Supporting Information

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SI Materials and Methods

Isolation of Endothelial Cells from the Synovial Tissue of the Joints of Arthritic Rats. The synovial tissue harvested from the ankle joints of arthritic rats was digested for 1 h at 37 °C in HBSS containing 3 mg/mL of collagenase 1A (Sigma-Aldrich), 1 mg/ mL of hyaluronidase IV-S (Sigma-Aldrich), 0.1 mg/mL of DNase II (Sigma-Aldrich), 1% FBS, and 5% Hepes. The digested tissue was filtered through a nylon mesh and washed extensively with PBS. Magnetic bead separation was used to isolate the $CD31^+$ endothelial cells. In brief, the suspended cells were incubated on ice with mouse anti-rat CD31 antibody (BD PharMingen) for 5 min, followed by the addition of rat anti-mouse IgG1 microbeads (Miltenyi Biotec). After 15 min, the cell suspension was loaded onto a MACS column placed in the magnetic field of a MACS separator (Miltenyi Biotec). The unlabeled cells were washed away, and the labeled cells were eluted and collected as the selected cell fraction.

Immunohistochemical Examination of Cells/Tissues. Endothelial cells from the synovial tissue and other control tissues were seeded on coverslips in M199 medium plus 1% BSA for 2 h, followed by the addition of fluorescein-conjugated peptide (5 μmol/L) onto the cells. After incubation on ice for 30 min, the cells were washed three times with PBS and then incubated with anti-rat CD31 antibody (1:100) overnight at 4 °C, followed by incubation with the secondary antibody, Alexa Fluor–conjugated goat anti-mouse IgG (1:1,000). The slides were washed three times with PBS, mounted with Prolong Gold anti-fade reagent, and examined under microscope. For detection of phage in tissues, rats were injected with 10^9 pfu of phage and perfused under anesthesia as described above. The hind paws of rats were removed and tissue sections were prepared as described below. An antibody against T7 phage was used for staining, followed by staining with peroxidase-conjugated secondary antibody.

Induction and Evaluation of AA. AA was induced in Lewis rats by immunizing them with 1 mg/rat of heat-killed *M. tuberculosis* H37Ra (Mtb; Difco) in 200 μL of mineral oil (Sigma-Aldrich) via s.c. injection at the base of the tail. Beginning on day 7 after immunization, the rats were observed regularly for signs of arthritis in the paws. The severity of arthritis was evaluated based on erythema and swelling as described previously (1). The highest score for each paw was 4, and the total maximum score for each rat was 16. The course of AA in the Lewis rat involved the following phases: incubation (days 0–8), onset (days 9–11), peak (days 15–18), and recovery (days 21–27).

Histopathological Evaluation of Hind Paw Joints of Rats. Hind paws of rats were harvested, skinned, and fixed with 10% phosphate-buffered formalin (Fisher Scientific) for 3–7 d (1). Then the paws were decalcified in formic acid (Fisher Scientific), embedded in paraffin, and sectioned longitudinally (820 microtome; Fisher Scientific). Tissue sections (5 μm) were placed on a glass slide, stained with H&E (Sigma-Aldrich), and assessed for morphological changes and cellular infiltrates.

Testing the Effects of Peptides on Tubule Formation. HUVECs (1×10^4) were cultured in 80 μL/well of growth factor-reduced Matrigel (BD Biosciences) in a 96-well plate in 100 μL/well of F-12K medium (with no supplements). These cells were then treated for 20 h with VEGF (R&D Systems) in the presence or absence of different concentrations of peptides (NQR, ADK, or RGD). Tubule formation was observed under a microscope, quantitated, and photographed.

Determining the Effects of Peptides on Cell Signaling. HUVECs were grown in F-12K medium in the presence of endothelial cell growth supplement (ECGS) (BD Biosciences) and 15% FBS. Confluent cells were starved in basal medium (without ECGS and FBS) for 6 h. Then the cells were pretreated for 1 h with different concentrations (10, 50, and 100 μg/mL) of a peptide (NQR, ADK, or RGD) in fresh basal medium, followed by treatment with VEGF (10 ng/mL) for another 20 min. The conditional medium was discarded, and the cells were lysed using a lysis buffer containing protease inhibitors and phosphatase inhibitors. The cell lysate was then centrifuged, and the resulting supernatant was used for analysis of signaling molecules using specific antibodies to human pERK1/2, ERK1/2, pAkt, Akt, and GAPDH (Cell Signaling Technology) by Western blot analysis (1). The intensity of the bands in the gel was quantified by densitometry. The levels of p-ERK1/2 and p-Akt were normalized to total ERK1/2 and Akt, respectively, and compared with cells in serum-free medium.

Statistical Analysis. Data are expressed as mean ± SEM of multiple experiments. ANOVA and the unpaired Student *t* test were used for statistical analysis. A P value < 0.05 was considered significant.

1. Rajaiah R, Puttabyatappa M, Polumuri SK, Moudgil KD (2011) Interleukin-27 and interferon-γ are involved in regulation of autoimmune arthritis. *J Biol Chem* 286: 2817–2825.

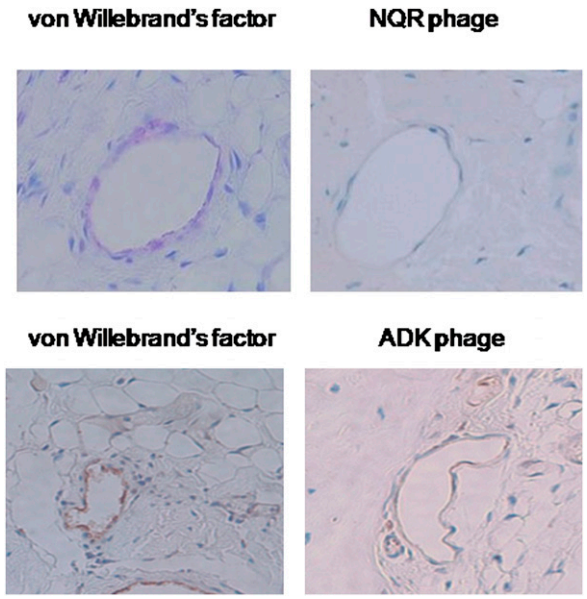

Fig. S1. Lack of binding of NQR/ADK-encoding phage to the uninflamed joints of naïve Lewis rats. Binding of the phage encoding the NQR peptide (*Upper*) or the ADK peptide (*Lower*) to the joints of naïve Lewis rats was visualized by immunohistochemical examination of the hind paw section using the anti-T7 phage antibody. Anti–von Willebrand factor staining was used as a marker for the vascular endothelium.

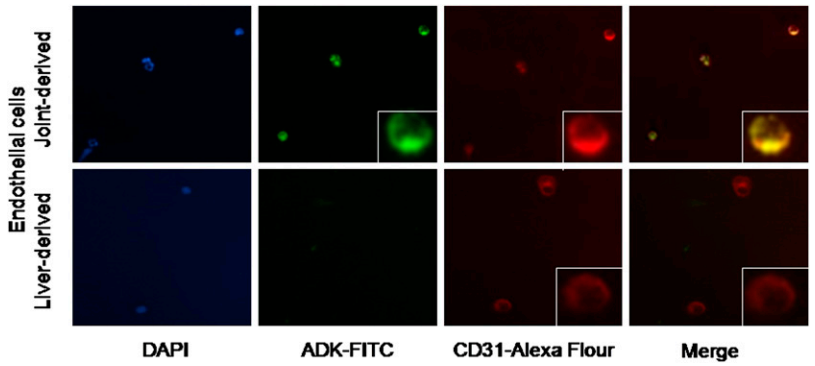

Fig. S2. The phage-encoded peptide ADK colocalizes with CD31 on endothelial cells of the inflamed joint. CD31-expressing endothelial cells were isolated from arthritic rat joints (*Upper*) or the liver (*Lower*) and stained with DAPI (blue), FITC-labeled peptide ADK (green), or Alexa Fluor 594–labeled anti-rat CD31 antibody (red). Also shown is the staining overlay (merge) of green and red (yellow). (*Inset*) Enlarged view of a single cell. (Original magnification, 20×.)

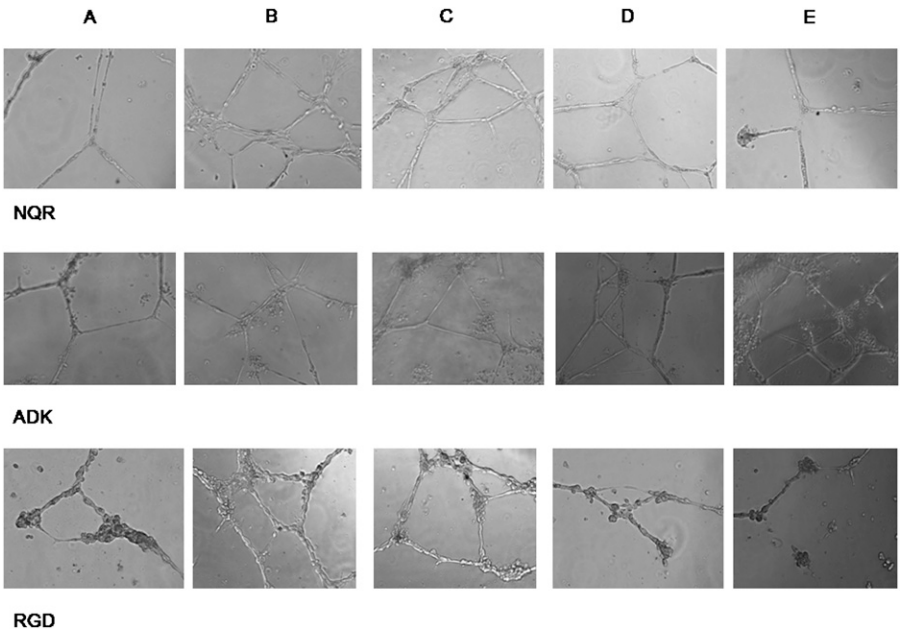

Fig. S3. Inhibitory effect of phage-encoded peptides on endothelial tube formation. HUVECs were cultured on Matrigel-coated wells for 24 h in the presence of VEGF (10 ng/mL) and various concentrations (0–100 μg/mL) of NQR peptide (*Top*), ADK peptide (*Middle*), or RGD peptide (*Bottom*), and the branches of vessels were counted. A representative set of results with each peptide is shown. A, basal medium; B, 0 μg/mL; C, 10 μg/mL; D, 50 μg/mL; E, 100 μg/mL.