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Blockade of Vascular Endothelial Growth Factor Receptor I (VEGF-RI), but not VEGF-RII, Suppresses Joint Destruction in the K/BxN Model of Rheumatoid Arthritis

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It was recently shown that vascular endothelial growth factor (VEGF), a growth factor for endothelial cells, plays a pivotal role in rheumatoid arthritis. VEGF binds to specific receptors, known as VEGF-RI and VEGF-RII. We assessed the physical and histological effects of selective blockade of VEGF and its receptors in transgenic K/BxN mice, a model of rheumatoid arthritis very close to the human disease. Mice were treated with anti-mouse VEGF Ab, anti-mouse VEGF-RI and -RII Abs, and an inhibitor of VEGF-RI tyrosine kinase. Disease activity was monitored using clinical indexes and by histological examination. We found that synovial cells from arthritic joints express VEGF, VEGF-RI, and VEGF-RII. Treatment with anti-VEGF-RI strongly attenuated the disease throughout the study period, while anti-VEGF only transiently delayed disease onset. Treatment with anti-VEGF-RII had no effect. Anti-VEGF-RI reduced the intensity of clinical manifestations and, based on qualitative and semiquantitative histological analyses, prevented joint damage. Treatment with a VEGF-RI tyrosine kinase inhibitor almost abolished the disease. These results show that VEGF is a key factor in pannus development, acting through the VEGF-RI pathway. The observation that in vivo administration of specific inhibitors targeting the VEGF-RI pathway suppressed arthritis and prevented bone destruction opens up new possibilities for the treatment of rheumatoid arthritis. The Journal of Immunology, 2003, 171: 4853–4859.

Rheumatoid arthritis (RA) is a painful and disabling disease of unknown origin. The hallmark of RA is synovial cell proliferation in response to inflammatory stimuli, leading to the formation of a very aggressive tissue called the rheumatoid pannus. Expansion of the pannus induces bone erosion and cartilage thinning, leading to loss of joint function. The rheumatoid pannus can thus be considered a local tumor.

One of the earliest phenomena observed in synovitis is the development of new vessels in the synovium, delivering nutrients, oxygen, and cells to the proliferating pannus (1). It was recently demonstrated that vascular endothelial growth factor (VEGF) plays a pivotal role in RA (1–3). VEGF is a growth factor for endothelial cells, it also increases vascular permeability. VEGF signals through two tyrosine kinase receptors. The angiogenic activity of VEGF is thought to be mainly mediated by VEGF receptor II (VEGF-RII; also known as flk-1/kinase domain region). The role of VEGF-RI (also known as flt1) is poorly understood, but it seems to be involved in inflammatory cell recruitment, inducing angiogenesis by producing proangiogenic mediators (4).

In human RA, VEGF is found in both synovial fluid and serum (3, 5–7). Immunohistochemical and in situ hybridization studies of synovial tissues have shown that VEGF is strongly expressed by subsynovial macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells, and synovial lining cells (7–11). The VEGF expression level in synovial fluid and tissues correlates with the clinical severity of human RA and with the degree of joint destruction (7, 10, 12). VEGF expression in diseased joints begins very early and persists throughout the disease course (7, 10). The synthesis of VEGF by inflammatory and synovial cells is induced by numerous cytokines (such as TNF, IL-1, TGF-β, and platelet-derived growth factor (PDGF)) (3–5), by oxidative stress (13), and by hypoxia (12, 14–16). TNF-α inhibitors, like other slow-acting anti-rheumatic drugs, reduce VEGF expression (7, 17) in RA.

Endothelial cells of pannus microvessels contain high levels of mRNAs for tyrosine kinase receptors VEGF-RI (flt1) and -RII (Flk-1/KDR) (4, 5). Functional VEGF-RII is overexpressed in the human RA synovium (18, 19). Osteoclast precursors and osteoclast cells express VEGF-RI on their membranes in vitro, and VEGF mediates the recruitment, chemotaxis, and proliferation of osteoclast precursor cells, leading to bone destruction in vivo (20). Nothing is known about VEGF-RII or -RII expression by synovial cells.

Based on promising results obtained in animal studies, angiogenesis inhibitors designed to control malignant tumor growth are currently undergoing clinical trials (21–25). Angiogenesis inhibitors could also potentially inhibit synovial growth in RA (19, 21, 25, 26), but no clinical trials have been conducted to date. Non-specific angiogenesis inhibitors have yielded significant clinical benefits in various animal models of arthritis, such as collagen-induced arthritis (CIA) and OVA-induced arthritis (26–30). In a...
rabbit model of adjuvant-induced arthritis of the knee, Storgard et al. (31) showed that intra-articular injections of a cyclic peptide antagonist of 
integrin αβ3, a pathway involved in angiogenesis, attenuated the disease. Likewise, administration of a soluble VEGF receptor transiently reduces the clinical manifestations of CIA (32). Abs against VEGF-RI can suppress blood vessel formation in tumor and in ischemic retinopathy as well as the growth of atherosclerotic plaques and articular inflammation in the CIA model by suppressing VEGF-RI-mediated inflammation (4).

Although widely used, the CIA and OVA-induced arthritis models do not perfectly mimic human RA, as they involve acute immunization with arthritogenic proteins, and their clinical presentations are not homogeneous. Kouskoff et al. (33) produced a transgenic animal model of RA, the K/BxN (or KR/Nodonese diabetic (NOD)) mouse model, by crossing αββ6 TCR-transgenic mice (KRN) with NOD mice. All offspring carrying the transgene spontaneously develop a form of polyarthritis that shares many features with the human disease. Disease onset is due to genetic factors and T cell presentation of the target Ag, and does not require prior immunization. The polyarthritis is chronic, aggressive, long-lasting, bilateral, symmetrical, and erosive, with joint destruction and physical deformations; importantly, synovial fluid can be sampled for analysis. The disease is triggered following chance recognition of a NOD-derived MHC class II molecule by the transgenic TCR (34). Disease progression is mediated by CD4+ T cells and B cells. Early neovascularization occurs in diseased mice (33, 35).

In our previous studies of the K/BxN mouse model, we found strong VEGF expression in arthritic joints. We also found that a nonspecific inhibitor of angiogenesis (called TNP-470) delayed arthritis and prevented bone destruction (35). In the present study we tested the capacity of specific anti-VEGF and anti-VEGF-RI and -RII neutralizing Abs and a VEGF-RI tyrosine kinase inhibitor to cure arthritis in the K/BxN mouse model on the basis of clinical and histological features. We also tested K/BxN synoviocytes for VEGF, VEGF-RI, and VEGF-RII mRNA and protein production in vitro.

Materials and Methods

Mice

The KRN/NOD transgenic mice used in this work were obtained by crossing KRN transgenic males (B10BR genetic background; provided by Dr. C. Benoist, Institut de Génétique et de Biologie Moléculaire, Illkirch, France) with female NOD mice (NOD/Ori Ico; purchased from IFFA CREDO, l’Abresle, France). Mice were bred and maintained in the animal facilities of Mouse Immunogenetics (Institut National de la Santé et de la Recherche Médicale, Unité 462, Saint Louis Hospital, Paris, France). (KRN/NOD)F1 offspring were bled on day 21 after birth, and those expressing the αββ6-TCR transgene were identified by flow cytometry. Controls were F1 offspring mice not expressing the transgene.

Flow cytometry

Flow cytometry was used to identify transgene-positive KRN/NOD F1 offspring on day 21 after birth. Whole blood was incubated on ice with PE-monoclonal anti-mouse CD4 T cell (0.5 mg) and FITC-monoclonal anti-mouse αββ6-TCR β-chain (0.5 mg) Abs (BD Pharmingen, Le Pont de Claix, France). Flow cytometry was performed after RBC lysis and washing steps. The presence of CD4+ Vβ6+ cells showed that the animal was KRN transgene positive. A FACScan flow cytometer (BD Biosciences, San Jose, CA) equipped with a 15-mW 488-nm argon laser was used. All results were obtained with a constant photomultiplier gain. Data were analyzed using CellQuest software (BD Biosciences). Nonspecific Ab binding was determined on cells incubated with the same concentration of an irrelevant Ab of the same isotype.

Anti-mouse VEGF and VEGF-R Abs

The neutralizing polyclonal goat anti-mouse-VEGF Ab was obtained from R&D Systems Europe (Abingdon, U.K.). Rat IgG1 mAbs against mouse VEGF-RI and -RII, called MF-1 and DC-101, respectively, were produced at ImClone Systems (New York, NY). Both are specific blocking Abs whose activity has been tested in various models (4, 23).

Synovial cells

Mice were killed on day 35 (1 wk after the onset of arthritis), and their joints were dissected. Pannus were collected and digested with collagenase. Synovial cell extracts were grown in RPMI 1640 medium (Bio-Whittaker Europe, Verniers, Belgium) containing 10% FCS (Abcys, Burlingame, CA). Cells were harvested after the second passage. The synovial cell phenotype was assessed by their morphological aspects and by immunohistochemical analysis. Cells were first incubated with rabbit anti-mouse vimentin mAb (ABR, Golden, CO) and rabbit anti-mouse epithelial membrane Ag (EMA; Biocare Resources, Newcastle, U.K.) and then with a biotin-labeled goat anti-rabbit Ig Ab; goat anti-mouse CD14 and CD16 (BioSource, Nivelles, Belgium) were followed by an FITC-labeled rabbit anti-goat Ab. The results showed that vimentin and EMA were abundant, whereas CD16 and CD14 were rare, in agreement with a fibroblastic synovial cell type.

VEGF-R RT-PCR

Total RNA was extracted from cultured synovial cells and from mouse lungs (positive control) using the TRIzol reagent (Invitrogen, Cergy Pontoise, France). Five micrograms of total RNA was subjected to RT-PCR as described previously (36). The sense primer for VEGF-RI (nucleotide position 1775) was 5′-GCC CATGACGGTCACTAGAAAGA-3′ and the antisense primer (position 2235) was 5′-TGAAGTTTGGAAAGCCAGGTG-3′. Amplification consisted of 35 cycles at 94°C for 1 min, 63°C for 2 min, and 72°C for 3 min. The sense primer for VEGF-RII (position 1834) was 5′-ATCACCAGCTCACAAG-3′, and the antisense primer (position 2260) was 5′-TGAGGTCATCCGCTCTAGAT-3′. Amplification consisted of 30 cycles at 94°C for 1 min, 60°C for 2 min, and 72°C for 3 min.

VEGF-R detection by indirect immunofluorescence

Mice were killed by neck dislocation on day 35. Joints were fixed for 12 h in 4% paraformaldehyde, decalcified in 5% nitric acid for 12 h, fixed again for 12 h in paraformaldehyde, and then embedded in paraffin. Slides were then deparaffinized in toluene, rehydrated in alcohol, washed in PBS with 1% BSA, incubated with rat anti-mouse VEGF-RI and -RII mAbs (dilution 1/50) or isotypic controls (IgG1) in and revealed with an FITC-conjugated goat anti-rat Ab (F5262; Sigma-Aldrich, St. Louis, MO).

Western blotting for VEGF-RI and -RII

An equal amount of synovial cell protein extract (20 μg) from arthritic and normal mice and mouse lungs (positive control) was solubilized in Laemmli buffer and then submitted to SDS-PAGE in 10% acrylamide gels. After transfer onto nitrocellulose, membranes were blotted with the primary Ab (anti-VEGF-RI or -RII at 1/1000; ImClone Systems), then with a secondary Ab (goat anti-rat peroxidase-labeled Ab, 1/2000; Invitrogen, Cergy Pontoise, France). Bands were visualized on an HP Scanjet 5500 (Hewlett-Packard, Palo Alto, CA), and the ratio between bands from arthritic mice and the positive control was calculated.

In vitro inhibition of endothelial cell proliferation by anti-VEGF and anti-VEGF-R

We used VEGF-induced endothelial cell proliferation to check the ability of all Abs to inhibit cell proliferation on the basis of bromodeoxyuridine incorporation, according to the manufacturer’s instructions (OncoGene Research Products, Boston, MA). Isotypic controls were used in each experiment. Anti-VEGF, anti-VEGF-RI, and anti-VEGF-RII Abs all inhibited endothelial cell proliferation in vitro.

Kinase assays

The tyrosine kinase inhibitor SU11657 was synthesized at SUGEN (South San Francisco, CA). This compound inhibits the kinase activity of VEGF-RI, VEGF-RII, PDGF-R, and c-Kit (see Table I). Biochemical kinase assays to evaluate the potencies of compounds to inhibit VEGF-RI, VEGF-RII, PDGF, and c-Kit receptors were conducted with GST/kinase domain chimeric proteins that were expressed in SF9 cells and purified on glutathione-agarose columns. In general, all kinase assays were performed at twice the Kd for ATP, so that the IC50 values were comparable. Kinase assays were conducted in either an ELISA or TR-FRET assay format. For the ELISA format, the purified protein was added to microtiter plate wells coated with poly-GluTyr (4/1), together with the test compound and the
Lymphocytes from K/BxN joints were stained with FITC-conjugated anti-CD4, R-PE-HL-1 anti-IgG1, PE-conjugated anti-CD3, and biotinylated anti-CD8, followed by streptavidin-phycocyanin. 

**Statistical analysis**

StatView software was used to calculate the mean and SD. Group means were compared by using ANOVA, followed by the Mann-Whitney non-parametric comparison test. A value of p < 0.05 was considered significant.

**Results**

VEGF-R1 and -R2 mRNAs and proteins are present in synovial cells from K/BxN joints

One week after the onset of arthritis (day 35) the synovial pannus from knee joints was collected and digested with collagenase, and synovial cells were grown in petri dishes. Cells were collected after two passages. VEGF-R1 and -R2 mRNAs were detected by RT-PCR. The expected 484-bp (VEGF-R1) and 450-bp (VEGF-R2) bands were readily detected (Fig. 1).

Indirect immunofluorescence detected both VEGF-R1 and -R2 on cultured cells and joint sections (Fig. 2). These cells coexpressed VEGF-R with vimentin and EMA, confirming their fibroblastic synovial phenotype (data not shown). The two receptors were identified in synovial cells by Western blotting analysis. VEGF-R1 and -R2 (150 and 151 kDa, respectively) were clearly present in synovial cells from two arthritic mice and in the positive lung control, but were barely detectable in cells from normal mice. The blots were scanned, and this showed that the VEGF-R1 and -R2 bands were both 20 times more intense in cells from arthritic mice than in the positive lung control (Fig. 3). Therefore, a

![FIGURE 1. VEGF-R1 and -R2 RT-PCR. To detect the two VEGF-Rs on synovial cells, VEGF-R1 and VEGF-R2 mRNAs were amplified by RT-PCR as described in Materials and Methods. Transcripts of the expected sizes were detected: 482 bp (VEGF-R1) and 448 bp (VEGF-R2). Lane a, the presence of VEGF-R1 mRNA in synovial cells from arthritic joints; lane b, positive controls for VEGF-R1 from mouse lungs; lane c, size ladder; lane d, positive controls for VEGF-R2 from mouse lungs; lane e, the presence of VEGF-R2 mRNA in synovial cells from arthritic joints.](https://www.jimmunol.org/content/jimmunol/119/6/4843/F1.large.jpg)
similar amount of both receptors was present in the cells from arthritic mice. As we have previously reported (35), VEGF was expressed by synovial cells from K/BxN mouse joints (data not shown).

**Time course of the AI in transgenic K/BxN F1 mice and K/BxN F1 nontransgenic mice (Fig. 4)**

Transgenic K/BxN F1 mice typically developed polyarthritis 27 ± 2 days after birth. Arthritis occurred in all transgenic mice, but not in nontransgenic mice. As expected in nontransgenic mice, which grew normally, the AI increased after birth and stabilized (day 35; 1130 ± 40) after the end of growth. In transgenic mice, the disease started with a very acute phase, characterized by joint effusion and florid synovitis, which gradually increased between days 27 and 36. The AI increased rapidly, reaching 1587 ± 96 on day 36 (p < 0.05 vs nontransgenic mice), in keeping with previous results (35). The disease remained active between days 36 and 60 after birth, with high AI values (mean daily AI during this period, 1596 ± 103). The AI value peaked on day 43 at 1678 ± 102. It declined from day 60 to day 110, but always remained significantly higher than the value in nontransgenic mice.

**Anti-VEGF transiently attenuates arthritis**

Intravenous anti-VEGF treatment (400 μg/injection) delayed the onset of clinical manifestations by about 1 wk compared with isotypic control-treated mice (Fig. 5). The mean AI was significantly lower in anti-VEGF-treated mice than in their placebo-treated counterparts during this period (days 28–35; p < 0.001). The effect of anti-VEGF Ab waned after day 35, as the AI curve joined that for isotypic control-treated mice. Similar results were obtained when treatment was administered via the i.p. route. No gain was observed with higher doses (800 μg/injection; data not shown).

**Anti-VEGF-RI and the tyrosine kinase inhibitor SU11657 strongly attenuate arthritis, contrary to anti-VEGF-RII (Fig. 6)**

Treatment with the anti-VEGF-RI mAb DC-101 was totally ineffective, as all clinical parameters (day of onset, AI, and weight) and histological variables (see below) were identical with those in the placebo group (Fig. 6B). In contrast, the anti-VEGF-RI mAb MF-1 delayed the onset of arthritis and attenuated the disease (Fig.

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**FIGURE 2.** Detection of VEGF-RI and -RII by indirect immunofluorescence. Slides (longitudinal sections of knee joints) were incubated with rat anti-mouse VEGF-RI and -RII mAbs (dilution 1/50) or IgG1 isotypic controls and revealed with an FITC-conjugated goat anti-rat Ab as described in Materials and Methods. a and b. Slides were treated with antiveGF-RI (a) and anti-VEGF-RII (b), respectively. VEGF-RI and -RII are indicated by the bright green spots in synovium and bone marrow. c and d. Slides were treated with isotypic controls for VEGF-RI (c) and VEGF-RII (d), respectively.

**FIGURE 3.** Equal amounts of cultured synovial cell protein extract (20 μg) from arthritic and normal mice and mouse lungs (positive control) were dissolved in Laemmli buffer and then submitted to SDS-PAGE in 10% acrylamide gels. After transfer onto nitrocellulose membranes, the membranes were probed with the primary Ab (anti-VEGF-RI and anti-VEGF-RII) and revealed with a peroxidase-labeled secondary Ab. Bands of the expected sizes (151 and 150 kDa) were observed in the arthritic mice and in the positive control. Bands were scanned with an HP scan jet 5500, and the ratio between bands from arthritic mice and the positive control was calculated. The results are representative of three experiments.

**FIGURE 4.** AI in transgenic and nontransgenic mice. The incidence and severity of arthritis were assessed daily in transgenic (○) and control nontransgenic (□) mice. Arthritis was quantified by measuring the thickness of each paw with an electronic caliper square as described in Materials and Methods. The sum of the measures of the four paws (in millimeters) was used as an AI. Results are expressed as the mean ± SD (for seven transgenic mice and eight nontransgenic mice). *p < 0.05. All the transgenic (100%) developed arthritis on day 27 ± 2, whereas nontransgenic mice had no signs of arthritis.

**FIGURE 5.** Effect of anti-mouse VEGF Ab treatment on the clinical course of arthritis in K/BxN transgenic mice. The incidence and severity of arthritis were assessed daily in anti-mouse-VEGF-treated (○; n = 9) and isotype-treated (□; n = 11) mice. Arthritis was quantified by measuring the thickness of each paw with an electronic caliper square. The sum of the measures of the four paws (in millimeters) was used as the AI. Results are expressed as the mean ± SD. *p < 0.05.
FIGURE 6. Effects of anti-VEGF-RI and anti-VEGF-RII Abs and the tyrosine kinase inhibitor SU11657 on the clinical course of arthritis in K/BxN transgenic mice. a and b, K/BxN transgenic mice were treated with anti-mouse VEGF-RI and -RII Abs or with an isotypic control as described in Materials and Methods. a, Anti-VEGF-RI-treated mice (○; n = 12) and isotypic control-treated mice (□; n = 11). *, p < 0.05. b, Anti-mouse VEGF-RII-treated mice (○; n = 11) and isotypic control-treated mice (□; n = 12). *, p < 0.05. c, K/BxN transgenic mice received oral SU11657 (○; n = 14) or placebo (□; n = 10) as described in Materials and Methods. *, p < 0.05. The incidence and severity of arthritis were assessed daily. Arthritis was quantified by measuring the thickness of each paw with an electronic caliper square. The sum of the measures of the four paws (in millimeters) was used as the AI. Results are expressed as the mean ± SD AI.

6A). The differences between anti-VEGF-RI-treated and isotypic control-treated mice were significant at every time point. In particular, the increase in AI from day 25 to day 33 in anti-VEGF-RI-treated mice was about half that observed in isotypic control-treated mice.

Treatment of transgenic mice with SU11657 almost completely abrogated all clinical signs of arthritis (Fig. 6C). SU11657 had the most potent suppressive effect on clinical parameters.

Anti-VEGF and anti-VEGF-RII attenuate the histological signs of arthritis

Qualitative analysis. The histological aspects of inflamed joints from placebo-treated and anti-VEGF-RII-treated mice are shown in Fig. 7. After 1 wk of treatment, isotypic control-treated mice had erosive acute arthritis, mainly in the tarsal and carpal joints. Articular spaces were filled with inflammatory material, synovial membranes were invaded by inflammatory cells, and tendon sheaths were inflamed. Multiple tendon ruptures were observed, and a rheumatoid-like pannus was present.

Mice treated with anti-VEGF-RI exhibited milder arthritis, with no erosion, no cartilage lesions, and no tendon rupture. Articular spaces were filled with edematous fluid devoid of inflammatory cells and altered polymorphonuclear cells. When present, the pannus was not hypertrophic or aggressive. Anti-VEGF-RII had no such protective effect.

Mice treated with SU11657 had very few histological manifestations of arthritis, apart from a thin pannus with a fibrous aspect. No bone or cartilage destruction was observed in these mice.

Mice treated with SU11657 had very few histological manifestations of arthritis, apart from a thin pannus with a fibrous aspect. No bone or cartilage destruction was observed in these mice.

Semiquantitative analysis. The median histological score was calculated for each mouse. Pairs of mice from the same litter were chosen for active and placebo treatments. Histological analysis focused on one anterior and one posterior limb of each animal, and each joint (anterior: shoulder, elbow, wrist, and metacarpophalangeal; posterior: hip, knee, ankle, and metatarsophalangeal) was analyzed and scored (Table II). Scores near 0 correspond to normal joints, scores between 10 and 20 correspond to acute nondestructive arthritis, and scores over 30 correspond to destructive arthritis. Placebo-treated mice had a mean ± SD score of 50 ± 7; mice treated with anti-VEGF-RII had a mean ± SD score of 47 ± 8 (NS
vs placebo). In contrast, mice treated with anti-VEGF-RI had a mean ± SD score of 12.4 ± 5 (p < 0.001 vs placebo), and mice treated with SU11657 had a mean ± SD score of 3.1 ± 2 (p < 0.001 vs placebo). The degradation of cartilage proteoglycan, as measured by Safranin O staining (positive staining surface per square millimeter of articular knee surface), was reduced by 45% in mice treated with anti-VEGF-RI and by 90% in mice treated with SU11657 compared with that in control mice. The number of CD31-positive cells (myeloid and endothelial cells) per unit area in mice treated with anti-VEGF-RI and SU11657 was reduced by 22% and 34%, respectively, compared with that in placebo-treated mice.

### Discussion

We tested a strategy to limit angiogenesis and inflammation in the K/BxN mouse model of arthritis, as angiogenesis seems to have a key role in pannus growth. We report for the first time that synovial cells contain VEGF-RI and -RII mRNAs and proteins, and that systemic delivery of an anti-VEGF-RI Ab in the K/BxN mouse model of rheumatoid arthritis delays the onset of arthritis, attenuates its intensity, and prevents joint destruction. The anti-VEGF Ab had only a transient protective effect. The fact that the anti-VEGF-RI Ab had a protective effect, whereas the anti-VEGF-RII Ab was totally ineffective, suggests that the VEGF-RI pathway is the main pathway involved in pannus proliferation and bone destruction.

The inhibition of inflammation and angiogenesis mediated by VEGF within the joint is an attractive therapeutic approach in arthritis (26–31) as VEGF (protein and mRNA) is present in human RA synovium and synovial fluid (10). In addition, we showed here that VEGF-RI and -RII mRNAs and proteins are present in synovial cells from K/BxN arthritic mice. The similar expression of VEGF-RI and -RII in synovial cells from arthritis mice suggests that the inefficacy of anti-VEGF-RII-neutralizing Ab is not related to a lower expression of VEGF-RII.

Previous studies have shown a transient clinical improvement in CIA and OVA-induced arthritis after treatment with a soluble VEGF-R (32). In a CIA model, treatment with anti-VEGF-RI reduces the incidence of joint disease by 60%, whereas anti-VEGF-RII is ineffective (4). The KRN/NOD (K/BxN) transgenic mouse model used here is the most reliable and accurate model of human RA currently available, and this is the first study to test specific neutralizing anti-VEGF and anti-VEGF-RI and -RII Abs in this model.

Angiogenesis is an early and important phenomenon in synovial pannus proliferation. The mechanisms triggering this angiogenesis are poorly understood, and numerous proangiogenic factors have been detected in RA synovial membranes (1–3, 7, 20). VEGF is one of the most powerful angiogenic agents known. VEGF exerts proangiogenic effects; its concentration is positively correlated with joint destruction and vascularization of the pannus, as observed by color Doppler echography (6). However, VEGF also exerts proinflammatory activities by recruiting hemopoietic cells from the bone marrow to the circulation. In the CIA model, synovial infiltration by inflammatory cells is reduced by anti-VEGF-RI Ab, whereas anti-VEGF-RII is ineffective (4). These authors suggested that although VEGF-RII may be the main pathway of angiogenesis in endothelial cells, many of the biological functions of VEGF-RI in arthritides may lie in the regulation of the migration of inflammatory cells, which themselves could induce the production of angiogenic mediators (4). Furthermore, Matsumoto et al. (20) recently detected VEGF-RI on preosteoclast cells and showed that the VEGF/VEGF-RI pathway might be involved in chemotaxis and the cell proliferation of preosteoclasts in arthritic joint destruction. Osteoclasts are derived from monocytes and macrophages, and these results are in accordance with the VEGF-induced migration of myeloid progenitors to the joints (4). In accordance with this perspective, we observed significant clinical and histological improvements in the K/BxN transgenic mouse model after treatment with anti-VEGF-RI, whereas anti-VEGF-RII Ab was ineffective. However, both receptor types were present on synovial cells, and both Abs blocked endothelial cell proliferation in vitro. These results suggest that the main pathway involved in VEGF-induced pannus proliferation is mediated by VEGF-RI-driven, and not by VEGF-RII-driven, angiogenesis in vivo. However, we cannot rule out the possibility that the differences between the two Abs in the K/BxN model were due to differences in the accessibility of the two Abs to their target in vivo.

After transiently diverging, the curves of the AI in anti-VEGF-treated and control mice came back together after a few days. A similar transient effect has been described with soluble VEGF-RI in a CIA model (32). It is feasible that the VEGF produced by joint cells, through autocrine and paracrine mechanisms, was rapidly captured by neighboring VEGF-Rs rather than by the neutralizing anti-VEGF Ab. Alternatively, other proangiogenic factors, such as placental growth factor may exert a compensatory mechanism (4). It can also be hypothesized that mice generated neutralizing Abs against goat anti-VEGF Abs.

The expression of VEGF-RI and -RII on synovial cells suggests that VEGF and its receptors also play a role in the extension of the rheumatoid pannus by inducing synovial proliferation as well as angiogenesis. Interestingly, some nonspecific angiogenesis inhibitors (such as TNP-470, an inhibitor of VEGF synthesis and metalloproteinases) are effective (35), suggesting that other pathways beside the VEGF-RI pathway should be studied in arthritis. The recent demonstration that both VEGF-RI and -RII are expressed on human synovial cells (37) strengthens our results.

Although it abrogated bone and cartilage destruction, anti-VEGF-RI did not totally suppress arthritis in K/BxN mice. This suggests that various cytokines have redundant effects on the angiogenic pathway involved in pannus proliferation. This treatment might be more effective if anti-VEGF-RI treatment is combined with other therapeutics such as TNF-α inhibitors. In this context, SU11657 might be a better inhibitor of the AI in K/BxN mice than was anti-VEGF-RI because it is a potent inhibitor of several kinases. Thus, it might inhibit signaling by other cytokines that are critical for the pathophysiology, in addition to that mediated by VEGF-RI. Although it is important for us to determine which of these explanations is correct if we are to develop better therapies for arthritis, the near-complete suppression of the disease is very encouraging for identifying appropriate therapeutic agents for this debilitating disease.

In conclusion, this study clearly shows the clinical and histological benefits of selective VEGF-RI blockade on cartilage and bone destruction in the K/BxN transgenic mouse model of arthritis,
the most reliable model of human RA. These results warrant clinical trials of specific VEGF-RI inhibitors for the treatment of arthritis.

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