Supplementary Methods

Animals

Flt3L$^{-/-}$ mice, maintained on a C57BL/6 background, were a kind gift of Prof. S.E. Jacobsen (University of Oxford, Oxford, United Kingdom). They were housed under specific pathogen-free conditions at the animal facility of the Academic Medical Center/University of Amsterdam (Amsterdam, The Netherlands). Feeding was *ad libitum*. The Institutional Animal Care and Use Committee of the Academic Medical Center approved all experiments.

Induction and assessment of CIA

Mice were immunized as previously described. Briefly, chicken CII (cCII; Sigma Chemical Co, St Louis, Missouri) was dissolved in 0.1 M acetic acid to a concentration of 2.0 mg/ml by overnight rotation at 4°C and mixed with an equal volume of Freund's complete adjuvant (2.5 mg/ml of *Mycobacterium tuberculosis*; Chondrex, Redmond, Washington, DC). The mice were immunized intradermally at the base of the tail with 100 µl of emulsion on day 0. The same injection was repeated on day 21. Mice were inspected three times a week for signs of arthritis by two independent observers who were not aware of the animal's genetic background. The severity of the arthritis was assessed using an established semiquantitative scoring system of 0–4 where 0=normal, 1=mild swelling, 2=moderate swelling, 3=swelling of all joints and 4=joint distortion and/or rigidity and dysfunction.(1) The cumulative score for all four paws of each mouse (maximum possible score 16) was used as the arthritis score to represent overall disease severity and progression in an animal. For the evaluation of incidence, mice were considered to have arthritis if the clinical arthritis score remained stable or increased by at least one point for two consecutive days. **All animals were sacrificed on acute phase, day 43 (experiment 1), or on chronic phase day 60 (experiment 2), of the**

disease. We used the terms acute and chronic in order to discriminate between induction (earlier phase- acute) and the effector phases (later phase- chronic) of CIA respectively. The onset of CIA usually occurs between days 28 and 35, and resolution of the arthritis occurs after several months, when the joints ankylose. The onset of arthritis was between days 28 and 33 in 80% of the mice utilized in this study.

Histological analysis

Two independent observers assessed the tissue for the degree of synovitis and cartilage degradation by microscopic evaluation, under blinded conditions, as described previously.(2;3) Synovitis and cartilage degradation in the knee joints were graded on a scale of 0 (no inflammation) to 3 (severely inflamed joint) based on the extent of infiltration by inflammatory cells into the synovium. Sections were also stained with safranin O-fast green to determine depletion of proteoglycans. Safranin O staining was scored with a semiquantitative scoring system of 0 (no loss of proteoglycans) to 3 (complete loss of proteoglycans) in the knee joint.(2)

Cell staining and flow cytometry

Single-cell suspensions were obtained from spleen, inguinal and axilary LNs. Spleen erythrocytes were removed. Cells were stained with the indicated fluorochrome-conjugated antibodies for surface markers and intracellular cytokines. The antibodies fluorescein isothiocyanate (FITC)–conjugated anti-CD44, allophycocyanin (APC)–conjugated anti-FoxP3, Alexa 700–conjugated anti-CD3; phycoerythrin (PE)–conjugated anti-CD25, eFluor Alexa780-conjugated anti-CD8, FITC-conjugated anti-CD44, PercP Cy5,5-conjugated anti-CD62L, PE–conjugated anti-ICOS, PerCP-Cy5.5–conjugated anti-B220,

FITC–conjugated anti-MHCII, PE-Cy7–conjugated anti-CD11C, Alexa 700–conjugated anti-CD11b, 488–conjugated IL-17, APC-conjugated anti-TNFα, PerCP-Cy5.5–conjugated anti-interferon-γ (IFNγ) and PE-conjugated anti-IL10 were obtained from eBiosciences. PE-Cy7–conjugated anti-CD4, APC-conjugated anti–IL-2, Alexa 488-conjugated anti–IL-4, and PE-conjugated anti–IL-10 were from BD Biosciences. FoxP3 intracellular stainings were performed according manufacturer's instructions/recommendations. For assessment of T cell cytokine expression, cells were stimulated for 2 hours with phorbol myristate acetate (PMA; 10 ng/ml;Sigma-Aldrich) and ionomycin (1 μM; Sigma-Aldrich). Brefeldin A (10 μg/ml; Sigma-Aldrich) was added for the final 4 hours of stimulation, and cells were harvested and stained with CD3, CD4 and CD8 antibodies. Cells were then fixed and permeabilized using Cytofix/Cytoperm (BD Biosciences) and labeled intracellularly with antibodies against the cytokines IL-2, IL-4, TNF-α, IFN-γ, IL-10 and IL-17. Cell surface markers and cytokine expression was monitored using Canto flow cytometer (BD Biosciences). **Doublet discrimination was performed by plotting the area (FL-A) of the fluorescence light pulse against the width (FL-W).**

Measurement of antigen-specific antibodies and their affinity in serum

A 96-well plate was coated with cCII (5 μg/ml; Chondrex Inc. Redmond, WA) and incubated overnight at 4°C. Non-specific binding was blocked with phosphate-buffered saline containing 1% bovine serum albumin for 1 h at room temperature. Serial dilutions of standard antibody solution (mouse anti-type II collagen IgG1 or IgG2a both 10 ng/ml, Chondrex) and serum samples were incubated for 1 h at room temperature, followed by the addition of horseradish peroxidase-conjugated rat anti-mouse IgG1 or IgG2a (BD Biosciences, San Jose,

CA). After 1 h of incubation at room temperature, tetramethylbenzidine substrate was added and the optical density at 450/540 nm was measured using a microplate reader.

T cell proliferation in vitro

CD4 T cells (OT-II T cells) were isolated from pooled LNs and spleen of OT-II mice using a $CD4^+$ T cell-positive isolation kit (Dynal, Invitrogen). Purity was determined by staining with CD3, CD4, and CD8. Purity was higher than 95%. For CFSE labeling, purified OT-II T cells were resuspended in PBS containing 2.5 mM CFSE (Molecular Probes) for 10 min at 37C. Bone marrow DCs (BMDC) were generated based on a modified protocol described previously.(4) In brief, 2×10^6 bone marrow cells were cultivated for 10 days in RMPI supplemented with 20 ng/ml rmGM-CSF (Fisher Scientific), 10% FCS, and 50 µM β-mercaptoethanol; one volume of fresh medium was added at day 3, and on days 6 and 9 one volume was replaced by fresh medium. Analysis of cells obtained with this procedure revealed that >90% expressed CD11c with 50%–60% showing high levels of MHCII. WT or $Flt3L^{-/-}$ BMDC were pulsed for 2 h at 37°C with 10ug OVA (Sigma). After washing, pulsed BMDCs were cultured with CFSE-labeled OT-II T cells in a 1:4 ratio in 200 ul RPMI 1640 supplemented with 10% FCS, 1 mM sodium pyruvate, 2 mM glutamine, and 50 mM 2-ME. After 3 days of culture, T cell proliferation was measured by FACS as a loss of CFSE staining. The percentages of dividing OT-II T cells were determined.

Reference List

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