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BRIEF REPORT

Therapeutic effect of anti-vascular endothelial growth factor receptor I antibody in the established collagen-induced arthritis mouse model

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Abstract Synovial angiogenesis plays an important role in the inflammation in rheumatoid arthritis (RA). Vascular endothelial growth factor (VEGF) is a key molecule in angiogenesis and binds to specific receptors, known as vascular endothelial growth factor receptor I (VEGF RI). In this study, we investigated the therapeutic efficacy of anti-VEGF RI antibody (Ab) on RA using a collagen-induced arthritis (CIA) mouse model. Twelve DBA/1 mice were divided into three groups. All mice except controls were injected with type II collagen. Mice in the anti-VEGF-RI-Ab-treated groups were injected on one posterior paw with 50 μg anti-VEGF RI Ab twice weekly for 3 weeks. Arthritis score and paw thickness were measured and histopathologic assessment of joint sections was performed by hematoxylin–eosin. The infiltration of $CD45^+$ inflammatory cells and neovascularization were evaluated by immunohistochemical staining. Anti-VEGF RI Ab significantly attenuated the arthritis severity and histopathologic findings in the CIA mice model. The infiltration of $CD45^+$ cells decreased in anti-VEGF-RI-Ab-treated joint tissues. Staining for CD31 revealed reduced synovial neovascularization after anti-VEGF RI Ab treatment. The data showing that in vivo administration of anti-VEGF RI Ab suppressed arthritis in established CIA mice suggest anti-VEGF RI Ab treatment may serve as a new therapeutic modality for RA.

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Introduction

Rheumatoid arthritis (RA) is a polyarticular inflammatory disease. A characteristic feature of RA is synovial proliferation, which leads to rheumatoid pannus with accompanying destruction of bone and cartilage. One of the early changes in the synovium of RA is neovascularization [1]. The inflammatory synovial cells should be supplied by oxygen and nutrients; therefore, synovial angiogenesis plays an important role in maintaining inflammation and promoting RA [2].

Angiogenesis needs several angiogenic factors. Vascular endothelial growth factor (VEGF), the most powerful angiogenic molecule, is associated with various physiological and pathological neovascularization, including synovial angiogenesis in RA [3]. In RA, VEGF is strongly expressed by synovial macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells, and synovial lining cells [4, 5]. VEGF expression begins very early and it also persists throughout the disease course [4, 5]. VEGF binds two types of receptors: vascular endothelial growth factor receptor I (VEGF RI) and vascular endothelial growth factor receptor II (VEGF RII) [6]. Both VEGF RI and VEGF RII are expressed on the cell surface of synovial cells and blood endothelial cells, and VEGF RII is the major mediator of endothelial cell mitogenesis and survival, as well as angiogenesis and microvascular permeability [4, 6]. However, in the collagen-induced arthritis (CIA) mice model and KRN/NOD (K/BxN) transgenic mice model, anti-VEGF RII treatment had no effects on preventing progression of arthritis [4, 7].

Lutten A et al. [7] showed that endothelial cells of synovial microvessels have high levels of tyrosine kinase receptor VEGF RI messenger RNA. They also reported that treatment with anti-VEGF RI before the onset of arthritis reduced the incidence of arthritis by 60% in a CIA mice model [7]. However, there was no report whether treatment with anti-VEGF RI has therapeutic effect on established CIA mouse. In this study, we investigated the therapeutic effects of anti-VEGF RI antibody (Ab) on the macroscopic and microscopic severity of arthritis in inflamed joints of mice with CIA.

Materials and methods

Induction of collagen-induced arthritis

All animals were treated in accordance with the guidelines and regulations for the use and care of animals at Yonsei University, Seoul, Korea. Twelve male DBA/1 mice at 8 weeks of age (SLC, Shizoka, Japan) were evenly divided into three groups (group 1: controls, group 2: untreated, group 3: 50 μg anti-VEGF-R1-Ab-treated). All mice except controls were given an intra-dermal injection of 100 μg of bovine type II collagen emulsified in complete Freund's adjuvant (Arthrogen-CIA, Redmond, WA, USA; 1:1, *w*/*v*) to the base of the tail. Two weeks later, the mice were given a booster intra-dermal injection of 100 μg bovine type II collagen in incomplete Freund's adjuvant (DIFCO, Detroit, MI, USA; 1:1, *v*/*v*). The control mice were treated with Freund's adjuvant without bovine type II collagen.

Treatment protocol for collagen-induced arthritis

The treatment with anti-VEGF RI Ab (R&D Systems, Minneapolis, MN, USA) began 5 weeks after the primary immunization (following full development of arthritis). Fifty-microgram anti-VEGF RI Ab was injected into only the left posterior paw twice weekly for 3 weeks and observed for one more week. Control and untreated mice received injections of the same volume of phosphate-buffered saline (Gibco BRL, Grand Island, NY, USA) into the left posterior paw twice per week during the anti-VEGF RI Ab treatment period.

Assessment of the arthritis severity

The mice were observed twice per week for 9 weeks after the primary collagen injection. The arthritis severity was evaluated by visual inspection. The left posterior paws in all of the mice and right posterior paws in anti-VEGF-RI-treated mice were evaluated. The evaluated paws were scored from 0 to 4 according to the following scale: 0=no signs of arthritis, 1=swelling and/or redness of the paw or one digit, 2=two joints involved, 3=more than two joints involved, and 4=severe arthritis of the entire paw and all digits. Paw thickness was measured with a Vernier caliper.

Histopathologic and immunohistochemical examination

The mice were anesthetized and sacrificed on day 63, and paw joints were removed for histopathologic examination after routine fixation, decalcification, and paraffin embedding of the tissue. Tissue sections prepared and stained with hematoxylin and eosin were analyzed and scored according to the three synovial membrane features (synovial lining cell layer, stroma cell density, and inflammatory infiltrate) [8]. Sections were sequentially incubated with specific antibodies directed against CD31 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and CD45 (Santa Cruz Biotechnology) followed by the appropriate secondary antibodies (ISU Abxis, Seoul, Korea). The number of blood vessels and the infiltration of $CD45^{+}$ cells per unit area were counted. All tissue samples were counterstained with hematoxylin.

Statistical analysis

The representative values were means of those obtained from each mouse in a group, and all values in the experimental groups were compared to controls and untreated CIA mice. All results and measurements are expressed as the mean± standard deviation. Statistical comparisons between two groups were evaluated using the Mann–Whitney *U* test. Statistical significance was set at the level of $p=0.05$. All analyses were performed using SPSS version 12.0.

Results

Anti-VEGF RI Ab treatment decreases the severity of established arthritis in mice with CIA

Anti-VEGF RI Ab treatment significantly mitigated the severity of arthritis in the mice with CIA compared to the untreated mice and non-injected side of the treated mice (Fig. 1). Both mean arthritis score and paw thickness in anti-VEGF-RI-Ab-treated mice were significantly lower than that of the untreated mice and the non-injected side of the treated mice (Fig. 2).

Anti-VEGF RI Ab reduces the inflammatory cell infiltration and angiogenesis in the inflamed joint

The mean synovitis score of the paw joints of untreated CIA mice was less than anti-VEGF-RI-Ab-treated mice but

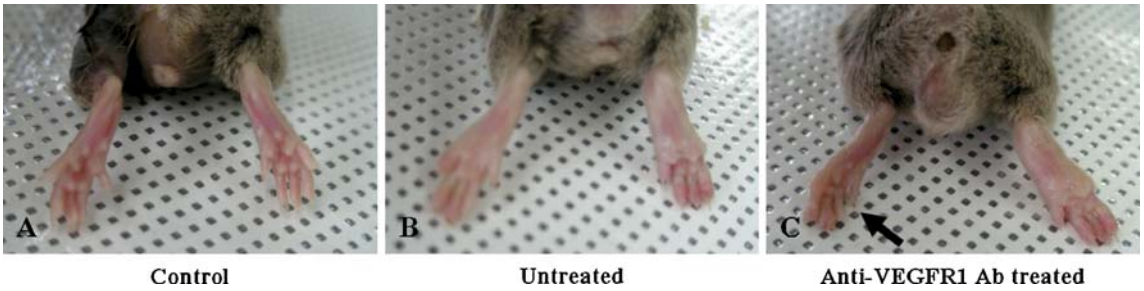

Fig. 1 Macroscopic arthritis in mice with CIA. Treatment with anti-VEGF RI Ab significantly mitigated the macroscopic severity of CIA compared to untreated mice with CIA. **a** Normal posterior paw of control mouse. **b** Pronounced swollen and erythematous paw of untreated mouse with CIA. **c** The *arrow* indicates the injection site of anti-VEGF RI Ab. The left paw with anti-VEGF RI Ab treatment shows decreased swelling and erythema compared to the untreated right paw

had no statistical significance (1.8±0.5 vs 0.8±0.5, p= 0.057). CD45 staining revealed more abundant accumulation of $CD45^+$ inflammatory cells in the joints of the paws in untreated mice than in the anti-VEGF-RI-Ab-treated mice (4.0±2.3 vs 0.0±0.0, p=0.029). Compared to untreated mice, staining for CD31 revealed reduced synovial vessels after anti-VEGF RI Ab treatment (1.5±0.6 vs 0.0± 0.0, p=0.029; Fig. 3).

Discussions

In this study, we found that anti-VEGF RI Ab has therapeutic effects on mice with CIA. Anti-VEGF RI Ab treatment reduced the inflammation in the joints of established CIA mice. The mean arthritis score, as well as mean paw thickness, in anti-VEGF-RI-Ab-treated CIA mice was significantly less than that of untreated mice. The finding that, within the same mouse, only the joints with anti-VEGF RI Ab treatment had decreased severity of arthritis while the untreated joints had persistent severe arthritis clearly supports the therapeutic effects of anti-VEGF RI on CIA mice. These effects appeared rapidly by 7–10 days after the first anti-VEGF RI Ab treatment and were maintained during the therapeutic period. In addition, anti-VEGF RI Ab significantly improved the histological findings by inflammatory cell infiltration and neovascularization in joint tissues of CIA mice compared to those of untreated mice.

In RA, VEGF is strongly expressed by synovial macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells, and synovial lining cells, and the concentration of VEGF is well correlated with joint destruction and clinical symptoms [5]. The biological effects of VEGF are mediated by two receptors, VEGF RI and VEGF RII, which differ considerably in signaling properties [6]. It is not clear which receptor pathway is dominantly involved in the pathogenesis of RA. VEGF RII is thought as a major mediator of endothelial cell mitogenesis and survival, as well as angiogenesis and microvascular permeability [4, 6]; however, anti-VEGF RII treatment had no effects on preventing progression of arthritis in the CIA mice model and KRN/NOD (K/BxN) transgenic mice model [4, 7]. These authors suggested that,

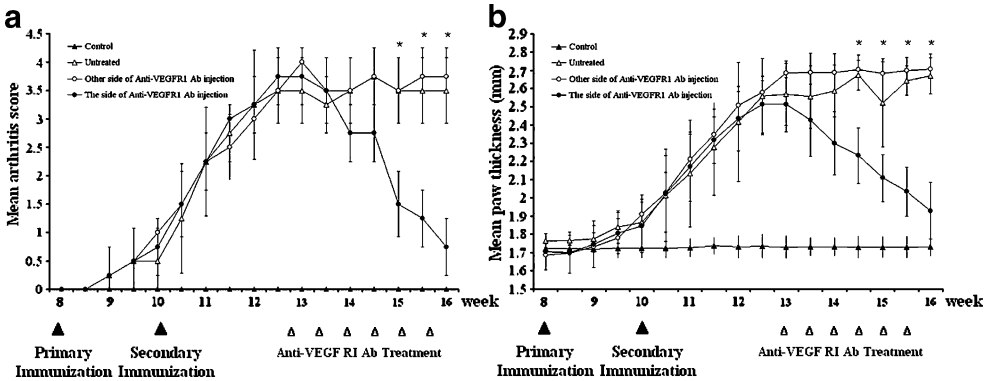

Fig. 2 Severity of arthritis in mice with CIA. **a** Mean arthritis score. After four injections, the arthritis score of the site with anti-VEGF RI Ab injection (*filled circles*) significantly decreased compared to that of untreated mice (*empty triangles*) and the site without anti-VEGF RI Ab injection (*empty circles*; p<0.05). **b** Mean paw thickness. After three injections, the paw thickness of the site with anti-VEGF RI Ab injection (*filled circles*) also significantly decreased compared to that of untreated mice (*empty triangles*) and the site without anti-VEGF RI Ab injection (*empty circles*; *p<0.05)

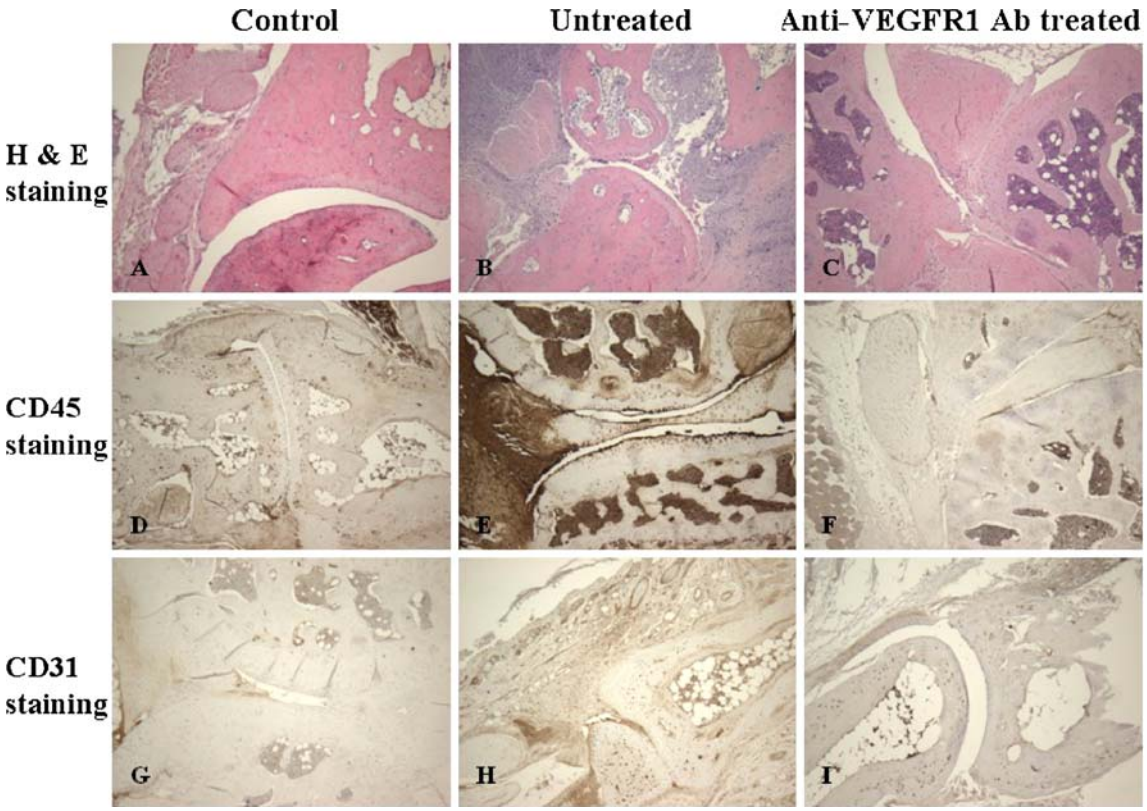

Fig. 3 Histopathologic findings and immunohistochemical staining for CD45 and CD31 in mice with CIA. *A–C*, histopathologic evaluation revealed severe inflammation in the joint sections of untreated CIA mice (*B*). In contrast, the extent of arthritis was significantly reduced in the joints of mice with anti-VEGF RI Ab treatment (*C*) such that they resembled the control mice (*A*; original magnification ×100, hematoxylin–eosin). *D–F*, CD45 staining revealed more abundant accumulation of $CD45^+$ inflammatory cells in the joints of the paw in untreated mice (*E*) than in the anti-VEGF-RI-Ab-treated mouse (*F*). *G–I*, staining for CD31 revealed markedly increased vessels in untreated mice (*H*) than in controls (*G*), and reduced synovial neovascularization was observed after anti-VEGF RI Ab treatment (*I*; original magnification ×100)

although VEGF RII may be an important pathway of angiogenesis in endothelial cells, the main pathway involved in VEGF-induced pannus proliferation is not mediated by VEGF-RII-driven angiogenesis in vivo [4, 7]. On the other hand, treatment with anti-VEGF RI before the onset of arthritis reduced the incidence of arthritis by 60% in a CIA mice model [7]. In K/BxN transgenic mice model, the treatment of anti-VEGF RI Ab for preventing arthritis was also effective [4]. These findings suggest that many of the angiogenic functions in RA may be involved in the VEGF-RI-driven pathway. The fact that VEGF RI is detected on pre-osteoclast cells supports that the VEGF RI pathway may be involved in chemotaxis and the proliferation of pre-osteoclasts in arthritic joint destruction [9].

Our study showed that treatment with anti-VEGF RI Ab has effects not only on preventing arthritis but also therapeutic effects on established arthritis in the CIA mice model. The joint inflammation and the synovial vasculature after anti-VEGF RI Ab injection were rapidly decreased in established arthritis. This finding is important in that angiogenesis has key roles not only in early arthritic development but also in the maintenance of persistent arthritis. Furthermore, the reports that VEGF expression begins very early and persists throughout the disease course suggest that the treatment with anti-VEGF RI Ab may be a good therapeutic modality in persistent arthritis [4, 5].

Synovitis score is not significantly decreased. We think these results are due to the relatively small numbers of sample size or the possibility that VEGF has other signal pathways besides the VEGF RI pathway in arthritis. Another possibility is that the anti-VEGF RI Ab treatment may not have a direct effect on other aspects of the joint histopathology except on endothelial biology through the VEGF pathway. However, we showed the therapeutic effects of anti-VEGF RI Ab on CIA in clinical and other microscopic aspects. The fact that anti-VEGF RI Ab was not systemic, one of the limitations of this study, paradoxically explained the effect of anti-VEGF RI Ab, in that the treated joint had good response compared to untreated joint. We used only one dose of anti-VEGF RI Ab. Further studies with different doses of anti-VEGF RI Ab will be needed to clearly identify the dose-dependant and systemic therapeutic effects. In

addition, an additive experiment using human RA synovial fibroblasts is warranted.

In conclusion, anti-VEGF RI Ab treatment significantly decreased the severity of arthritis and improved the histological findings in established CIA mice. Anti-VEGF RI Ab also reduced the neovascularization and infiltration of inflammatory cells in inflamed joints. The findings showing that in vivo administration of anti-VEGF RI Ab suppressed arthritis on established CIA mice suggest anti-VEGF RI Ab treatment may serve as a new and additional therapeutic modality for RA.

Disclosures None.

References

1. Taylor PC, Sivakumar B (2005) Hypoxia and angiogenesis in rheumatoid arthritis. Curr Opin Rheumatol 17:293–298
2. Maruotti N, Cantatore FP, Crivellato E et al (2006) Angiogenesis in rheumatoid arthritis. Histol Histopathol 21:557–566
3. Sone H, Kawakami Y, Sakauchi M et al (2001) Neutralization of vascular endothelial growth factor prevents collagen-induced arthritis and ameliorates established disease in mice. Biochem Biophys Res Commun 281:562–568
4. De Bandt M, Ben Mahdi MH, Ollivier V et al (2003) Blockade of vascular endothelial growth factor receptor I (VEGFR-RI), but not VEFF-RII, suppresses joint destruction in the K/BxN model of rheumatoid arthritis. J Immunol 171:4853–4859
5. Ballara S, Taylor PC, Reusch P et al (2001) Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. Arthritis rheum 44:2055–2064
6. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
7. Luttun A, Tjwa M, Moons L et al (2002) Revascularization of ischemic tissues by PIGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat Med 8:831–840
8. Krenn V, Morawietz L, Burmester GR et al (2006) Synovitis score: discrimination between chronic low-grade and high-grade synovitis. Histopathology 49:358–364
9. Matsumoto Y, Tanaka K, Hirata G et al (2002) Possible involvement of the vascular endothelial growth factor-Flt-1-focal adhesion kinase pathway in chemotaxis and cell proliferation of osteoclast precursor cells in arthritis joints. J Immunol 168:5824–5831