signaling (Dahmen et al., 1998; Boulanger et al., 2003b). A crystal structure of GP130 in complex with IL-6 showed that GP130 can engage cytokines through both site 2 and site 3 interactions (Boulanger et al., 2003b). While the first two CHR domains of GP130 (D2 and D3) form the primary interface at site 2, the Ig domain (D1) of GP130 at site 3 bridges a second cytokine and is responsible for the lateral hexameric complex. In our structure of the IL-27 cytokine recognition complex, we observe a third prong of density extending from the core complex consistent with the arrangement of D1–D3 in the GP130 crystal structure (Boulanger et al., 2003b). In the IL-27-receptor signaling complex, D1 of GP130 contacts p28 at site 3 and bridges EBI3 in the heterodimeric cytokine (Fig 2A and E). Specifically, the aromatic side chain of p28 W195 could pack against GP130 Y116. Indeed, biochemical data confirm that GP130 engages p28 at site 3 with the equivalent residue in the human p28 (W197) playing a key role (Rousseau et al., 2010).

**Figure 1. Structure of the IL-27 receptor recognition complex.**

A, B CryoEM reconstruction (A) and atomic model (B) of the IL-27 receptor recognition complex. The complex consists of the IL-27 heterodimeric cytokine, p28 (blue) and EBI3 (purple), bound to two signaling receptors, GP130 (red) and IL-27Rα (yellow). EBI3 occupies site 1 on p28, while IL-27Rα occupies site 2. GP130 binds p28 at site 3 via domains 1 and 2 (D1, D2). The canonical cytokine recognition site on GP130 between domains D2 and D3 is unoccupied.

### Kinetic drivers of IL-27 signaling complex assembly

To understand the kinetic drivers underpinning assembly of the IL-27 signaling complex, we defined the binding affinities for each of the subcomponents using surface plasmon resonance (SPR; Fig 3A–E). First, we set out to identify which of the two signaling receptors bound the IL-27 heterodimer with higher affinity. We immobilized biotinylated IL-27Rα or GP130 on a streptavidin SPR surface and passed a range of IL-27 concentrations to measure rates of association and dissociation (Fig 3A and B). We found that IL-27Rα has a higher affinity (KD: 0.28 nM) for the heterodimeric cytokine than GP130 (KD: 2.1 nM; Fig 3E), in agreement with biochemical data showing that GP130 engages the low-affinity binding site 3 (Rousseau et al., 2010). These results suggest a two-step binding mode for IL-27 complex formation, where IL-27 binds IL-27Rα with high affinity in a first step, and subsequently recruits GP130 with lower affinity to form the signaling complex. We next wanted to understand the roles of EBI3 and p28 in the kinetics of complex formation. As we were unable to produce recombinant EBI3, we focused our analysis on p28. We quantified the binding affinity of p28 for each signaling receptor immobilized to the streptavidin SPR chip (Fig 3C...
Figure 2. Interaction interfaces of the complex.

A Overview of IL-27 interactions with signaling receptors. Ribbon representation of the complex colored according to protein components: p28 (blue), EBI3 (purple), IL-27Rα (yellow), GP130 (red). Individual domains of GP130 (D1, D2, and D3) are labeled.

B The hinge between the two CHR domains of EBI3 form a hydrophobic groove (Y39, P40, F96, F159, and Y209) that is filled by W93 of p28. Residue EBI3: D205, which is important for assembly of the heterodimeric cytokine (Rousseau et al., 2010), is also present in the binding interface and could form a salt bridge with p28:R217.

C IL-27Rα binds site 2 of p28 at the apex of the elbow between its two CHR domains. The loops of IL-27Rα form a pocket comprised of residues IL-27Rα: W151, P152, and P153 in which the aromatic side chain of p28:Y48 could slot into.

D The orientation of IL-27Rα at site 2 is stabilized by a second interaction interface with EBI3, which is dominated by electrostatic complementarity between the two domains (IL-27Rα: E187, D133, E141, E137, and EBI3: R176, R169, K192).

E Site 3 of p28 is occupied by the bend between the CHR domain 2 (D2) and the immunoglobulin (Ig) domain D1 of GP130. While this interface is not well resolved, p28:W195, which is essential for GP130-mediated signaling (Rousseau et al., 2010), is facing D1. The key residues that mediate the interactions at interfaces are shown as sticks.
Figure 3.
and D). We observed only weak binding of p28 to IL-27Rα (K<sub>a</sub>: 1.7 μM) and no binding to GP130 at the concentrations tested (Fig 3E). These data are supported by our structural observations that the GP130 binding site is formed by the cleft between p28 and EBI3 and are consistent with weak signaling responses elicited by p28 when compared to IL-27 in CD8<sup>+</sup> T cells (Fig 3F and G). Taken together, these data support a stabilizing role of EBI3 in formation of the IL-27 signaling complex.

IL-27 is a model system for signaling by heterodimeric cytokines, including IL-12 and IL-23. A comparison of the heterodimeric IL-23 receptor complex (Data ref: Glassman et al, 2021a; Glassman et al, 2021b) with our structure shows that EBI3 and the IL-12β subunit (p40) overlay with site 1 (Fig 4A). In addition, we observe that the GP130 density extending away from the core IL-27 complex has similarities to the IL-23 signaling receptor. The arrangement of these extracellular domains likely directs the orientation of intracellular regions that activate the JAK/STAT pathway. Therefore, it may be possible that the bend between D2 and D3 of GP130 in the signaling receptor is a topological requirement for activation by IL-12, IL-23, and IL-27.

IL-27 is a central member of the IL-6 family of cytokines that signal through GP130 (Hunter & Kastelein, 2012). The diversity in cellular responses within the family stems in part from the strict transcriptional control of cytokines secreted by different cell types (Taka & Kishimoto, 1997). Although IL-27 is a heterodimeric cytokine, there are some instances whereby the two components are independently expressed (Devergne et al, 1996; Maaser et al, 2004). In the absence of EBI3, p28 can act as an antagonist for IL-6-mediated GP130 signaling (Stumhofer et al, 2010). Our biophysical data show that binding of p28 alone to GP130 is negligible (Fig 3). A comparison of the IL-6 cytokine recognition complex (Data ref: Boulanger et al, 2003c) with our structure shows that binding of EBI3 to site 1 overlaps with site 1 of the IL-6 (Fig 4A). Previous studies have reported that p28 can bind IL-6Rα (Garbers et al, 2013). Though signaling by the putative p28:IL-6Rα is weaker compared to IL-6 or IL-27, it may be possible that IL-6Rα engages
p28 at site 1 with low affinity to produce weak agonistic or antagonistic activities in certain contexts. 

Chain sharing is a common theme among cytokine receptors (Vignali & Kuchroo, 2012) and contributes to the layers of complexity derived from relatively few building blocks. Although both IL-27 and IL-6 engage GP130, they do so through different interaction interfaces. One important difference between these two structures occurs at site 2 (Fig 4A). While the two CHR domains of GP130 (D2 and D3) bind IL-6 at site 2, the equivalent position in the IL-27 system is occupied by IL-27Rα. Unlike the IL-6 co-receptor (IL-6Rα), the intracellular domains of GP130 and IL-27Rα both associate with JAKs proteins and activate signaling upon dimer formation. In the case of IL-27, the cognate co-receptor IL-27Rα could fill an analogous role to GP130 at site 2 in the IL-6-signaling complex. In both systems, site 1 is occupied by nonsignaling proteins: EB3 and IL-6Rα. Our structural and biophysical data support role for auxiliary proteins at site 1 to enhance the stability of the signaling complex, while receptors occupying site 2 are responsible for signal transduction.

Signaling through the JAK/STAT pathway requires dimerization of signaling receptors. How that dimerization is achieved, and how the geometry of the signaling complex contributes to the functional diversity of the cascade, remains an open question. There is a wide range of oligomeric assemblies observed for GP130-cytokine complexes (Boulanger et al, 2003b; Wang et al, 2009). For IL-6, dimerization of GP130 occurs through a hexameric assembly (2 copies of the IL-6:IL-6Rα:GP130 complex). Within the hexamer, GP130 bound to IL-6 site 2 dimerizes with a second copy bound to site 3 of the additional cytokine (Fig 4B). In the IL-27 signaling complex, dimerization of IL-27Rα and GP130 may occur from receptors occupying sites 2 and 3 from the same cytokine, though additional cytokine-receptor stoichiometries cannot be ruled out. Indeed, the primary interaction interface observed in the IL-6:GP130 complex occurs at the apex of a bend between GP130 CHR domains (D2 and D3). In the IL-27 complex, this region is likely unoccupied and available to engage other cytokines. In addition, IL-27 has been shown to inhibit signaling by oncostatin M (OSM), an IL-6 family cytokine (Baker et al, 2010). Based on our structure, IL-27 immunosuppressant activities may be achieved by blocking inflammatory cytokines such as OSM from binding GP130 at site 2 in a signaling competent conformation. Understanding how mixtures of cytokines influence signaling assemblies and downstream cascades will inform new strategies in protein engineering for fine-tuning cytokine responses.

Materials and Methods

Protein expression and purification

Murine IL-27 was cloned as a linker-connected single-chain variant (p28 + EB13) as described in Oniki et al (2006) (Fig EV1A). In the text, we refer to this variant only as IL-27. Murine IL-27Rα (amino acids 28–224) and GP130 (amino acid 23–319) ectodomains were cloned and expressed as described in Martinez-Fabregas et al (2019). Briefly, protein sequences were cloned into the pAcGP67-A vector (BD Biosciences) in frame with an N-terminal gp67 signal sequence, driving protein secretion, and a C-terminal hexahistidine tag. Baculovirus stocks were produced by transfection and amplification in Spodoptera frugiperda (Sf9) cells grown in SF900III media (Invitrogen), and protein expression was carried out in suspension Trichoplusia ni (High Five) cells grown in InsectXpress media (Lonza).

Protein purification was carried out using the method described in Spangler et al (2019). Hi-Five cells were pelleted with centrifugation at 1,000 × g, and impurities from the remaining media were removed by a precipitation step through addition of Tris pH 8.0, CaCl2, and NiCl2 to final concentrations of 200, 50, and 1 mM, respectively. The precipitate formed was then removed through centrifugation at 9,000 × g. Nickel-NTA agarose beads (Qiagen) were added to the clarified media and the target proteins purified through batch binding followed by column washing in PBS buffer for murine IL-27Rα and GP130 or PBS-Hi buffer (PBS buffer supplemented to 500 mM of NaCl and 5% glycerol, pH 7.2) for murine IL-27sc. Elution was performed using PBS or PBS-Hi buffer plus 200 mM imidazole. Final purification was performed by size exclusion chromatography on an ENRich SEC 650 10 × 300 column (Biorad), equilibrated in PBS or PBS-Hi buffers. Concentration of the purified sample was carried out using 30 kDa Millipore Amicon-Ultra spin concentrators. Recombinant proteins were purified to greater than 98% homogeneity. For cryoEM studies, IL-27Rα, GP130, and IL-27 were mixed in a 1:1:1 ratio and subsequently purified by size-exclusion chromatography.

To generate biotinylated proteins for surface plasmon resonance studies, the GP130 sequence was subcloned into the pAcGP67-A vector with a C-terminal biotin acceptor peptide (BAP)–LNDIFEAQ-KIEWHW followed by a hexa-histidine tag. The purified protein was biotinylated with BirA ligase following a previously described protocol (Spangler et al, 2019). IL-27Rα was N-terminal biotinylated in vitro using EZ-Link Sulfo-NHS-SS-Biotin (Pierce) at pH 6.5.

Surface plasmon resonance

Surface plasmon resonance experiments were performed to determine the binding affinity of the recombinantly produced IL-27 and p28 to IL-27Rα and GP130. These were carried out on a Biacore T100 instrument (T200 sensitivity enhanced). C-terminal biotinylated mouse GP130 and mouse IL-27Rα were immobilized onto a SA sensor chip (GE Healthcare) at levels of ~100 response units (RU). The immobilization was performed in 10 mM HEPES, 150 mM NaCl, 0.02% (v/v) TWEEN-20, pH 7.2 buffer. Analysis runs were performed at 25°C in 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) TWEEN-20, pH 7.2, and 0.5% BSA. Mouse IL-27 binding was tested by injecting a 1:3 dilution series of inhibitor from a top concentration of 100 nM, and at a flow rate of 30 µl/min. Mouse p28 binding was tested in a similar manner, but from a top concentration of 20 µM and with a 1:2 dilution series. In both cases, the association time was set to 120 s, while the dissociation time was 600 s for IL-27 and 60 s for p28. Data analysis was performed using Biacore T200 Evaluation Software 3.0.

Phospho-flow analysis

For phospho-flow analysis of STAT1 and STAT3, mouse CD8 T cells were purified from a wild-type spleen, kindly provided by the Cantrell’s laboratory (Dundee), and were plated at a density of 2 × 105 cells per well in 50 µl in a 96-well V bottom plate. Cells were left unstimulated or stimulated with 3-fold serially diluted IL-
CryoEM sample preparation and data collection

Preformed IL-27:IL-27Rα:GP130 complex sample was diluted to 0.1 mg/ml and vitrified in liquid ethane using Vitrobot Mark IV (Thermo Fisher Scientific). Lacey Carbon Au 300 grids (EMS) were glow discharged in air using a Cressington 208 for 60 s prior to application of the sample. Four microlitre of sample were applied to the grids at a temperature of 20°C and a humidity level of 100%. Grids were then immediately blotted (force −2, time 2 s) and plunged-frozen into liquid ethane cooled to liquid nitrogen temperature.

Grids were imaged using a 300 kV Titan Krios transmission electron microscope (Thermo Fisher Scientific) equipped with K3 camera (Gatan) operated in super resolution mode. Movies were collected at 81,000× magnification and binned by two on the camera (calibrated pixel size of 1.06 Å/pixel) with a total dose of 50 electrons per Å² using EPU (Thermo Fisher Scientific, version 2.11.1.11) automated data software. The first dataset was collected at 0 degree tilt and consisted of 28,437 image stacks taken over a defocus range of −0.5 to −2.25 μm in 0.25 μm increments. As initial processing of the first dataset showed that the particles adopted a favored orientation, we used the cryoEF software to estimate the appropriate tilt angle for data collection and acquisition of missing views. Although the recommended tilt angle reported by cryoEF (Naydenova & Russo, 2017) was 37°, due to sample drift at high tilt, we collected 6,612 image stacks at 35° and 21,474 image stacks at 30°. Tilted images were taken over a defocus range of −1.5 to −2.7 μm in 0.3 μm steps. Movie stacks were collected with similar conditions as dataset-1. A summary of imaging conditions is presented in Table EV1.

CryoEM data processing

Dataset-1, which comprised 28,437 micrographs, was pre-processed using cryoSPARC (v.3.3.1; Punjani et al., 2017) patch motion correction and patch CTF. The micrographs were manually curated to remove movies with substantial drift and crystalline ice, resulting in a total of 17,228 micrographs going on for further processing. Deep learning models used in Topaz (v.0.2.5; Bepler et al., 2020) were used to automatically pick particles from these micrographs within the cryoSPARC workflow. We used the Topaz pre-trained model (ResNetNet16) for particle picking in the first instance. During the initial 2D classification, rare views were combined and used as input for a second round of Topaz training. Particles from both rounds were combined and duplicates were removed. A total of 3,575,367 particles were extracted at 2.12 Å/pixel (bin by 2) and subject to iterative 2D classification to remove ice contamination, carbon edges and broken particles, resulting in a total of 627,181 particles from dataset-1.

Dataset-2, which comprised 28,086 tilted image stacks, was pre-processed in cryoSPARC and curated as described for dataset-1. Of these 18,838 micrographs were used for particle picking in Topaz. A total of 4,652,258 particles were extracted at 2.12 Å/pixel (bin by 2) and subject to iterative 2D classification to remove ice contamination, carbon edges, and broken particles, resulting in a total of 1,184,217 particles from dataset-2.

Particles from dataset-1 and dataset-2 were merged and 1,811,398 particles were re-extracted at 1.06 Å/pixel and used to create an initial model using cryoSPARC’s ab initio reconstruction procedure with three classes. One class showed structural features consistent with the 2D classes and was used as an initial model for further processing. The total dataset (1,811,398 particles) was subjected to heterogeneous refinement with 5 classes. Each of these underwent nonuniform refinement and particles pertaining highest resolution class (496,334 particles) was taken forward. Of these, particles underwent further 2D classification implementing a probability threshold of 0.8 applied to each class. The probability threshold is defined as a value between 0 and 1 and excludes particles with smaller 2D class posterior probabilities compared with probabilities from the particles.alignments2D. The final 203,411 particles went into a round of nonuniform refinement followed by local refinement. All nonuniform refinement runs included optimization of per particle defocus and optimization of per group CTF parameters. The final map resolution 4.0 Å was assessed using the gold standard FSC at a threshold of 0.143 and locally filtered using cryoSPARC’s own implementation.

Model building and refinement

An initial model of IL-27 was generated by rigid body fitting each protein into the local resolution filtered maps sharpened using a B-factor of −100. Rigid body fitting was performed in Chimera (Petersen et al., 2004). The models used were murine p28 (AlphaFold Protein Structure Database Q8K316, 2022, https://alphafold.ebi.ac.uk/entry/Q8K316), murine IL-27Rx (AlphaFold Protein Structure Database O70394, 2022, https://alphafold.ebi.ac.uk/entry/O70394), murine EBI3 (AlphaFold Protein Structure Database O35228, 2022, https://alphafold.ebi.ac.uk/entry/O35228), and murine GP130 (AlphaFold Protein Structure Database Q00560, 2022, https://alphafold.ebi.ac.uk/entry/Q00560). Alphafold models were trimmed to reflect the domain boundaries in the constructs used and known glycans were added using the carbohydrate module in Coot (Emsley et al., 2010; Fig EV4).

The initial model of IL-27 was refined into the cryoEM map using ISOLDE (v.1.3; Croll, 2018) implemented in ChimeraX (v.1.3; Petersen et al., 2021). During refinement, we applied adaptive distance restraints to each subunit. A loop region of p28 (residues 178–194)
was removed to prevent steric clashes with GP130. The atomic models were refined using phenix.real_space_refine in Phenix (v.1.20.1-4487; Afonine et al., 2018) with secondary structure, reference model, and geometry restraints. B-factors were refined in Phenix. Model FSC validation tools and the overall quality of the model were assessed in the map using the cryoEM validation tools in Phenix and MolProbity (Williams et al., 2018; Table EV1). The map-model FSC was calculated using Refmac5 (Murshudov et al., 2011) and reported at a threshold of 0.5. Interface residues were defined by the server Pisa (Krissinel & Henrick, 2007).

Data availability

Data supporting the findings of this manuscript are available from the corresponding authors upon reasonable request. The datasets produced in this study are available in the following databases:

i) IL-27 receptor complex cryoEM map: Electron microscopy database EMD-14427 (http://www.ebi.ac.uk/EMDB-EMD-14427)

ii) IL-27 receptor complex structural model: Protein Data Bank PDB-7Z0L (https://doi.org/10.2210/pdb7Z0L/pdb) accession numbers for the EM map and models of the IL27-receptor recognition complex reported in this paper are EMD-14427 and PDB ID 7Z0L.

Expanded View for this article is available online.

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Author contributions

Yibo Jin: Formal analysis; investigation; writing – review and editing. Paul K Fyfe: Formal analysis; investigation; writing – review and editing. Scott Gardiner: Formal analysis; investigation; writing – review and editing. Stephan Wilmes: Formal analysis; investigation. Doryen Bubeck: Conceptualization; formal analysis; supervision; writing – original draft; writing – review and editing. Ignacio Moraga: Conceptualization; formal analysis; supervision; funding acquisition; writing – original draft; writing – review and editing.

In addition to the CRediT author contributions listed above, the contributions in detail are:

YJ conducted cryoEM work. SG and DB built and refined atomic models of the complex. PKF recombinantly expressed proteins and perform SPR studies. SW conducted signaling studies. DB and IM conceived the ideas. YJ, SG and DB analyzed cryoEM data. DB and IM wrote the manuscript. SG and YJ generated the figures. All authors assisted with manuscript editing.

Disclosure and competing interests statement

The authors declare that they have no conflict of interest.

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