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ORIGINAL ARTICLE

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Imatinib mesylate both prevents and treats the arthritis induced by type II collagen antibody in mice

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Abstract Rheumatoid arthritis (RA) is a chronic inflammatory disease that is associated with joint destruction. Imatinib mesylate (imatinib) is an inhibitor that specifically targets a set of protein tyrosine kinase, such as abl, c-kit, and platelet-derived growth factor receptor (PDGFR) and it is widely used to treat chronic myeloid leukemia (CML). The purpose of the present study is to determine whether imatinib can provide benefit in the arthritis induced by anti-collagen type II antibody (CAIA) in mice, a model that provides an opportunity to study the effector inflammatory phase of arthritis without involving the priming phase of the immune responses. Mice treated with intraperitoneal administration of imatinib (1 or 10 mg/kg) prior to the development of CAIA displayed significant reductions in the severity of CAIA as assessed by arthritis score, histology, and synovial PDGF and vascular endothelial growth factor expression. In addition, treatment of the mice that had developed CAIA with intraperitoneal administration of imatinib (1 or 10 mg/kg) inhibited the progression of arthritis as assessed by those parameters. These results suggest that imatinib prevents and treats CAIA. Imatinib may thus have both a preventive and therapeutic potential for the joint inflammation at the effector stage of RA.

Key words Anti-collagen type II antibody-induced arthritis (CAIA) · Imatinib mesylate · Rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the synovial tissue in multiple joints associated with autoantibody production such as anti-collagen type II antibody (OMIM #180300). Rheumatoid arthritis synovial tissue is characterized by synovial hyperplasia with an increased number of synovial fibroblasts and infiltrating inflammatory cells consisting predominantly of macrophages and lymphocytes.[1,2]

Imatinib mesylate (imatinib) is a signal transduction inhibitor that specifically targets protein tyrosine kinases such as abl, c-Kit, and platelet-derived growth factor receptor (PDGFR), and it is widely used to treat chronic myeloid leukemia (CML) and c-kit-positive gastrointestinal stromal tumors.[3,4] Recent case reports describe severe RA patients treated with imatinib, who show marked improvement in the joint pain, disease activity, and serum C-reactive protein (CRP) levels.[5,6] Consistent with these clinical findings, in vitro studies showed that imatinib inhibited platelet-derived growth factor (PDGF)-stimulated proliferation of rheumatoid synovial fibroblasts.[7,8] Furthermore, Ando et al. recently showed that imatinib inhibited osteoclastogenesis and joint destruction, but not the joint inflammation, in a rat model of collagen-induced arthritis (CIA).[9]

In the current study, we aimed to determine whether imatinib can inhibit joint inflammation using a mouse model of anti-collagen type II antibody-induced arthritis (CAIA), a representative model for the effector inflammatory phase of RA.[10–13] Our results suggest that imatinib has both preventive and therapeutic effects on CAIA, suggesting that imatinib may be useful for the prevention and treatment of the effector phase of RA.

Materials and methods

Reagents

Imatinib mesylate (STI571: Gleevec) capsules were from Novartis Pharma (Basel, Switzerland). The contents of one

capsule were dissolved in 10 ml of phosphate-buffered saline (PBS), centrifuged, filtered, and used as 100 mg/ml stock solution.

Induction of arthritis

Female 4–5-week-old BALB/c mice were purchased from SLC (Tokyo, Japan) and kept under specific pathogen-free conditions. The arthritogenic anti-collagen type II monoclonal antibody (mAb) cocktail obtained from Chondrex (Seattle, WA, USA) contains four mAbs (F10, A2, D8, and D1) in equal amounts. To establish anti-collagen type II antibody-induced arthritis,[10] the mice were intravenously injected with 2 mg per mouse of anti-collagen type II mAb cocktail (day 1) and 3 days later (day 4) with 50 μg per mouse of lipopolysaccharide (LPS) (Chondrex). For the CAIA prevention studies, imatinib (1 or 10 mg/kg per mouse) or a control vehicle (PBS) were intraperitoneally administered on day 5 and thereafter every day until sacrifice. For the CAIA treatment studies, imatinib (1 or 10 mg/kg per mouse) or a control vehicle (PBS) were intraperitoneally administered into the mice that had already developed arthritis on day 7 (average arthritis score of 12) and thereafter every day until sacrifice. The animal experiments were approved by the Institutional Review Board of the University of Yamanashi.

Clinical evaluation of arthritis

Starting on day 1 after mAb injection, the mice were blindly inspected for disease progression. The clinical severity of the disease was scored using a scoring system based on the number of inflamed joints in forepaws, hindpaws, and ankles, inflammation being defined by swelling: 0, normal; 1, slight swelling; 2, mild swelling; 3, moderate swelling; and 4, severe swelling. All paws and ankles were graded, thus resulting in a maximal clinical score of 24 per mouse, and then were expressed as the mean arthritic index on a given day.

Histology

The hind paws (torsocrural joint) were removed postmortem on day 10 (for prevention studies) or day 12 (for treatment studies) after mAb injection, fixed, and decalcified. The decalcified paws were embedded in paraffin, sectioned, and stained with hematoxylin–eosin or toluidine blue for evaluation of mast cells. For immunohistochemistry, the paws were immediately dissected and frozen in OCT compound in liquid nitrogen. The samples were stored at –80°C until cryosectioning. The sections were stained with anti-PDGF-A and -VEGF (vascular endothelial growth factor) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) through the use of peroxidase-based Vectastain ABC kits with DAB substrate (Vector Laboratories, Burlingame, CA, USA).

Data analysis

The data are summarized as the mean ± SD. The unpaired Student's *t*-test was used for the statistical analysis of the results. $P < 0.05$ was considered to be significant.

Results

To determine the effects of imatinib on arthritis, we used a mouse model of arthritis which was developed by the injection of monoclonal antibodies (mAbs) against type II collagen followed by the subsequent injection of bacterial LPS to reduce the threshold of the arthritogenic dose of mAbs and the required number of mAb clone (collagen type II antibody-induced arthritis; CAIA).[10] The development of this arthritis model, in particular in the early phase, is independent of T cells and B cells[11,12] and it is dependent on interleukin (IL)-1β and tumor necrosis factor (TNF)-α,[13] with neutrophils and macrophages being the major mediator of this inflammation.[10] Therefore, the CAIA model provides an opportunity to study the effector inflammatory phase of RA without involving the priming phase of the immune responses. Wild-type mice receiving control PBS developed the arthritis after the injection of anti-type II collagen mAbs (day 1) plus LPS, beginning on day 5 after the administration of the mAbs, with subsequent increasing severity of inflammation until day 10 (Fig. 1A,B).

For the CAIA prevention studies, mice received imatinib (1 mg/kg, 10 mg/kg) or control vehicle (PBS) on day 5 prior to the development of CAIA and thereafter every day. The mice receiving imatinib (1 or 10 mg/kg) did not show any clinical manifestations of arthritis as judged by the clinical scoring (Fig. 1A,B). Consistent with the clinical findings, a histological examination of the hind paws on day 10 showed a significantly reduced cellular infiltration and synovial hyperplasia in the mice receiving 1 mg/kg and 10 mg/kg imatinib (Fig. 1C). The expression of PDGF-A and VEGF in the synovial tissue was up-regulated after the induction of arthritis, which was reduced by the preventive treatment with both 1 mg/kg and 10 mg/kg imatinib (Fig. 1D). Thus, imatinib effectively prevented the development of arthritis induced by anti-collagen type II antibody.

To determine the therapeutic effects of imatinib on the established arthritis, imatinib (1 mg/kg) was intraperitoneally injected into the mice with a peak of arthritis (day 7) and thereafter every day. The mice treated with PBS beginning from day 7 showed persistent arthritis at least until day 12 whereas in the mice receiving imatinib (1 mg/kg), the arthritis improved from day 7 and thereafter as measured by the clinical scoring (Fig. 2A,B). A histological examination of the hind paws on day 12 also showed a significantly reduced cellular infiltration and synovial hyperplasia in the mice receiving 1 mg/kg imatinib (Fig. 2C). The expression of PDGF-A and VEGF in the synovial tissue was up-regulated after the induction of arthritis, which was reduced by the therapeutic treatment with 1 mg/kg imatinib (Fig.

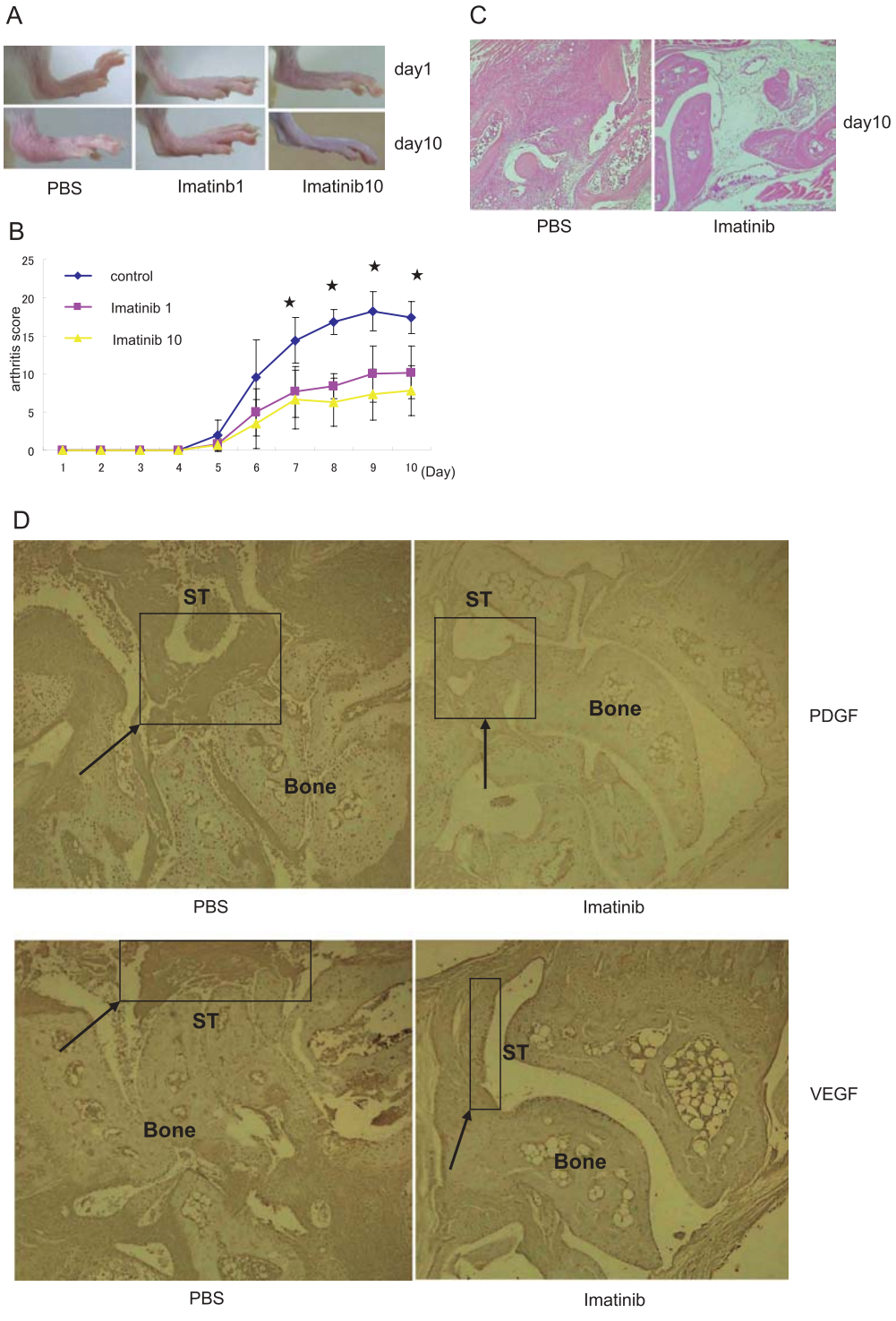

Fig. 1A–D. Imatinib mesylate prevented the development of arthritis induced by anti-collagen type II antibody in mice. The mice were intraperitoneally injected with 2 mg per mouse of anti-collagen type II monoclonal antibody (mAb) cocktail (day 1) and 3 days later (day 4) with 50 µg per mouse of lipopolysaccharide (LPS). Imatinib mesylate (1 mg and 10 mg/kg per mouse) or a control vehicle (phosphate-buffered saline; PBS) were intraperitoneally administered on day 5 and thereafter every day. **A** Representative photographs showing the fore and hind paws of the mice treated or untreated with imatinib mesylate (1 mg/kg and 10 mg/kg per mouse) on day 10. **B** Clinical scoring measured during the course of study. Values represent the mean ± SD of three mice per group. *$P <$ 0.05 compared with corresponding control. Similar results were obtained from at least three independent experiments. **C** A histological examination of the hind paws (torsocrural joint) of the mice treated or untreated with imatinib mesylate (10 mg/kg per mouse) on day 10. Representative photographs of hematoxylin–eosin staining are shown. **D** Immunohistochemical examination of the hind paws (torsocrural joint) of the mice treated or untreated with imatinib mesylate (10 mg/kg per mouse) on day 10. Representative photographs of immunohistochemical staining with anti-VEGF (*left panels*) and anti-PDGF-A (*right panels*) antibodies are shown. Please note that the intensity of the positive staining (*brown area*) in the synovial tissue decreased after the treatment of imatinib. *PBS*, phosphate-buffered saline; *PDGF*, platelet-derived growth factor; *VEGF*, vascular endothelial growth factor; *ST*, synovial tissue

2D). Thus, imatinib effectively reversed the established arthritis induced by anti-collagen type II antibody.

Because imatinib was shown to inhibit signaling pathways predominantly in mast cells[14] and mast cells are suggested to contribute to the RA pathgenesis,[1,2] we examined the presence of mast cells in synovial tissue of the CAIA model. Toluidine blue staining revealed that mast cells were hardly observed in the normal joint tissue whereas mast cells were significantly present in the synovial tissue after the induction of arthritis (Fig. 3 and data not shown).

Discussion

Although the etiology of RA remains unknown, macrophages, B cells, mast cells, and synovial fibroblasts become

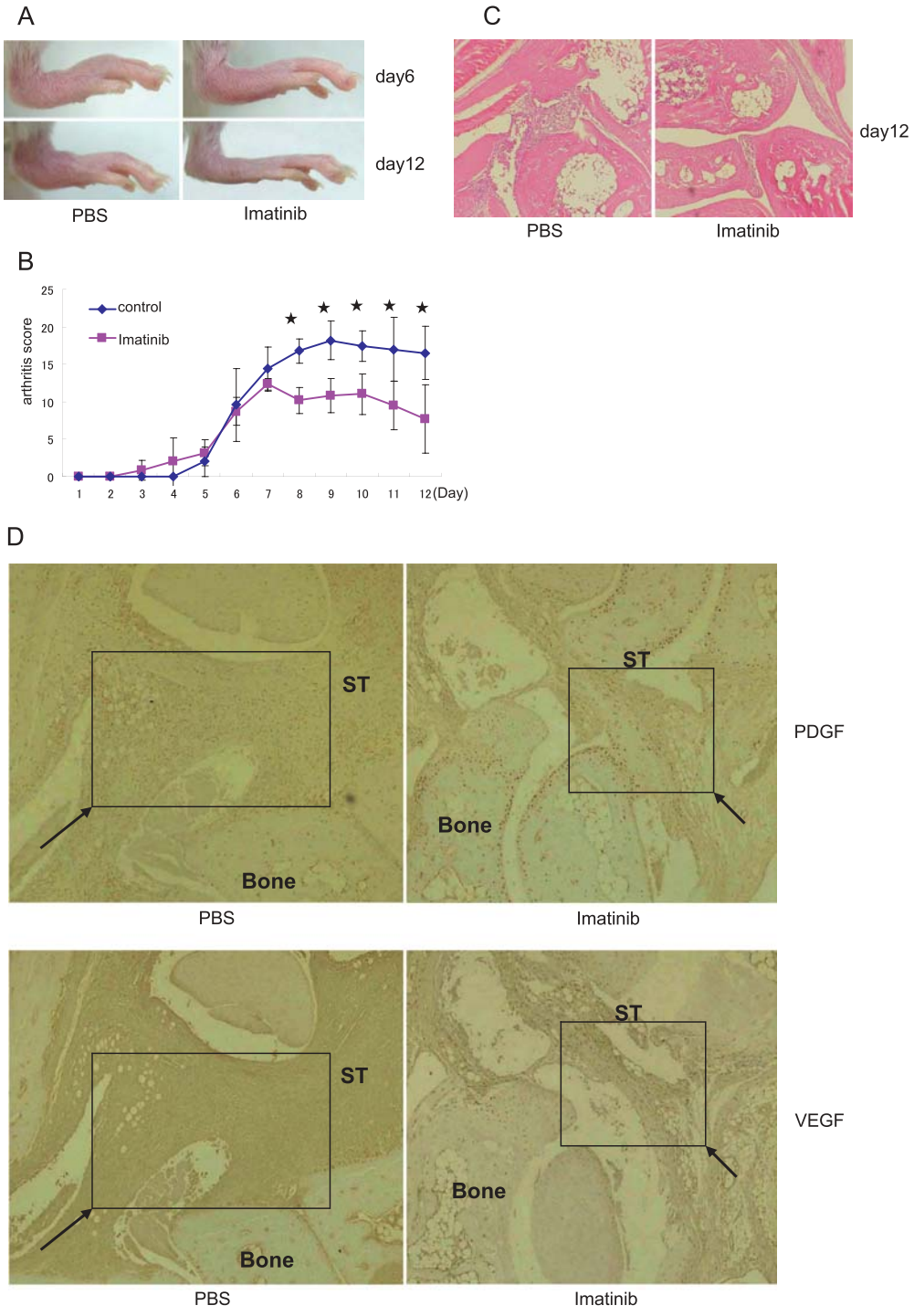

Fig. 2A–D. Imatinib mesylate reversed the established arthritis induced by anti-collagen type II antibody in mice. The mice were intraperitoneally injected with 2 mg per mouse of ant-collagen type II mAb cocktail (day 1) and 3 days later (day 4) with 50 µg per mouse of LPS. Imatinib mesylate (1 mg and 10 mg/kg per mouse) or a control vehicle (PBS) were intraperitoneally administered on day 7 and thereafter every day. **A** Representative photographs showing the fore and hind paws of the mice treated or untreated with imatinib mesylate (1 mg/kg and 10 mg/kg per mouse) on day 12. **B** Clinical scoring measured during the course of study. Values represent the mean ± SD of three mice per group. *$P <$ 0.05 compared with corresponding control. Similar results were obtained from at least three independent experiments. **C** A histological examination of the hind paws (torsocrural joint) of the mice treated or untreated with imatinib mesylate (10 mg/kg per mouse) on day 12. Representative photographs of hematoxylin–eosin staining are shown. **D** Immunohistochemical examination of the hind paws (torsocrural joint) of the mice treated or untreated with imatinib mesylate (10 mg/kg per mouse) on day 12. Representative photographs of immunohistochemical staining with anti-VEGF (*left panels*) and anti-PDGF-A (*right panels*) antibodies are shown. Please note that the intensity of the positive staining (brown area) in the synovial tissue (*ST*) decreased after the treatment of imatinib

activated in and contribute to synovial inflammation and joint destruction.[1,2] Recent studies have shown that imatinib inhibits multiple signaling pathways implicated in RA pathogenesis in vitro, including mast cell c-Kit signaling and TNF-α release, macrophage c-Fms activation and cytokine production, and synovial fibroblast PDGFR signaling and proliferation.[7–9,14] These cellular responses are also involved in the development of CAIA.[10] Thus, it is most likely that imatinib prevents and treats CAIA by inhibiting these signaling pathways that drive synovial inflammation.

Platelet-derived growth factor and VEGF are overexpressed in RA synovial tissue.[15–17] In addition, PDGF is a potent stimulator of synovial hyperplasia in RA[16,18] and the inhibition of VEGF signaling also suppressed acute arthritis in an animal model.[19,20] Our findings that imatinib inhibited synovial PDGF and VEGF expression in CAIA (Figs. 1D and 2D) thus also support the notion that imatinib may be useful for the treatment of RA.

Ando et al. recently showed that imatinib inhibited osteoclastogenesis and joint destruction, but not the joint

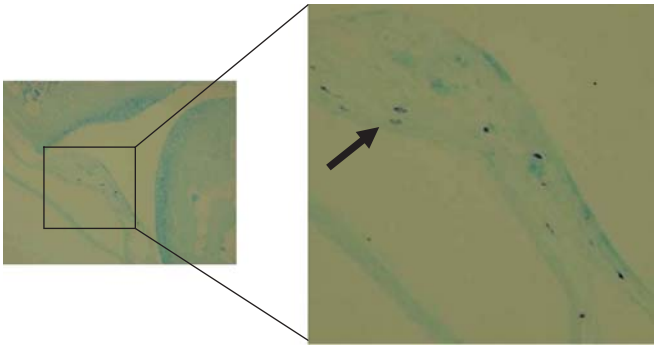

Fig. 3. Toluidine blue staining of the synovial tissue of anti-collagen type II antibody-induced arthritis. The mice were intraperitoneally injected with 2 mg per mouse of ant-collagen type II mAb cocktail (day 1) and 3 days later (day 4) with 50 μg per mouse of LPS. The hind paws (torsocrural joint) were removed postmortem on day 10, fixed, and decalcified. The decalcified paws were embedded in paraffin, sectioned, and stained with toluidine blue for evaluation of mast cells. Representative photographs are shown. *Arrows* indicate positive staining (mast cells)

inflammation, in a rat model of collagen-induced arthritis (CIA).[9] In contrast, most recently, Paniagua et al. reported that imatinib efficiently reduced synovitis as well as pannus formation and joint erosion in mouse CIA.[14] The discrepancy between the two studies might be due to the usage of different species. In the current study, we used CAIA model that develops joint inflammation independent of T cells and B cells. This model has advantage over CIA model in that we can evaluate the effects of imatinib on T-cell- and B-cell-independent effector inflammatory phase of RA. Therefore, our results suggest that imatinib can prevent and treat inflammation at the effector stage of RA.

It is not yet clear whether mast cells contribute to the development of CAIA. The current findings that the number of mast cells increased after the induction of CAIA (Fig. 3) and imatinib efficiently prevented and treated CAIA may suggest that mast cells are also an important component involved in the CAIA model. This point will be investigated in our future studies.

In summary, we demonstrate that imatinib efficiently prevents and treats CAIA, a representative model for inflammatory phase of RA. Our results thus suggest that imatinib may have beneficial effects not only on joint destruction but also for joint inflammation in RA.

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References

1. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell 1996;85:307–10.
2. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.
3. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344:1031–7.
4. Demettri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472–80.
5. Miyachi K, Ihara A, Hankins RW, Murai R, Maehiro S, Miyashita H. Efficacy of imatinib mesylate (STI571) treatment for a patients with rheumatoid arthritis developing chronic myelogenous leukemia. Clin Rheumatol 2003;22:329–32.
6. Eklund KK, Joensuu H. Treatment of rheumatoid arthritis with imatinib mesylate: clinical improvement in three refractory cases. Ann Med 2003;35:362–7.
7. Sandler C, Joutsiniemi S, Lindstedt KA, Juutilainen T, Kovanen PT, Eklund KK. Imatinib mesylate inhibits platelet derived growth factor stimulated proliferation of rheumatoid synovial fibroblasts Biochem Biophys Res Commun 2006;347:31–5.
8. Kameda H, Ishigami H, Suzuki M, Abe T, Takeuchi T. Imatinib mesylate inhibits proliferation of rheumatoid synovial fibroblast-like cells and phosphorylation of Gab adapter proteins activated by platelet-derived growth factor. Clin Exp Immunol 2006;144: 335–41.
9. Ando W, Hashimoto J, Nampei A, Tsuboi T, Tateishi K, Ono T, et al. Imatinib mesylate inhibits osteoclastogenesis and joint destruction in rats with collagen-induced arthritis (CIA). J Bone Miner Metab 2006;24:274–82.
10. Nandakumar KS, Svensson L, Holmdahl R. Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. Am J Pathol 2003;163:1827–37.
11. Nandakumar KS, Backlund J, Vestberg M, Holmdahl R. Collagen type II (CII)-specific antibodies induce arthritis in the absence of T or B cells but the arthritis progression is enhanced by CII-reactive T cells. Arthritis Res Ther 2004;6:R544–50.
12. Wang J, Fathman JW, Lugo-Villarino G, Scimone L, von Andrian U, Dorfman DM, et al. Transcription factor T-beta regulates inflammatory arthritis through its function in dendritic cells. J Clin Invest 2006;116:414–21.
13. Kagari, T, Doi H, Shimozato T. The importance of IL-1β and TNF-α, and the noninvolvement of IL-6, in the development of monoclonal antibody-induced arthritis. J Immunol 2002;169: 1459–66.
14. Paniagua RT, Sharpe O, Ho PP, Chan SM, Chang A, Higgins JP, et al. Selective tyrosine kinase inhibition by imatinib mesylate for the treatment of autoimmune arthritis. J Clin Invest 2006;116: 2633–42.
15. Bucala R, Ritchlin C, Winchester R, Cerami A. Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts. J Exp Med 1991;173:569–74.
16. Cheon H, et al. Platelet-derived growth factor-AA increases IL-1β and IL-8 expression and activates NF-κB in rheumatoid fibroblast-like synoviocytes. Scand J Immunol 2004;60:1121–9.
17. Kasama T, Shiozawa F, Kobayashi K, Yajima N, Hanyuda M, Takeuchi HT, et al. Vascular endothelial growth factor expression by activated synovial leukocytes in rheumatoid arthritis: critical involvement of the interaction with synovial fibroblasts. Arthritis Rheum 2001;44:2512–24.
18. Butler DM, Leizer T, Hamilton JA. Stimulation of human synovial fibroblast DNA synthesis by platelet-derived growth factor and fibroblast growth factor. Differences to the activation by IL-1. J Immunol 1989;142:3098–103.
19. Fava RA, Olsen NJ, Spencer-Green G, Yeo KT, Yeo TK, Berse B, et al. Vascular permeability factor/endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue. J Exp Med 1994;180:341–6.
20. Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, et al. Revascularization of ischemic tissues by PIGF treatment, and inhibition of tumor angiogenesis, arthritis, and atherosclerosis by anti-Flt1. Nat Med 2002;8:831–40.