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

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Abstract

Interleukin 27 (IL-27) is a heterodimeric cytokine that elicits potent immuno-suppressive responses. Comprised of EBI3 and p28 subunits, IL-27 binds GP130 and IL-27R α receptor chains to activate the JAK/STAT signalling 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) to solve the structure of the IL-27 receptor recognition complex. Our data show how IL-27 serves as a bridge connecting IL-27R α with GP130 to initiate signalling. While both receptors weakly bind the p28 component of the heterodimeric cytokine, EBI3 stabilizes the complex by binding a positively charged surface of IL-27R α . 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.

Introduction

IL-27 is an immunosuppressive cytokine involved in resolving T cell-mediated inflammation. IL-27 inhibits Th-17 responses ¹⁻³ and induces differentiation of T regulatory cells ⁴. T cell stimulation by IL-27 promotes secretion of the anti-inflammatory cytokine, IL-10, further contributing to a reduction in the inflammatory response ⁵. 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 ^{6,7}. Elevated levels of IL-27 gene signatures are found in cancer and are associated with poor prognoses ⁸. Understanding how IL-27 engages its cellular receptors to form a signalling 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) ⁹. Grouped within the IL-12 family of heterocytokines, p28 and EBI3 share sequence homology with two components of IL-12: p35 and p40, respectively ¹⁰. 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 ¹¹. These differences can be attributed to the engagement of GP130 by IL-27, which is shared across the IL-6 cytokine family ¹².

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 ¹³⁻¹⁷. 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 ^{18,19}. 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 ²⁰, Leukemia

inhibitory factor (LIF)²¹ and Ciliary neurotrophic factor (CNTF)²². The N-terminal immunoglobulin (Ig) domain of GP130 comprises an additional cytokine-binding interface that recognises an epitope at site 3, resulting in higher order assemblies with different stoichiometries^{20,23}. In the hexameric GP130:IL-6 signalling complex, two molecules of GP130 dimerize to trigger JAK/STAT activation²³. Here, the two low affinity site 3 interfaces are necessary to stabilize the complex and trigger receptor activation by IL-6. For GP130 complexes with LIF and CNTF cytokines, site 3 is occupied by the co-receptor LIF-R^{22,24}. Structural details for how GP130 forms a heterodimeric signalling complex by binding site 3 of a cytokine remains unresolved.

Here, we report a 3.8 Å resolution cryo-EM structure of IL-27 in complex with cytokine binding domains of GP130 and its co-receptor IL-27R α . This structure reveals a modular assembly mechanism that differs from those described for other members of the IL-12 and IL-6 families. IL-27 binds with high affinity to IL-27R α at site 2, which is further stabilized by electrostatic interactions between IL-27R α and EBI3. Subsequently, GP130 is recruited to the heterodimeric cytokine in a second step. Our structural and biochemical data explain how IL-27 coordinates two signalling receptors to modulate T cell mediated inflammation. Our results provide a blueprint for developing new therapeutics that target and tune IL-27 responses.

Results

Molecular architecture of the IL-27 cytokine recognition complex

IL-27 signals through dimerization of the cognate receptor IL-27R α and the shared receptor GP130²⁵. Current models for the assembly of complexes mediated by IL-27 have been largely based on structural principles derived from the IL-12/IL-23 or IL-6 systems. However, given differences in signalling outcomes between IL-27 and these two families, together with the growing importance of IL-27 as a therapeutic target, we used cryoEM to solve the structure of the IL-27 cytokine recognition complex.

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 linker²⁵. 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-27R α (Supplementary Fig. 1). We then used cryoEM to solve the structure of the complete IL-27 cytokine-recognition complex (Fig. 1, Supplementary Fig. 2 and Supplementary Fig.3). We used the ab initio reconstruction protocols within cryoSPARC²⁶ to generate an initial model of the complex. Maps were further refined using a combination of homogenous and heterogenous refinement procedures. The final map was refined to a reported resolution of 3.8 Å using the gold standard FSC 0.143 cut-off, however an analysis of the angular distribution of particles indicated strong preferential orientations which impacted the quality of the map in some directions. We therefore limit our interpretation of the structure to the assignment of individual domains with models derived from Alpha fold predictions²⁷ refined with strong adaptive distance restraints and geometric restraints imposed (Supplementary Table 1). The chirality of the helical bundle central to the p28 cytokine was used to assign the handedness of the reconstruction.

The overall structure of the IL-27 cytokine recognition complex exhibits a classical architecture with a helical cytokine bundle central to the assembly (Fig. 1). For the IL-27 complex, the helical bundle is sandwiched by two 'L'-shaped densities, analogous to other cytokine

complexes. However, unlike previous structures, the IL-27 cytokine recognition complex has an additional prong that contacts the back face of the heterodimeric cytokine.

Principle binding interfaces for IL27 cytokine recognition

Cytokines from the IL-12/IL-6 family have three highly-conserved principle binding interfaces: site 1, site 2 and site 3²³. For the IL-27 recognition complex, site 1 is occupied by the second half of the heterodimeric cytokine, EBI3 (Figs. 1a-c). EBI3 is comprised of two Fibronectin type III (FNIII) domains bent into an 'L'-shaped arrangement. Docking and minimal refinement of the EBI3 Alpha fold model into the map show that the bend of the EBI3 elbow is formed by a cluster of aromatic residues (EBI3: Y39, P40, F96, F159, and Y209), which create a hydrophobic groove recognized by p28:W93 (Fig. 1c). This feature of knobs and holes shape recognition is consistent with other cytokine binding interfaces²³. Our p28:EBI3 interface is supported by mutagenesis studies showing that equivalent residues in the human cytokine (EBI3:F97; p28:W97) are essential for cytokine complex formation²⁸. The same study identified an aspartic acid on EBI3 that also influences stability of the heterodimer²⁸. In our model, the equivalent murine residue (EBI3:D205) is nearby an arginine on p28 (p28:R217) and may form an additional salt bridge to stabilize the orientation of the heterodimer (Fig. 1c).

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)²⁰. To understand how GP130 recognizes chemically unique cytokines, we next investigated the IL-27 site 2 interface. By 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-27R α (Figs. 1a, b). Here we find that the knob and hole pattern is encoded by aromatic residues of IL-27R α (IL-27R α : P153, P152, and W151), which form a pocket to bind a tyrosine sidechain extending from p28 (p28:Y48) (Fig. 1d). In the IL-6:GP130 cytokine recognition complex, the equivalent tyrosine on IL-6 slots into a hydrophobic groove formed by the interface between D2 and D3 of GP130²⁰.

The position of EBI3 at site 1 and IL-27R α at site 2 are coordinated by a second interaction interface. EBI3 directly contacts the second CHR domain of IL-27R α . By contrast to the

hydrophobic knob and hole recognition motifs observed for p28, this secondary interface is dominated by electrostatic interactions (Figs. 1a, e, and Supplementary Fig. 4). Positively charged residues of EBI3 (EBI3: R169, R176, K192) face a negatively charged surface of IL-27R α (IL-27R α : 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 signalling^{20,29}. A crystal structure of GP130 in complex with IL-6 showed that GP130 can engage cytokines through both site 2 and site 3 interactions²⁰. 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. Due to the poor angular distribution of particle orientations and resulting resolution anisotropy, we were unable to model this density. Based on our biochemical characterization of the complex showing that all components are present (Supplementary Fig.1), we conclude that some or all of this density could be accounted for by GP130. Indeed, biochemical data confirm that GP130 engages p28 at site 3²⁸.

Kinetic drivers of IL- 27 signalling complex assembly

To understand the kinetic drivers underpinning assembly of the IL-27 signalling complex we defined the binding affinities for each of the subcomponents using surface plasmon resonance (SPR). First, we set out to identify which of the two signalling receptors bound the IL-27 heterodimer with higher affinity. We immobilized biotinylated IL-27R α or GP130 on a streptavidin SPR surface and passed a range of IL-27 concentrations to measure rates of association and dissociation (Figs. 2 a,b). We found that IL-27R α has a higher affinity (K_D : 0.29 nM) for the heterodimeric cytokine than GP130 (K_D : 3.1 nM), in agreement with biochemical data showing that GP130 engages the low-affinity binding site 3²⁸. These results suggest a two-step binding mode for IL-27 complex formation, where IL-27 binds IL-27R α with high affinity in a first step, and subsequently recruits GP130 with lower affinity to form the

signalling 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 signalling receptor immobilized to the streptavidin SPR chip (Fig. 2c, d). We observed only weak binding of p28 to IL-27R α (K_D : 2.8 μ M) and no binding to GP130 at the doses tested, consistent with weak signalling responses elicited by p28 when compared to IL-27 in CD8 T cells (Fig. 2e, f). Taken together, these data support a stabilizing role of EBI3 in formation of the IL-27 signalling complex.

Discussion

IL-27 is a model system for signalling by heterodimeric cytokines, including IL-12 and IL-23. A comparison of the heterodimeric IL-23 receptor complex (PDB: 6WDQ)³⁰ with our structure shows that EBI3 and the IL-12 β subunit (p40) overlay with site 1 (Fig. 3a). In addition, we observe that the GP130 density extending away from the core IL-27 complex has similarities to the IL-23 signalling 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 signalling receptor is a topological requirement for activation by IL-12, IL-23 and IL-27.

IL-27 is also a central member of the IL-6 family of cytokines that signal through GP130³¹. The diversity in cellular responses within the family stems in part from the strict transcriptional control of cytokines secreted by different cell types³². Although IL-27 is a heterodimeric cytokine, there are some instances whereby the two components are independently expressed^{33,34}. In the absence of EBI3, p28 can act as an antagonist for IL-6-mediated GP130 signalling¹³. Our biophysical data show that binding of p28 alone to GP130 is negligible (Fig. 2). A comparison of the IL-6 cytokine recognition complex (PDB:1P9M) with our structure shows that binding of EBI3 to site 1 overlaps with site 1 of the IL-6 (Fig. 3a). Previous studies have reported that p28 can bind IL-6R α ¹⁷. Though signalling by the putative p28:IL-6R α is weaker compared to IL-6 or IL-27, it may be possible that IL-6R α 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 receptors⁹ 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. 3a). 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-27R α . Unlike the IL-6 co-receptor (IL-6R α), the intracellular domains of GP130 and IL-27R α both associate with JAKs proteins and activate signalling upon dimer formation. In the case of IL-27, the cognate co-receptor IL-27R α could fill an analogous role to GP130 at site 2 in the IL-

6-signaling complex. In both systems site 1 is occupied by non-signalling proteins: EBI3 and IL-6R α . Our structural and biophysical data support role for auxiliary proteins at site 1 to enhance the stability of the signalling complex, while receptors occupying site 2 are responsible for signal transduction.

Signalling through the JAK/STAT pathway requires dimerization of signalling receptors. How that dimerization is achieved, and how the geometry of the signalling 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^{20,23}. For IL-6, dimerization of GP130 occurs through a hexameric assembly (2 copies of the IL-6:IL-6R α :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. 3b). In the IL-27 signalling complex, dimerization of IL-27R α 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 signalling by oncostatin M (OSM), an IL-6 family cytokine³⁵. 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 signalling competent conformation. Understanding how mixtures of cytokines influence signalling assemblies and downstream cascades will inform new strategies in protein engineering for fine-tune cytokine responses.

Material and methods

Protein expression and purification

Murine IL-27 was cloned as a linker-connected single-chain variant (p28+EBI3) as described in ³⁶ (Supplementary Fig. 1a). In the text we refer to this variant only as IL-27. Murine IL-27R α (amino acids 28-224) and GP130 (amino acid 23-319) ectodomains were cloned and expressed as described in ³⁷. 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 *Trichoplusiani ni* (High Five) cells grown in InsectXpress media (Lonza).

Protein purification was carried out using the method described in ³⁸. Hi-Five cells were pelleted with centrifugation at 2000 rpm, 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 200mM, 50mM and 1mM respectively. The precipitate formed was then removed through centrifugation at 6000 rpm. Nickel-NTA agarose beads (Qiagen) were added to the clarified media and the target proteins purified through batch binding followed by column washing in HBS buffer for murine IL-27R α and GP130 or HBS-Hi buffer (HBS buffer supplemented to 500mM NaCl and 5% glycerol, pH 7.2) for murine IL-27sc. Elution was performed using HBS or HBS-Hi buffer plus 200mM imidazole. Final purification was performed by size exclusion chromatography on an ENrich SEC 650 10 x 300 column (Biorad), equilibrated in HBS or HBS-Hi buffers. Concentration of the purified sample was carried out using 30kDa Millipore Amicon-Ultra spin concentrators. Recombinant proteins were purified to greater than 98% homogeneity. For cryoEM studies, IL-27R α , 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)–

LNDIFEAQKIEWHW followed by a hexa-histidine tag. The purified protein was biotinylated with BirA ligase following a previously described protocol ³⁸. IL-27R α was N-terminal biotinylated in vitro using EZ-Link Sulfo-NHS-SS-Biotin (Pierce) at pH 6.5.

Surface plasmon resonance

Surface plasmon resonance was used to determine the binding affinity of the recombinantly produced IL-27 and p28 to IL-27R α and GP130. Biotinylated IL-27R α and GP130 were immobilised onto the chip surface with streptavidin. Series S Sensor SA (GE Healthcare) chips were primed in 10 mM HEPES, 150 mM NaCl, 0.02% TWEEN-20, prior to immobilisation of the biotinylated receptor. Analysis runs were performed in 10 mM HEPES, 150 mM NaCl, 0.05% TWEEN-20 and 0.5% BSA. A Biacore T100 (T200 Sensitivity Enhanced) was used for measurement and Biacore T200 Evaluation Software 3.0 was used for data analysis.

Phospho-flow analysis

For phospho-flow analysis of STAT1 and STAT3, mouse CD8 T cells purified from a wildtype spleen were plated at a density of 2×10^5 cells per well in 50 μ L in a 96-well V bottom plate. Cells were left unstimulated or stimulated with 3-fold serially diluted IL-27sc or p28 variants (50 μ L per well) for 15 minutes at 37°C before fixation with 2% paraformaldehyde for 10 minutes at room temperature. Cells were washed in PBS and permeabilised in ice-cold 100% methanol and incubated on ice for a minimum of 30 minutes. Cells were fluorescently barcoded as previously described ^{37,39}. Briefly, a panel of 16 combinations of two NHS-dyes (Pacific Blue and DyLight800, Thermo) was used to stain individual wells on ice for 35 minutes before stopping the reaction by washing in PBS/0.5% BSA. Once barcoded, the 16 populations were pooled together for antibody staining. Cells were stained with anti-pSTAT3^{Alexa488} (Biolegend #651006) and anti-pSTAT1^{Alexa647} (Cell Signalling Technologies #8009). During acquisition, individual populations were identified according to the barcoding pattern and pSTAT3^{Alexa488} and pSTAT1^{Alexa647} MFI was quantified for all populations. MFI was plotted and sigmoidal dose response curves were fitted using Prism software (Version 7, GraphPad).

CryoEM sample preparation and data collection

Pre-formed IL-27:IL-27R α :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 seconds prior to application of the sample. 4 μ L 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 seconds) and plunge-frozen into liquid ethane cooled to liquid nitrogen temperature.

Grids were imaged using a 300kV a Titan Krios transmission electron microscope (Thermo Fisher Scientific) equipped with K3 camera (Gatan) operated in super resolution mode. Movies were collected at 81,000x magnification and binned by two on the camera (calibrated pixel size of 1.06 Å/pixel). Images were taken over a defocus range of -0.5 μ m to -2.25 μ m with a total accumulated dose of 50 electrons per Å² using EPU (Thermo Fisher Scientific, version 2.11.1.11) automated data software. A summary of imaging conditions is presented in Supplementary Table 1.

CryoEM data processing

28,437 micrographs were pre-processing using cryoSPARC (v.3.3.1)²⁶ patch motion correction and patch CTF. The datasets 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)⁴⁰ were used to automatically pick particles from these micrographs within the cryoSPARC workflow. We used the Topaz pre-trained model (ResNet16) 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. Of these, 628,386 particles were used to create an initial model using cryoSPARC's ab initio reconstruction procedure with three classes. By comparing the reconstructions, it was obvious

that one class represented a subcomponent of the complex. Particles from the other two classes (486,648 particles) showing similar structural features were merged and refined using nonuniform refinement. Particles were then re-extracted at 1.06 Å/pixel and movement within the ice was corrected using cryoSPARC's own implementation of local motion correction. The dataset was then subjected to heterogeneous refinement using six classes. All six classes were individually refined using homogeneous refinement. The three classes that refined to the highest resolution were pooled (289,489 particles) and were further refined using local refinement procedures manually centered on the core. The final map resolution 3.8 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 locally sharpened map generated by DeepEMhancer⁴¹. Rigid body fitting was performed in Chimera⁴². The models used were murine p28 (AlphaFold entry ID: Q8K3I6), murine IL-27Rα (AlphaFold entry ID: O703940), murine EBI3 (AlphaFold entry ID: O35228), and murine GP130 (AlphaFold entry ID: Q00560). Alpha fold models were trimmed to reflect the domain boundaries in the constructs used.

The initial model of IL-27 was refined into the cryoEM map using ISOLDE (v.1.3)⁴³ implemented in ChimeraX (v.1.3)⁴⁴. 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)⁴⁵ 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 MolProbity⁴⁶ (Supplementary Table 1).

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Figure legends

Figure 1. CryoEM reconstruction (a) and 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 signalling receptors, GP130 (red) and IL-27R α (yellow). EBI3 occupies site 1 on p28, while IL-27R α occupies site 2. (c-e) Interaction interfaces of the complex. (c) The hinge between the two CHR domains of EBI3 form a hydrophobic groove composed of aromatic residues (Y39, P40, F96, F159 and Y209) that is filled by W93 of p28. Residue EBI3: D205, which is important for assembly of the heterodimeric cytokine²⁸, is also present in the binding interface and could form a salt bridge with p28:R217. (d) IL-27R α binds site 2 of p28 at the apex of the elbow between its two CHR domains. The loops of IL-27R α form an aromatic pocket comprised of residues IL-27R α : W151, P152 and P153 in which the aromatic side chain of p28:Y48 slots into. (e) The orientation of IL-27R α at site 2 is stabilized by a second interaction interface with EBI3, which is dominated by electrostatic complementarity between the two domains (IL-27R α : E187, D133, E141, E137 and EBI3:R176, R169, K192). Key residues that likely mediate interactions at interfaces are shown as sticks.

Figure 2. Biophysical analysis of the assembly of the IL-27 receptor complex. (a-d)

For SPR measurements, IL-27R α or GP130 were immobilized on the chip surface by biotin-streptavidin interaction and IL-27 or p28 were flowed across the chip in solution. (a, b). Kinetic charts for IL-27R α (a) and GP130 (b) with inserts for affinity curves. Concentrations used are shown on the curves. K_{on} , K_{off} and K_D values are shown. (c, d). Equilibrium charts for IL-27R α (c) and GP130 (d) with inserts for affinity curves. Dose response curves for pSTAT1 (e) and pSTAT3 (f) in resting mouse CD8⁺ T cells. Cells were stimulated with mIL-27 or p28 for 15 minutes with the indicated doses. Data shown are the mean of four biological replicates with error bars depicting standard error of the mean.

Figure 3. Model for how the IL-27 receptor recognition complex compares with IL-23 and IL-

6 cytokine families. (a) (top panel) Surface rendering of the IL-23 receptor recognition complex (PDB: 6WDQ) coloured according to protein components: IL-23 receptor (red), IL-12 β (p40)

(purple), IL-23p19 (blue), IL-12R β 1 (orange). (middle panel) A comparison with IL-27 receptor complex shows that both GP130 (red) and IL-23 receptor protrude from the core cytokines, orienting domains similarly towards the membrane. Both EBI3 (purple) and the other component of the IL-23 heterocytokine, p40, occupy the site 1 epitope. (bottom panel) Surface rendering of the IL-6 receptor recognition complex (PDB: 1P9M) colored according to protein components: IL-6 (blue), IL-6R α (purple), GP130 (red). (middle panel) A comparison with the IL-27 receptor complex shows that the nonsignaling components of both complexes (EBI3 and IL-6R α) occupy site 1, while GP130 binds in a different way. For IL-6, GP130 binds site 2 through an interaction site located between the CHR domains, D2 and D3. While site 2 is of IL-27 is occupied by IL-27R α . **(b)** Surface rendering of the hexameric IL-6 assembly (PDB: 1P9M) colored according to protein components: IL-6 (blue), IL-6R α (purple), GP130 (red). GP130 bound to site 2 of one IL-6 molecule bridges a second cytokine by binding at site 3 to stabilize the complex.

Data Availability

Data supporting the findings of this manuscript are available from the corresponding authors upon reasonable request. The accession numbers for the EM map and models of the IL27-receptor recognition complex reported in this paper are XXX and XXX.

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Author Contributions

Y.J. conducted cryoEM work. S.G. and D.B. built and refined atomic models of the complex. P.K.F. recombinantly expressed proteins and perform SPR studies. S.W. conducted signalling studies. D.B. and I.M. conceived the ideas. Y.J., S.G and D.B. analyzed cryoEM data. D.B. and I.M. wrote the manuscript. S.G. and Y.J. generated the figures. All authors assisted with manuscript editing.

Competing Interests Statement

The authors declare that there are no competing interests.

a **b** **c** **d** **e** **Figure 1**

Figure 2

a**IL-23** **IL-27** **IL-6** **b****IL-6 hexameric assembly** **Figure 3**