# Supporting Information

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## SI Materials and Methods

**Mice.** Eight- to 12-week-old, sex- and age-matched mice were used for all experiments in compliance with federal laws, institutional guidelines and have been approved by the Institutional Review Board and the Institutional Animal Care and Use Committee at the Rockefeller University. Inbred wild-type (WT) strains (NOD, C57BL/6J, BALB/cJ, 129 × 1/SvJ, CBA/J, and FVB/NJ), knockout strains on C57BL/6 background (IL-2, STAT6, TLR4, and Rag1), and knockout strains on BALB/c background (IL-4Rα) were obtained from Jackson Laboratories. Outbred WT strains (ICR and Swiss Webster) were obtained from Taconic Farms. IL-4Rα fl/fl LysMcre mice on BALB/c background (1) were generated by Frank Brombacher (University of Cape Town) and provided by Ajay Chawla (University of California, San Francisco). Fc gamma receptor (FcγR)-chain–deficient (2) and FcγRIIB deficient (3) mice were previously generated in this laboratory and bred to generate FcγR-deficient (FcγR/RIIB double-knockout) mice, lacking all classic activating and inhibitory FcγRs. IL-4Rα$^{+/-}$ mice were generated by mating WT and IL-4Rα$^{-/-}$ BALB/c mice. KRN TCR C57BL/6 mice (4) were generated and provided by Diane Mathis and Christophe Benoist (Harvard Medical School) and bred with NOD mice to generate K/BxN mice (5).

K/BxN serum was prepared and transferred as described (6), with the modification that C57BL/6 mice were administered one i.v. dose of 200 μL of serum, and BALB/c mice were administered 100 μL. IL-4 (Peprotech) was administered 30 min before K/BxN serum as a single i.v. injection of a cytokine:anticytokine complex [1:5 weight ratio: 2.5 or 5 μg of IL-4 and 12.5 or 25 μg of anti–IL-4 (clone 11B11; Biolegend)] with prolonged in vivo half-life as described (7, 8). Severity of arthritis was scored by several means: clinical examination of each paw (0, no swelling; 3, maximal swelling) was performed, adding up to a total clinical score (0–12) per mouse; increased paw volume was compared with noninjected mice by measuring the displaced fluid of all four paws immersed in a tube with a defined volume of fluid; functionality of paws (upside-down test) was measured by the average time (in seconds) mice hung upside down clinging to a mesh; H&E staining of paraffin-embedded paws were performed and scored by the Tri-Institutional Laboratory of Comparative Pathology and Genetically Engineered Mouse Phenotyping Service (Memorial Sloan–Kettering Cancer Center, New York). No K/BxN-mediated inflammation was seen in FcγR-deficient mice, and only low inflammation was seen in TNF-α receptor (TNF-αR)–deficient mice, showing their central role in this model. To address whether signaling downstream of these receptors were directly responsible for the IL-4Rα up-regulation seen during K/BxN-mediated inflammation, we had to use a mixed bone marrow (BM) chimeric approach.

BM transfer experiments were performed by transferring $10^6$ BM cells (either from one donor or a 1:1 mix of BMs) 4 h after lethally irradiating mice. Mice were kept on antibiotics (Neomycin trisulfate in drinking water and sulfatrim chow) for the week leading up to the transfer, as well as for 2 wk after irradiation/BM transfer. Experiments were performed 8 wk after irradiation/BM transfer. For surgical procedures, mice were anesthetized, and spleen was cauterized under sterile conditions. Peritoneum was then closed by sutures, and the skin was closed by staples. Sham-operated mice were used as control. Mice were kept on antibiotics (Neomycin trisulfate in drinking water and sulfatrim chow) for the week leading up to the surgery and were kept on it for >2 wk. Experiments were performed 4 wk after surgery. Anti-sheep IgG hybridomas were generated by standard techniques, by fusing SP2/0 myeloma cells with splenocytes from WT BALB/c immunized and boosted twice with 100 μg of sheep IgG (Jackson) in complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA), respectively. Three days before fusion, mice were given an i.v. boost with 10 μg of antigen in PBS. Three IgG1 clones (2E4, 2G4, and 3B10) were identified, subcloned, and shown to strongly bind nonoverlapping epitopes on sheep IgG by not competing with each other's binding.

Mice were also injected as indicated with TLR4-specific LPS [*Salmonella* Minnesota R595 (Re); Enzo Life Sciences; which, in contrast to many LPS preparations, do not engage TLR2), anti-CD3 (Low Endotoxin, Azide-Free clone 17A2; Bilegend). Poly(inosinic:cytidylic) acid (InvivoGen), Zymosan (InvivoGen), Alum (Thermo), IFA (BD Difco), and CFA (BD Difco), as well as preformed IgG immune complexes (IgGic) made by mixing sheep IgG (Jackson ImmunoResearch) with mouse IgG1 anti-sheep IgG clones 2E4, 2G4, and 3B10 (described above) at a 1:1:1:1 molar ratio.

**Flow Cytometry and PCR.** Single cell suspensions were prepared from peripheral blood and paws from mice and red blood cells lysed with lysis buffer (BD Biosciences). Paw cells were prepared by cutting isolated paws into small pieces in cold PBS with 10 μM EDTA, followed by filtration through a 70-μm mesh. Cells were Fc-blocked (clone 2.4G2) and stained with indicated antibodies, followed by analysis using a FACSCalibur or a LSR-II (BD Biosciences). Antibodies used (from BD Biosciences and Biolegend) were as follows: CD4 (clone GK1.5), CD8 (53-6.7), TCR-β (H57-597), B220 (RA3-6B2), CD11b (M1/70), GR-1 (RB6-8C5), CD49b (Dx5), CD45.1 (A20), CD45.2 (104), IL-4Rα (M1), or isotype control (R35-95). Also, dead cells were excluded by using 7-aminoactinomycin D (BD Biosciences) or DAPI (Invitrogen). RNA from FACS-sorted blood cells (using a FACSAria II; BD Biosciences) was prepared with RNeasy Mini kits (Qiagen) and reverse-transcribed by using the Verso cDNA synthesis kit (Thermo). Quantitative PCR was performed in a 7300 real-time PCR system (Life Technologies) with IL-4Rα–FAM and ACTB (beta-actin)–VIC primers (Applied Biosystems) in the same 20-μL reaction. IL-4Rα gene expression levels were determined by normalization to beta-actin levels.

**Ex Vivo Stimulations.** LPS serum was generated by injecting WT mice i.p. with 10–20 μg of LPS [*Salmonella* Minnesota R595 (Re); Enzo Life Sciences] and collecting sera 20 h later. Sera were pooled and stored in aliquots at −80 °C (activity, however, is not lost after several months of storage at 4 °C). Proteinase K treatment of LPS serum was performed by using Proteinase K–Agarose from Tritirachium album (Sigma; dissolved in 4 mL of Milli-Q water). Then, 400 μL of slurry was added to spin columns (Thermo) and washed three times with PBS before adding 40 μL of serum and incubating for 90 min at 37 °C with some movement. LPS serum was also incubated at the indicated temperatures for 45 min in a heat block. Organs (epididymal fat pads, lung, spleen, liver, kidneys, and BM) from LPS-injected WT mice were collected and cut into small pieces in DMEM with 10% FBS, antibiotics, glutamine, and beta-mercaptoethanol (Gibco). Organs were washed and cultured for 8 h before cell-free supernatant was collected by centrifugation. Organs from 10 mice were cultured in 50 mL of medium in T-175 flasks (BD).

Ex vivo stimulation of blood cells were performed by collecting blood from WT, TLR4$^{-/-}$, or myeloid differentiation primary response protein 88 (MyD88)/Toll/IL-1 resistance domain-

containing adaptor-inducing IFN-β (TRIF)$^{-/-}$ mice, followed by two rounds of red blood cell lysis with a lysis buffer (BD Biosciences). Cells equivalent to 50 μL of blood were plated in 96-well plates in 200 μL of DMEM with 10% FBS, antibiotics, glutamine, and beta-mercaptoethanol (Gibco). LPS serum, control serum, or supernatant from organs was added to cells, reaching a final concentration of 0.5% (serum) or 10% (supernatant from organs). After 18 h, IL-4Rα expression on indicated viable cell populations was analyzed by flow cytometry. Serum and lung supernatant was also preincubated with an anti-IL21 antibody (eBioscience; functional grade, clone FFA21) for 15 min before being added to cells at a final concentration of 50 μg/mL. WT blood cells were incubated with 10 or 1 μg/mL LPS [*Salmonella* Minnesota R595 (Re), Enzo Life Sciences].

Thioglycolate-elicited macrophages were isolated by collecting peritoneal exudate cells from mice injected i.p. with 1 mL of a sterile 3% thioglycolate solution (Difco fluid Thioglycolate medium; BD) 4 d earlier. Cells were dissolved in DMEM with 10% FBS, antibiotics, glutamine, and beta-mercaptoethanol (Gibco). A 0.5-mL cell suspension (1–5 × $10^6$ cells per mL) was plated in 24-well plates. After 2–4 h, nonadherent cells were removed, and fresh medium was added to cells. Experiments were performed with the adherent cells after being rested overnight.

Western blot for STAT6 phosphorylation was performed by using TLR4$^{-/-}$ thioglycolate-elicited macrophages exposed to 10% lung supernatant or not for 20 h. Cells were washed two times with serum-free medium (DMEM with antibiotics, glutamine, and beta-mercaptoethanol; Gibco) and kept in the same medium for 5 h before being exposed to mouse IL-4 (Peprotech) at concentrations of 20, 2, and 0.2 ng/mL After 15 min, medium was removed, and cells were lysed with 100 μL of radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors (Pierce). The supernatant was cleared by centrifugation and heated to 70 °C for 10 min in SDS loading buffer and reducing agent (Invitrogen). Samples with equal protein concentrations were then run on 4–12% BisTris gels (Invitrogen) and blotted to PVDF membranes (Immobilon-p; Millipore). After blocking (Pierce protein-free blocking buffer), membranes were stained with an anti–phospho-STAT6 antibody (MA1-41052; Thermo; diluted 1:500) followed by detection with a goat anti-mouse IgG HRP (Santa Cruz; dilution 1:5,000) and ECL substrate (Thermo). Membranes were stripped (Restore plus; Thermo) for 10 min and reprobed with a HRP-conjugated anti-actin antibody (Abcam). In between incubations with antibodies (1–2 h), membranes were thoroughly washed with PBS plus 0.2% Tween.


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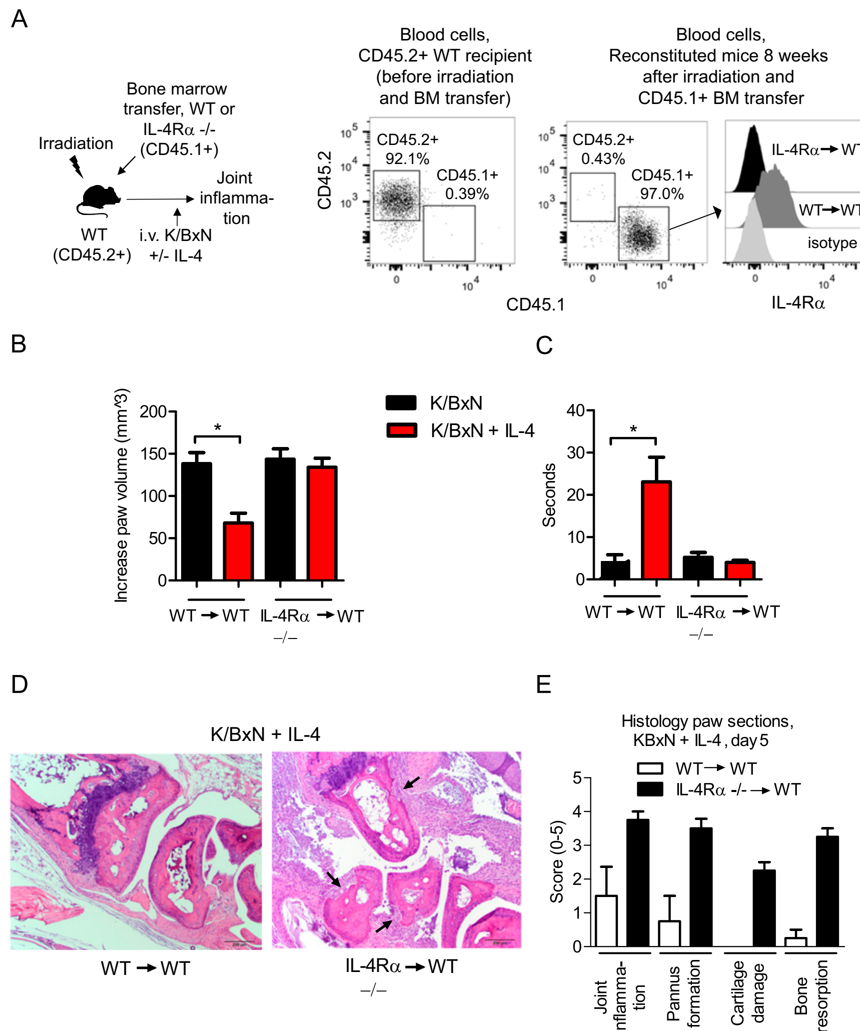


**Fig. S1.** BM transfer experiment (WT or IL-4Rα$^{-/-}$→WT). (*A*) Outline of BM transfer experiment where CD45.2+ WT mice were irradiated with 900 rad and reconstituted with either WT (WT→WT) or IL-4Rα$^{-/-}$ (IL-4Rα$^{-/-}$→WT) CD45.1+ BM cells. Eight weeks after irradiation/BM transfer, mice were injected with K/BxN and IL-4 or control. Flow cytometry plots show blood cells of recipient (CD45.2+) mice before and 8 wk after irradiation and transfer of WT or IL-4Rα$^{-/-}$ CD45.1+ BM. (*B*) Increase of the volume of all four paws at day 5 after K/BxN and IL-4 (red) or control (black) injection, compared with the volume of non-injected mice. (*C*) Upside-down test: A functional assay testing the ability of mice, injected with K/BxN and IL-4 (red) or control (black) 4 d earlier, to hang upside-down holding on to a mesh. Data are presented as the average time in seconds. (*D*) H&E staining of the intercarpal joint of BM-transferred mice injected with K/BxN and IL-4 5 d earlier. Arrows indicate areas of cartilage damage and bone resorption. (*E*) Blinded pathology scores (0–5) of H&E-stained paw sections of BM-transferred mice injected with K/BxN and IL-4 5 d earlier. *$P < 0.05$ by Mann–Whitney u test comparing IL-4–treated and control-treated mice at day 5 (*B*) and day 4 (*C*).

**Fig. S2.** IL-4Rα expression on hematopoietic cells. (*A*) Flow cytometry analysis of IL-4Rα expression on blood cells of different naïve mice strains. Data are shown as increase (percent MFI signal) of IL-4Rα expression compared with an isotype control. (*B*) IL-4Rα mRNA levels of sorted blood cell populations from naïve BALB/c mice. Data are shown as fold difference (percent) compared with neutrophil levels. (*C*) IL-4Rα expression on blood lymphocytes and CD11b+ cells in IL-4Rα fl/fl and IL-4Rα fl/fl LysMcre$^{+/-}$ mice 8 d after K/BxN injection. Samples from K/BxN-injected mice were used because CD11b+ cells in naïve mice express very low levels of IL-4Rα.

**Fig. S3.** IL-4Rα expression in K/BxN-injected mice. (*A*) Flow cytometry plots showing cells infiltrating the paws during K/BxN inflammation. (*B*) IL-4Rα mRNA levels of sorted blood cell populations from K/BxN-injected mice. Data are shown as fold change (percent) comparing indicated cells from naïve (–) and 5 d after K/BxN injection (K/BxN). (*C* and *D*) IL-4Rα expression on paw CD11b+ cells on day 5 following K/BxN or control injection in WT and IL-2$^{-/-}$ mice (*C*) and WT and STAT6$^{-/-}$ mice (*D*). (*E*) Flow cytometry plot showing blood cells from mixed BM chimeric mice 8 wk after 900-rad irradiation and transfer of a 1:1 mix of BM cells from CD45.1+ and CD45.2+ mice. (*F* and *G*) IL-4Rα expression on blood CD11b+ cells on day 5 following K/BxN or control injection, gated as described in *E*. (*F*) CD45.1+ WT and CD45.2+ TNF-αR$^{-/-}$ mixed BM chimeras. (*G*) CD45.1+ WT and CD45.2+ FcγR$^{-/-}$ mixed BM chimeras.

**Fig. S4.** IL-4Rα$^{+/-}$ mice. (*A*) IL-4Rα expression on indicated blood cell population in WT, IL-4Rα$^{+/-}$, and IL-4Rα$^{-/-}$ mice injected with K/BxN serum. Samples from K/BxN-injected mice were used because neutrophils and inflammatory monocytes express very low levels of IL-4Rα in naïve mice. (*B*) Upside-down test: A functional assay testing the ability of mice, injected with K/BxN and IL-4 (red) or control (black) 4 d earlier, to hang upside-down holding on to a mesh. Data are presented as the average time in seconds. $^{*}P < 0.05$ by Mann–Whitney u test comparing IL-4–treated and control-treated mice at day 4.

**Fig. S5.** Inflammation up-regulates IL-4Rα selectively on myeloid cells. (*A* and *B*) IL-4Rα expression on blood neutrophils (*A*) and lymphocytes (*B*) 20 h after the injection of indicated reagents in WT mice as described in Table 1. (*C*) Preformed IgG immune complexes were made by mixing sheep IgG and three anti-sheep IgG monoclonal mouse IgG1 antibodies (clones 2E4, 2G4, and 3B10; generated by immunizing mice with sheep IgG) at a 1:1:1:1 molar ratio. Injecting these immune complexes at the indicated dose resulted in an FcγR3-dependent IgG anaphylaxis with a dramatic transient drop in core body temperature.

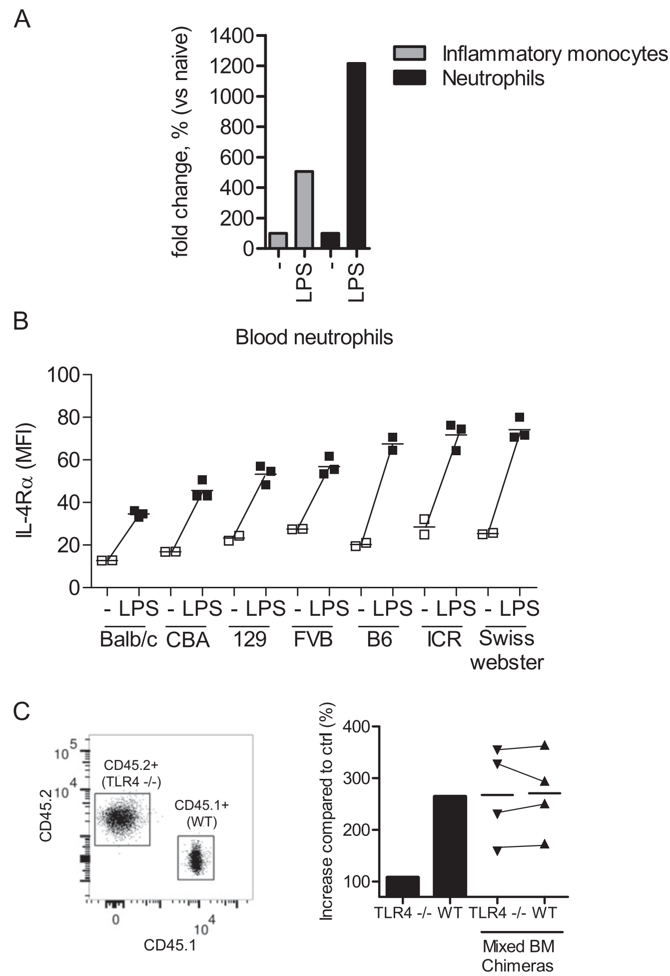


**Fig. S6.** LPS-mediated IL-4Rα up-regulation on blood myeloid cells. (*A*) IL-4Rα mRNA levels of sorted blood cell populations from LPS-injected mice. Data are shown as fold change (percent) comparing indicated cells from naïve (–) and 20 h after LPS injection (LPS). (*B*) IL-4Rα up-regulation on blood neutrophils 20 h after injection of LPS in different mice strains. Lines connect the IL-4Rα expression in naïve (–) and LPS-injected (LPS) mice from the same strain. (*C*) IL-4Rα expression levels on WT and TLR4$^{-/-}$ myeloid blood cells in LPS-injected 1:1 mixed BM chimeric mice, irradiated and reconstituted 8 wk earlier with CD45.1+ WT and CD45.2+ TLR-4$^{-/-}$ BM cells. (*Right*) Lines connect cells of indicated genotype in individual mice and are compared with TLR4$^{-/-}$ and WT mice, also injected with LPS.

**Fig. S7.** Ex vivo stimulation of blood cells with serum from LPS-injected mice. (*A*) Pooled sera from LPS- or control-injected mice were added, at two different concentrations, to ex vivo cultures with MyD88/TRIF$^{-/-}$ blood cells. At 18 h later, IL-4Rα expression on myeloid cells was measured by flow cytometry. (*B*) Control or LPS serum (0.5%), as well as two different concentrations of LPS, was added to WT blood cells and incubated for 18 h. IL-4Rα expression on myeloid cells was then measured by flow cytometry. (*C*) LPS and control serum was incubated at the indicated temperatures for 45 min. The sera were then added to ex vivo cultures with TLR4$^{-/-}$ blood cells, and the ability to up-regulate IL-4Rα on myeloid cells was measured by flow cytometry 18 h later. (*D*) Sham-operated or splenectomized mice were injected with control or LPS 4 wk after the operation, and sera were collected 20 h after the injection. The ability of the sera to up-regulate IL-4Rα on myeloid cells from TLR4$^{-/-}$ blood cells was then tested in the described ex vivo culture system. (*E*) TLR4$^{-/-}$ blood cells were incubated ex vivo with LPS serum, with control or a neutralizing anti–IL-21 antibody, as well as with recombinant IL-21 for 18 h, and IL-4Rα expression on myeloid cells was measured by flow cytometer.