

ONLINE SUPPLEMENTARY METHODS

Antigen-induced arthritis (AIA)

Adult (8-12 week old) mice were immunized with a subcutaneous injection of 100 µl mBSA (1 mg/ml; Sigma-Aldrich), emulsified in an equal volume of Complete Freund's Adjuvant (CFA). Mice also received an intraperitoneal administration of heat-inactivated *Bordetella pertussis* toxin (160 ng; Sigma-Aldrich). After 7 days, mice were re-immunized with a second identical subcutaneous administration of mBSA in CFA. Twenty-one days after the initial immunization, inflammatory arthritis was triggered by intra-articular administration of 10 µl mBSA (10 mg/ml) into the right knee joint. Animals were monitored daily for arthritis development by measuring knee joint diameters using a POCO 2T micrometer (Kroeplin). Mice were culled at defined time-points and joint pathology assessed histologically. Accumulative arthritic scores were generated according to the severity of synovial infiltration, exudate, hyperplasia, and joint erosion (see below).

Histopathology and immunohistochemistry

Knee joints were fixed in neutral buffered formalin (10% v/v) and decalcified in 10% (v/v) formic acid at 4°C before embedding in paraffin. Midsagittal serial sections (7 µm thick) were stained with haematoxylin, safranin-O and fast green. Two independent observers, blinded to the experimental groups, scored the sections for subsynovial inflammation (0 = normal, to 5 = ablation of adipose tissue due to leukocyte infiltrate), synovial exudate (0 = normal, to 3 = substantial number of cells with large fibrin deposits), synovial hyperplasia (0 = normal 1-3 cells thick, to 3 = over three layers thick with overgrowth onto joint surfaces with evidence of cartilage/bone erosion), and cartilage/bone erosion (0 = normal, 3 = destruction of a significant part of the bone). The aggregate score for all parameters is presented as the arthritic index (AI).

Synovial T-cell infiltration was detected in paraffin sections using a rabbit antibody to murine CD3 (DakoCytomation). Antigen unmasking was achieved by heating in 10 mM sodium citrate buffer containing 0.05% Tween 20 (95°C, 40 min). Endogenous peroxidase and biotin activity was blocked using 3% H₂O₂ and an avidin/biotin blocking kit (Vector Laboratories) respectively. Sections were incubated in 10% (v:v) swine serum for 1 hour before overnight antibody incubation at 4°C. Antibody labelling was detected using a biotinylated swine anti-rabbit secondary antibody (DakoCytomation), the Vectastain ABC kit and diaminobenzidine chromagen (Vector Laboratories). Two independent observers scored the degree of CD3 staining from 0 (no staining) to 4 (large number of positive cells) as described previously¹³.

Flow cytometry

For intracellular cytokine detection, *in vitro* cell cultures or single cell suspensions prepared from inguinal lymph nodes were treated with PMA (50 ng/ml), ionomycin (500 ng/ml) and monensin (3 μ M) for 4 hours at 37°C. Cells were stained with antibodies to cell surface CD4 (clone RM4-5), fixed and permeabilised using Cytofix/Cytoperm (BD Biosciences), before cytokine detection using anti-IFN- γ (XMG1.2) and anti-IL-17A (TC11-18H10.1). Cells were acquired on a CyAn ADP (Beckman Coulter) or FACSCanto II flow cytometer (BD Biosciences) and analysed using FlowJo (Tree Star Inc.).

Enzyme-linked immunosorbent assay (ELISA).

IL-17A levels were quantified in cell culture supernatants using a commercial murine IL-17A ELISA (Duoset kit from R&D Systems).