Supporting Information

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SI Materials and Methods

Sequence Alignments, Molecular Modeling, and Docking. Orthologs of IL-27p28, ciliary neurotrophic factor (CNTF), cardiotrophin-like cytokine (CLC), neuropoietin (NP), IL-6, IL-12p35, IL-23p19, EBI3, CNTFRα, IL-6Rα, and IL-12p40 were retrieved from the Swissprot database. The multiple sequence alignments of cytokines and receptors were obtained using T-Coffee (1). IL-27p28 and EBI3 were modeled using Modeller (2) based on multiple sequence alignments and secondary structure predicted with PSIPRED (3). For IL-27p28, structural coordinates of human CNTF [Protein Data Bank (PDB) accession no. 1CNT] and IL-6 (PDB: accession no. 1P9M) were selected as molecular templates. CD loop models built using RosettaAbinitio and RosettaFragments softwares were integrated during the molecular modeling process and evaluated using Verify3D (nihserver.mbi.ucla.edu/Verify_3D/). The coordinates of an additional α-helix predicted in the AB loop were computed with the Biopolymer module of Insight II (Accelrys). For EBI3, the D1 domain was modeled on the basis of the structure of IL-6Rα (PDB: 1P9M) and of the IL-12/p40 subunit for the EF loop (PDB: accession no. 1F45); the D2 domain was based on the CNTFRα structure (PDB: accession no. 1UC6); and the angle between D1 and D2 and their assembly was based on the overall structure of IL-6Rα. All of the models were energy-minimized and quality-verified using the Profiles-3D and Procheck softwares (nihserver.mbi.ucla.edu/SAVES/). IL-27p28 and EBI3 were manually superimposed onto their respective homologs in the IL-6–IL-6Rα complex (PDB accession no.: 1P9M) to build preliminary models. These models were further refined with the molecular docking program HEX 5.0 (4) to optimize structural complementarities. The best scoring solution was energy-minimized with CHARMM (5) using the 100 steepest descent steps, followed by adapted basis Newton-Raphson minimization steps until a convergence gradient of 0.001 was reached. All of the models are available upon request.

Site-Directed Mutagenesis, Fusion Protein, and Bicistronic Expression Vectors. For the hyper human IL-27–protC construct (WT IL-27, W197A IL-27), the cDNA encoding human EBI3 was first amplified by PCR with an oligonucleotide encoding the $(G4S)_2$ flexible polypeptide in 3′ and cloned into the pcDNA3.1 vector (Invitrogen). The cDNA encoding the mature form of human IL-27p28 tagged with a C-terminal protein C epitope was cloned into the pcDNA3.1 vector containing the modified EBI3 cDNA. The hyper mouse IL-27–protC constructs were obtained by the same method (WT and W195A mIL-27). For the generation of a bicistronic expression vector (EBI3-p28 protC), the cDNAs encoding human IL-27p28 tagged with a protein C epitope and human EBI3 were cloned into the pIRES vector (Clontech). For the human soluble IL-27R construct, the cDNA encoding the extracellular portion of IL-27R was cloned in-frame upstream of the Myc-His tag in the pcDNA3.1/Myc-His vector. These constructs were subjected to site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis kit (Stratagene).

Cells, Reagents, and Protein Purification. Cos-7, HEK-293, and EB1 Epstein–Barr virus-transformed B cells were grown in RPMI medium 1640 supplemented with 10% FCS. The cytokine-dependent erythroleukemia TF-1 cell line was grown in the same culture medium supplemented with 1 ng/mL of human GM-CSF. The anti-protein C (HPC4) mAb and affinity matrix were purchased from Roche Diagnostics. The anti-V5 HRP mAb was from Invitrogen. The anti–tetra-His mAb was purchased from Qiagen. The anti-Flag M2, anti-Flag M2 HRP mAbs, and ConA were from Sigma-Aldrich. Phospho-Tyr701-STAT-1 and phospho-Tyr705-STAT-3 monoclonal antibodies were purchased from Cell Signaling. Polyclonal antibodies against STAT-1 and STAT-3 were obtained from Santa Cruz Biotechnology. The anti-EBI3 mAb was a kind gift of O. Devergne (UMR CNRS 8147, Hôpital Necker, Paris). Goat anti-rabbit peroxidase-labeled immunoglobulins were from Invitrogen. Recombinant human IL-27 with a poly-histidine tag (IL-27-His), IL-6, recombinant mouse IL-27, IL-6, and a Quantikine ELISA kit mIL-27 were purchased from R&D Systems. The Cos-7 cell line was transfected by the DEAE-dextran method with appropriate cDNAs and incubated for a 72-h culture period. HEK-293 cells were stably transfected with hyper mouse WT and W195A mIL-27 cDNAs and were cultured in 293 SFM II medium (Invitrogen). IL-27–type proteins were purified by affinity chromatography on an anti-protC affinity matrix, followed by an anionic HPLC column step.

Immunoprecipitation and Protein Phosphorylation Analysis. Cell supernatants were immunoprecipitated using anti-Flag M2 mAb at 10 μg/mL Complexes were isolated using beads coupled to protein A and subjected to Western blot analysis using an anti-V5 HRP mAb. Membranes were stripped and reblotted with an anti-Flag M2 HRP mAb. Supernatants containing IL-27R were incubated with WT and W197A EBI3-p28protC overnight at 4 °C. Complexes were isolated using nickel agarose beads followed by SDS/PAGE and Western blot. To analyze Δ_{13}E forms of IL-27, the samples were incubated with an anti-protein C (HPC4) mAb at 10 μg/mL Complexes were immunoprecipitated with protein A beads and subjected to Western blot analysis using anti-protein C and anti-EBI3 mAbs. For the STAT tyrosine phosphorylation assay, TF-1, EB1 cells or mouse splenocytes were stimulated for 10 min with the indicated cytokine. Cell lysates were analyzed by Western blot. For the inhibition assay, cells were incubated with increasing concentrations of human or mouse mutants of IL-27 for 30 min at 4 °C before activation.

Proliferation Assay and Flow Cytometry Analysis. TF-1 cells were seeded in 96-well plates at a concentration of 5.10^3 cells/well in RPMI medium 1640 containing 5% FCS and incubated for 72 h. Thymidine incorporation was measured as described (6). For the binding analyses of the W197A IL-27 mutant, EB1 cells were incubated for 30 min at 4 °C with WT or W197A IL-27, followed by an additional 30-min incubation step with the HPC4 mAb before adding a phycoerythrin-conjugated anti-mouse antibody. For the competing inhibition study, EB1 cells were incubated with increasing concentrations of W197A IL-27 for 30 min at 4 °C before addition of IL-27-His for 30 min. Then, an anti–tetra-His mAb was added to the cells for an additional 30-min incubation step before using a phycoerythrin-conjugated anti-mouse antibody for labeling. Fluorescence was analyzed using a FACScalibur flow cytometer (Beckton Dickinson).

Mouse Model of ConA-Induced Liver Injury. Eight-week-old male BALB/c mice obtained from Janvier were injected in the tail vein with 200 μg W195A mIL-27 or PBS. Fifteen minutes later, mice were injected with 3 mg/kg ConA or with PBS. Blood was collected by cardiac puncture and livers were harvested.

Real-Time PCR Analysis. RNAs were extracted using an RNeasy minikit (Qiagen) and reversed-transcribed into cDNA using random hexamer primers and SuperScript II reverse transcriptase (Invitrogen). The ΔCt method was used for quantification and housekeeping genes were used for multiple normalization as described previously (7).

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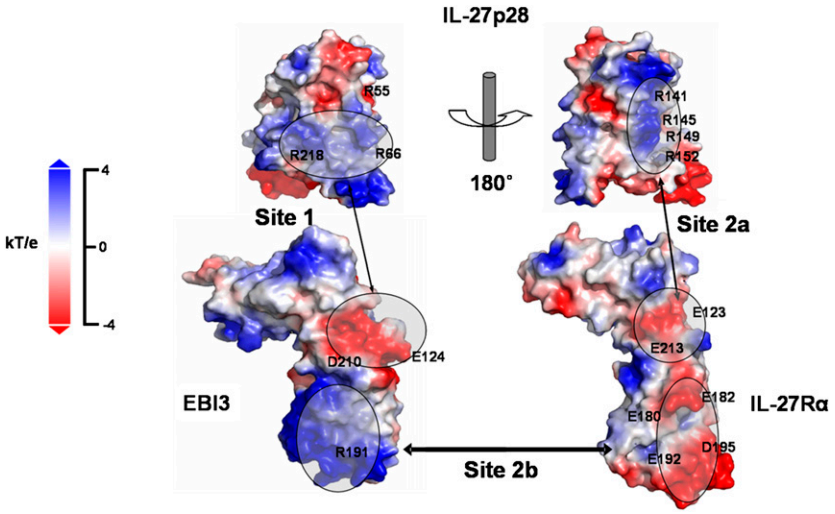

Fig. S1. Comparison of the Connolly surfaces of IL-27p28, EBI3, and IL-27R site 1, site 2a, and site 2b. The surfaces were collared according to their electrostatic potentials as computed with APBS (1). Sites 1, 2a, and 2b are delimited by circled areas on the cytokine/receptor surfaces.

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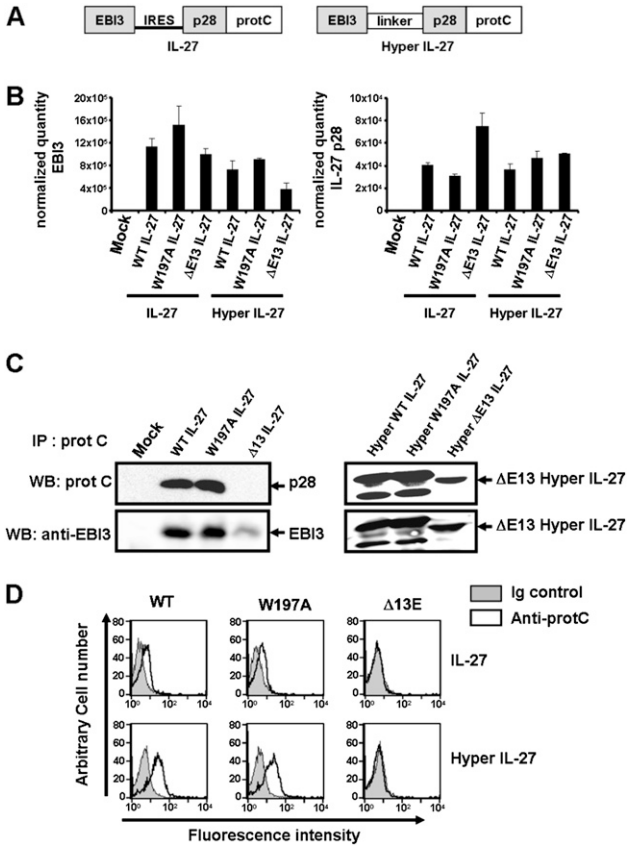

Fig. S2. IL-27p28 with 13 sequential glutamic acids deleted in the CD loop (aa 164 to aa 176) (ΔE13 IL-27) altered its secretion. Two ΔE13 IL-27 protC pcDNA3.1 constructs (hyper IL-27) and EBI3-IRES-p28 (IL-27) were synthesized. Mutagenesis PCRs on hyper IL-27 and IL-27 protC pcDNA3.1 were performed using the QuikChange Lightning Site-Directed Mutagenesis kit from Stratagene. Plasmids were purified (QIAprep Spin Miniprep kit, Qiagen) and ΔE13-deleted IL-27p28 ORFs and mutations were verified by sequencing (Quick Start kit, Beckman Coulter). (*A*) Expression of EBI3 (*Left*) and p28 (*Right*) in cell lysates were analyzed in qPCR. Results are expressed in relative quantity normalized with housekeeping genes. (*B*) Cell supernatants were analyzed by Western blot after an immunoprecipitation step of IL-27p28. (*C*) Binding of WT, W197A, and Δ13E IL-27 and hyper IL-27 on EB1 cells was analyzed by flow cytometry using an anti-protC mAb. Ig control histograms are in gray. (*D*) Binding of WT and modified forms of IL-27 and hyper IL-27 to EB1 cells was monitored by flow cytometry using an anti-tag mAb.