undergone surgical intervention (Table 2). In patients with rheumatic diseases, pneumothorax complication, in general, tends to be recurrent and intractable, because the most patients have underlying pulmonary lesions and glucocorticoid therapy. Furthermore, prolonged chest tube use may increase the risk of infection to these patients. Thus, surgical intervention to pneumothorax complicated in patients with rheumatic diseases may be considered at the early stage[17], especially when the pneumothorax is recurrent or respond poorly to nonsurgical procedure.

Acknowledgment

We wish to thank Ms Sonoko Sakurai for secretarial assistance.

References

1) McCune WJ, et al. : Plumonary Manifestatons of Systemic Lupus Erythematous. Wallace DJ, Hahn BH, Eds. Dubois' Lupus Erythmatousus 7th ed. Lippincott Williams & Wikins, Philadelphia, pp258, 678–699, 2007.
2) Jang KA, et al. : Subcutaneous emphysema with spontaneous pneumomediastinum and pneumothorax in adult dermatomyositis. *J Dermatol.* **26** : 125–127, 1999.
3) Shimamoto K, et al. : Three cases of polymyositis/dermatomyositis complicated with pneumomediastinum. *Nihon Rinsho Meneki Gakkai Kaishi.* **31** : 56–61, 2008. (in Japanese, Abstract in English)
4) Bouros D, et al. : Pleural involvement in systemic autoimmune disorders. *Respiration.* **75** : 361–371, 2008.
5) Ayzenberg O, et al. : Bilateral pneumothoraces and pleural effusions complicating rheumatoid lung disease. *Thorax.* **38** : 159–160, 1983.
6) Zeuner M, et al. : Spontaneous pneumothorax in a patient with systemic sclerosis. *Clin Rheumatol.* **15** : 211–213, 1996.
7) Ng SC, Tan WC : Bilateral spontaneous pneumothorax in systemic sclerosis—report of two cases. *J Rheumatol.* **17** : 689–691, 1990.
8) Lang B, et al. : Progressive systemic sclerosis presenting with spontaneous pneumothorax. *J Rheumatol.* **16** : 254–256, 1989.
9) Dines DE, et al. : Nontuberculous pulmonary parenchymal conditions predisposing to spontaneous pneumothorax. Report of four cases. *J Thorac Cardiovasc Surg.* **53** : 726–732, 1967.
10) Edwards WG Jr, Dines DE : Recurrent spontaneous pneumothorax in diffuse scleroderma. Report of a case. *Dis Chest.* **49** : 96–98, 1966.
11) Israel MS, Harley BJ. Spontaneous pneumothorax in scleroderma. *Thorax.* **11** : 113–118, 1956.
12) Kono H, et al. : Pneumomediastinum in dermatomyositis : association with cutaneous vasculopathy. *Ann Rheum Dis.* **59** : 372–376, 2000.
13) Tong SQ, et al. : Clinical analysis of pneumomediastinum complicated in polymyositis and dermatomyositis. *Chinese medical journal.* **86** : 624–627, 2006.
14) Wicke C, et al. : Effects of steroids and retinoids on wound healing. *Arch Surg.* **135** : 1265–1270, 2000.
15) Beer HD, et al. : Glucocorticoid–regulated gene expression during cutaneous wound repair. *Vitam Horm.* **59** : 217–239, 2000.
16) Hamada K, et al. : Cyclophosphamide–induced late–onset lung disease. *Intern Med. 2003 Jan* ; **42**(1) : 82–88. Review.
17) Sawkar LA, Easom HF : Recurrent spontaneous pneumothoraces in systemic lupus erythematosus. *Chest.* **60** : 604–605, 1971.
18) Richards AJ, et al. : Diffuse pulmonary fibrosis and bilateral pneumothoraces in systemic lupus erythematosus. *Postgrad Med J.* **51** : 851–855, 1975.
19) Passero FC, Myers AR : Hemopneumothorax in systemic lupus erythematosus. *J Rheumatol.* 7 : 183–186, 1980.
20) Masuda A, et al. : Recurrent pneumothoraces and mediastinal emphysema in systemic lupus erythematosus. *J Rheumatol.* **17** : 544–548, 1990.
21) Paira SO, Roverano S : Bilateral pneumothorax and mediastinal emphysema in systemic lupus erythematosus. *Clin Rheumatol.* **11** : 571–573, 1992.
22) Nishitsuji M, et al. : A case of systemic lupus erythematodes with hemosputum and pneumothorax probably resulting from pulmonary infarction and pulmonary angitis. *Nihon Kokyuki Gakkai Zasshi* **36** : 71–77, 1998.
23) Yen JH, et al. : Systemic lupus erythematosus complicated with recurrent spontaneous pneumothorax—acase report. *Kaohsiung J Med Sci.* **17** : 540–544, 2001.
24) Wilhelm M, Van Why SK : Pneumothoraces complicating systemic lupus erythematosus with

nephritis. *Pediatr Nephrol.* **17**: 261–263, 2002.
25) Maeda R, et al.: Systemic lupus erythematosus with multiple lung cysts. *Interact Cardiovasc Thorac Surg.* **8**: 701–702, 2009.
26) Buyon JP: Systemic lupus erythematous: Clinical and Laboratory Features. Klippel JH, Ed, Primer on the Rheumatic Disease. 12th ed. Arthris Foundation, Atlanta, pp335–345, 2001.

ARTHRITIS & RHEUMATISM
Vol. 62, No. 6, June 2010, pp 1641–1649
DOI 10.1002/art.27450

Adiponectin Stimulates Prostaglandin E_2 Production in Rheumatoid Arthritis Synovial Fibroblasts

Natsuko Kusunoki,[1] Kanako Kitahara,[1] Fumiaki Kojima,[2] Nahoko Tanaka,[1] Kaichi Kaneko,[1] Hirahito Endo,[1] Toru Suguro,[1] and Shinichi Kawai[1]

Objective. **Adipokines may influence inflammatory and/or immune responses. This study was undertaken to examine whether adiponectin affects the production of prostaglandin E_2 (PGE_2) by rheumatoid arthritis synovial fibroblasts (RASFs).**

Methods. **Synovial tissue was obtained from patients with RA who were undergoing joint replacement surgery. Fibroblast-like cells from the third or fourth passage were used as RASFs. Expression of adiponectin receptor messenger RNA (mRNA) and protein was detected. PGE_2 (converted from arachidonic acid) was measured by enzyme-linked immunosorbent assay (ELISA). Expression of mRNA and protein for cyclooxygenase 2 (COX-2) and membrane-associated PGE synthase 1 (mPGES-1), key enzymes involved in PGE_2 synthesis, was detected in RASFs. The effects of RNA interference (RNAi) targeting the adiponectin receptor genes and the receptor signal inhibitors were examined. The influence of adiponectin on NF-κB activation in RASFs was measured with an ELISA kit.**

Results. **Adiponectin receptors were detected in RASFs. Adiponectin increased both COX-2 and mPGES-1 mRNA and protein expression by RASFs in a time- and concentration-dependent manner. PGE_2 production by RASFs was also increased by the addition of adiponectin, and this increase was inhibited by RNAi for the adiponectin receptor gene, or coincubation with the receptor signal inhibitors. Enhancement of NF-κB activation by adiponectin as well as by interleukin-1β was observed in RASFs.**

Conclusion. **Our findings indicate that adiponectin induces COX-2 and mPGES-1 expression, resulting in the enhancement of PGE_2 production by RASFs. Thus, adiponectin may play a role in the pathogenesis of synovitis in RA patients.**

Adipose tissue has long been considered to be a structural component of many organs and a site for energy storage. Recently, however, some studies have demonstrated that the major cellular component of adipose tissue, the adipocyte, has the ability to synthesize and release physiologically active molecules such as adiponectin, leptin, and resistin, as well as cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) (1). These molecules are called adipokines or adipocytokines. Several adipokines, such as adiponectin, may play a central role in the regulation of insulin resistance (2), as well as being involved in many aspects of inflammation and immunity (3,4).

Rheumatoid arthritis (RA) is characterized by extensive inflammation and proliferation of the synovium in various joints. Since proinflammatory cytokines, such as TNFα, IL-1β, and IL-6, play a central role in the pathophysiologic mechanisms of RA, novel strategies that neutralize these cytokines by using monoclonal antibodies or soluble receptors have recently been developed as new treatments for RA (5). Although blockade of these cytokines is beneficial, it is not curative and the effect is only partial, with failure to respond being common (5). Therefore, it seems possible that other proinflammatory cytokines may contribute to the pathophysiology of inflammation in RA patients.

Supported in part by research grants to Dr. Kawai from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (grant 17590477), the Ministry of Health, Labor, and Welfare of Japan, and Toho University Graduate School of Medicine (Research Promotion grant 07-04).

[1]Natsuko Kusunoki, PhD, Kanako Kitahara, MD, PhD, Nahoko Tanaka, MD, Kaichi Kaneko, MD, Hirahito Endo, MD, PhD, Toru Suguro, MD, PhD, Shinichi Kawai, MD, PhD: Toho University School of Medicine, Tokyo, Japan; [2]Fumiaki Kojima, PhD: Asahikawa Medical College, Asahikawa, Japan.

Address correspondence and reprint requests to Shinichi Kawai, MD, PhD, Department of Internal Medicine, Division of Rheumatology, Toho University School of Medicine, 6-11-1 Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan. E-mail: skawai@med.toho-u.ac.jp.

Submitted for publication June 6, 2009; accepted in revised form February 25, 2010.

Some previous studies provoked our interest in the role of adiponectin in the pathogenesis of arthritis. For instance, the concentration of adiponectin in RA synovial fluid was shown to be significantly higher than in that of patients with osteoarthritis (OA) (6–8). Moreover, serum and plasma concentrations of adiponectin are higher in RA patients than in healthy controls (7,9) and are significantly correlated with the C-reactive protein level (9). Ehling et al (10) showed that adiponectin exists in cells from the synovial lining layer and in articular adipose tissue. Furthermore, adiponectin induces proinflammatory molecules, such as IL-6 and matrix metalloproteinase 1, in RA synovial fibroblasts (RASFs). Moreover, adiponectin enhances the expression of monocyte chemoattractant protein 1 and IL-6 by RASFs (11). Recently, Giles et al (12) reported that adiponectin may represent a mechanistic link between low adiposity and increased radiographic damage in RA. The results of these studies suggest that adiponectin might play a role in the pathogenesis of RA.

In the synovial tissue of RA patients, we previously found that proinflammatory cytokines, such as IL-1β, increased the expression of cyclooxygenase 2 (COX-2) and membrane-associated PGE synthase 1 (mPGES-1), resulting in increased production of prostaglandin E_2 (PGE_2) (13). We also found that PGE_2 was a strong enhancer of IL-1β–induced mPGES-1 expression in RASFs (14). In the present study, we examined the effects of adiponectin on these key enzymes that contribute to the inflammatory response of RASFs.

MATERIALS AND METHODS

Materials. Recombinant human adiponectin, which was composed of 3 isoforms (low, middle, and high molecular weight), was purchased from Biovendor Laboratory Medicine. It was dissolved in deionized water to prepare a stock solution. Recombinant human IL-1β was purchased from R&D Systems and was dissolved in sterile phosphate buffered saline (PBS) containing 0.1% (volume/volume) bovine serum albumin to prepare a stock solution. Mouse anti-human COX-1 monoclonal antibody was purchased from Wako Pure Chemical Industries. Rabbit anti-human COX-2 polyclonal antibody, rabbit anti-human mPGES-1 polyclonal antibody, and rabbit anti-human cytosolic PGES (cPGES) polyclonal antibody were obtained from Cayman Chemical. Rabbit anti-human GAPDH polyclonal antibody, goat anti-human adiponectin receptor 1 (AdipoR1) polyclonal antibody, and goat anti-human AdipoR2 polyclonal antibody were obtained from Santa Cruz Biotechnology. Horseradish peroxidase (HRP)–conjugated goat anti-rabbit IgG, HRP-conjugated goat anti-mouse IgG, and HRP-conjugated donkey anti-goat IgG were purchased from Jackson ImmunoResearch Laboratories. ECL Western blotting detection reagent was purchased from GE Healthcare UK, and polyvinylidene difluoride membrane (Immobilon-P) was obtained from Millipore. Compound C (6-[4-(2-Piperidin-1-yl-ethoxy)-phenyl)]-3-pyridin-4-yl-pyrazolo[1,5-a] pyrimidine) was purchased from Merck. MK886 (1-[(4-chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-α,α-dimethyl-5-(1-methylethyl)-1H-indole-2-propanoic acid, sodium salt) was purchased from Sigma-Aldrich. RPMI 1640 medium, penicillin/streptomycin solution, fetal bovine serum (FBS), and 0.25% trypsin/EDTA were obtained from Invitrogen. PBS was purchased from Takara Shuzo. All other chemicals were purchased from Wako Pure Chemical Industries.

Cell culture. RASFs were prepared from synovial tissue as previously described (15). RA and OA tissue specimens were obtained from patients undergoing total knee replacement surgery who fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for RA or OA (16,17). The protocol for this study was approved by the Toho University Ethics Committee, and all patients gave written consent for the use of their tissue in the present research. Synovial tissue was digested for 2 hours with 0.25% (weight/volume) bacterial collagenase (Immuno-Biological Laboratories) and then was suspended in RPMI 1640 medium with 10% (v/v) FBS, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. The cells were incubated at 37°C in 5% CO_2 for several days, after which nonadherent cells were removed. Fibroblast-like adherent cells from the third or fourth passages were used as RASFs. The concentration of RASFs was 2.5×10^6 cells/75-cm^2 flask.

Reverse transcription–polymerase chain reaction (RT-PCR). Cells were seeded in culture medium containing 10% (v/v) FBS, and total RNA was extracted with an RNeasy Mini kit according to the recommendations of the manufacturer (Qiagen). Reverse transcription was performed with a SuperScript first-strand synthesis system for RT-PCR according to the recommendations of the manufacturer (Invitrogen), with 1 μg of total RNA from the cells as a template. Equal amounts of each reverse-transcribed product were amplified by PCR with HotStar *Taq* polymerase (Qiagen). The primer sequences and numbers of cycles were as follows: for AdipoR1 (35 cycles), sense 5′-CCCTGACTGGCTAAAGGACA and antisense 5′-CAGTACAGCCGCCTTCTAGG; for AdipoR2 (35 cycles), sense 5′-TTTGGAGCCCATTTTAGAGG and antisense 5′-TCAACCAGCCTATCTGCCCTA; and for β-actin (28 cycles), sense 5′-CCTCGCCTTTGCCGATCC and antisense 5′-GGATCTTCATGAGGTAGTCAGTC. After initial denaturation at 95°C for 15 minutes, PCR involved amplification for a variable number of cycles of 30 seconds at 95°C, 30 seconds at 56°C, and 45 seconds at 72°C, followed by elongation for 5 minutes at 72°C. The amplified complementary DNA (cDNA) fragments were resolved by electrophoresis on a 2% (w/v) agarose gel, and were detected under ultraviolet light using LAS-3000 (Fujifilm) after staining the gel with ethidium bromide.

Real-time PCR. To evaluate the expression of messenger RNA (mRNA) for AdipoR1, AdipoR2, COX-2, and mPGES-1, real-time PCR was performed using real-time TaqMan technology with a Sequence Detection System model 7000 according to the recommendations of the manufacturer (Applied Biosystems). Cells were cultured under various conditions in medium containing 1% (v/v) FBS, and extraction of total RNA and synthesis of cDNA were performed as de-

scribed above. The specific probes for AdipoR1, AdipoR2, COX-2, and mPGES-1 were obtained from TaqMan Gene Expression Assay (Applied Biosystems). The ID numbers of the products were Hs00360422_m1 for AdipoR1, Hs00226105_m1 for AdipoR2, Hs00153133_m1 for COX-2, and Hs00610420_m1 for mPGES-1. The threshold cycle was calculated from a standard curve. Expression of the target mRNA was normalized to the expression of β-actin mRNA.

Western blot analysis. Cells (at a density of $5 \times 10^4/cm^2$) were cultured under various conditions in medium containing 1% (v/v) FBS. Subsequently, the cells were lysed in Tris buffered saline (TBS) containing 0.1% (w/v) sodium dodecyl sulfate (SDS) for COX and PGES as reported previously (14). For AdipoR1 and AdipoR2, the cells were lysed in Triton lysis buffer containing 50 m*M* Tris HCl (pH 8.0), 150 m*M* NaCl, 10 m*M* EDTA, 1% Triton X-100, and a protease inhibitor cocktail (Pierce Biotechnology) as reported previously (18). The protein content of the lysates was determined with the bicinchoninic acid protein assay reagent (Pierce Biotechnology), using bovine serum albumin as the standard. Then cell lysates were adjusted to 10 μg of protein and were applied to SDS polyacrylamide gel (10–15% [w/v]) for electrophoresis. Next, the proteins were electroblotted onto Immobilon-P polyvinylidene difluoride membranes with a semidry blotter (Atto). After the membranes had been blocked in 10 m*M* TBS containing 0.1% (v/v) Tween 20 (TBST) and 5% (w/v) skim milk, the primary antibody (goat anti-human AdipoR1 antibody, goat anti-human AdipoR2 antibody, rabbit anti-human GAPDH antibody, mouse anti-human COX-1 antibody, rabbit anti-human COX-2 antibody, rabbit anti-human mPGES-1 antibody, or rabbit anti-human cPGES antibody) was added at a dilution of 1:200 (AdipoR1, AdipoR2, GAPDH, COX-1, and COX-2) or 1:500 (mPGES-1 and cPGES) in TBST, and incubated for 1.5 hours. After the membranes had been washed with TBST, the secondary antibody (HRP-conjugated donkey anti-goat antibody, HRP-conjugated goat anti-rabbit antibody, or HRP-conjugated goat anti-mouse antibody) was added (at a dilution of 1:10,000 or 1:5,000 in TBST) and incubation was performed for 1 hour. After further washing with TBST, protein bands were detected with enhanced chemiluminescence Western blotting detection reagents (GE Healthcare UK) using LAS-3000 (Fujifilm).

Measurement of PG levels in culture medium. Cells were plated in 24-well plastic plates (1×10^5/well) and cultured for 18 hours under various conditions in medium containing 1% (v/v) FBS in an atmosphere of 5% CO_2. After washing with PBS, 3 μ*M* arachidonic acid (Cayman Chemical) was added to each well. After incubation for 30 minutes, the culture medium was harvested using a syringe and filtered through a 0.22-μm filter (Millipore). Then PGE_2 concentrations in the medium were measured by an enzyme-linked immunosorbent assay (ELISA) kit according to the recommendations of the manufacturer (Cayman Chemical). Experiments using RASFs and OASFs were conducted in triplicate wells, and PGE_2 concentration was measured in triplicate.

Inhibition of adiponectin with antiadiponectin antibody. Antiadiponectin antibody was used to neutralize adiponectin as described previously (18). Adiponectin was incubated with mouse antiadiponectin monoclonal antibody (Millipore), mouse monoclonal IgG_1 negative control (Millipore), or PBS and Protein G–Sepharose beads (GE Healthcare UK) at 4°C overnight. The supernatant was collected and added to RASFs cultured in 96-well plates (2×10^4/well) for measurement of PGE_2 levels in culture medium. After 18 hours of incubation, PGE_2 production from arachidonic acid was analyzed as described above.

RNA interference (RNAi) with adiponectin receptors. An RNAi assay was performed to down-regulate the expression of AdipoR1 or AdipoR2 by RASFs. Small interfering RNA (siRNA) for AdipoR1 and AdipoR2 (Stealth RNAi) were purchased from Invitrogen. For gene knockdown experiments, RASFs were plated in 10-cm plastic dishes (3×10^5/dish) in RPMI 1640 medium with 10% (v/v) FBS and cultured for 18 hours. Then the medium was changed to serum-free RPMI 1640 medium, and the cells were transfected with siRNA (10 pmoles/ml) for adiponectin receptors or with control siRNA (10 pmoles/ml; Invitrogen) using Lipofectamine RNAiMAX according to the recommendations of the manufacturer (Invitrogen). After 72 hours, the cells were replated into 35-mm plastic dishes for PCR or into 96-well plastic plates for PGE_2 ELISA and receptor protein analyses.

Receptor protein analyses. RASFs, which were transfected with siRNA for AdipoR1, AdipoR2, or negative control were plated into 96-well plates (2×10^4/well) for cell-based ELISA (R&D Systems) and cultured for 18 hours. The cells were fixed with 4% formaldehyde for 20 minutes at room temperature. After washing, cells were blocked for 1 hour at room temperature. Cells were incubated overnight at 4°C with primary antibody (anti-AdipoR1 antibody, anti-AdipoR2 antibody, or anti-GAPDH antibody). Alkaline phosphatase–conjugated secondary antibody and HRP-conjugated secondary antibody were added to the wells, and incubation at room temperature for 2 hours was carried out. After incubation, fluorogenic substrates for each secondary antibody were added to the wells. Fluorescence was measured according to the recommendations of the manufacturer. Experiments were performed using triplicate samples from each of 3 patients.

Analysis of nuclear translocation of NF-κB. RASFs were incubated without serum for 18 hours, and then were incubated with or without adiponectin (2 μg/ml) or IL-1β (1 ng/ml) for 3 hours. Next the cells were lysed, and nuclear extracts were obtained with a Nuclear Extract Kit according to the recommendations of the manufacturer (Active Motif). These nuclear extracts were diluted and applied to an NF-κB Family Transcription Factor Assay Kit (Active Motif). Nuclear translocation of NF-κB subunits was measured by ELISA using antibodies for each subtype of NF-κB.

Statistical analysis. Data are expressed as the mean ± SEM. Groups were compared using the Kruskal-Wallis test or Tukey's multiple comparison test. *P* values less than 0.05 were considered significant.

RESULTS

Detection of adiponectin receptor expression in RASFs. To determine whether the 2 adiponectin receptors were expressed by RASFs, we performed RT-PCR and Western blotting. Messenger RNA for both adiponectin receptors, AdipoR1 and AdipoR2, was ex-

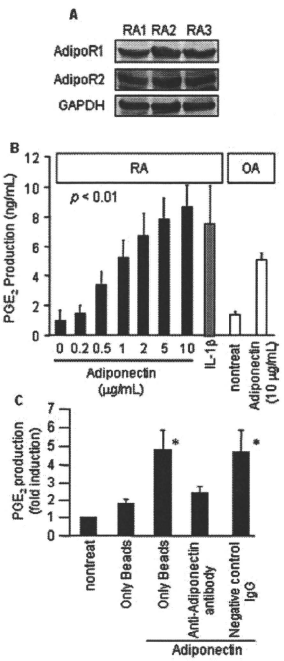

Figure 1. A, Western blot analysis of lysates of rheumatoid arthritis synovial fibroblasts (RASFs) for adiponectin receptor 1 (AdipoR1), AdipoR2, and GAPDH. RA1 = RA patient 1. **B,** Prostaglandin E_2 (PGE_2) production by RASFs and osteoarthritis synovial fibroblasts (OASFs) treated with various concentrations of adiponectin. RASFs and OASFs were incubated with adiponectin (at the indicated concentrations) or with interleukin-1β (IL-1β; 1 ng/ml) for 18 hours. The concentration of PGE_2 in the culture medium was measured by enzyme-linked immunosorbent assay (ELISA). Bars show the mean and SEM from 3 patients with RA and 3 patients with OA. Significance across groups was evaluated by Kruskal-Wallis test. **C,** Inhibition of adiponectin-induced PGE_2 production by antiadiponectin antibody. Adiponectin was incubated overnight at 4°C with negative control IgG, antiadiponectin antibody, or phosphate buffered saline (PBS) and Sepharose beads. PBS incubated alone (nontreat) and PBS incubated with Sepharose beads were used as negative controls. Supernatant was collected and added to cultured RASFs. The PGE_2 concentration was measured by ELISA. Bars show the mean and SEM (n = 3). * = $P <$ 0.05 versus treatment with PBS alone, by Tukey's multiple comparison test.

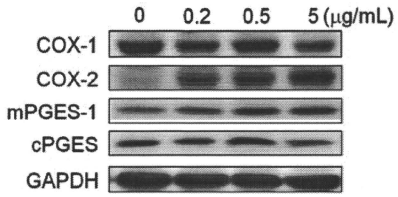

Figure 2. Effect of adiponectin on expression of cyclooxygenase 1 (COX-1), COX-2, membrane-associated prostaglandin E synthase 1 (mPGES-1), and cytosolic PGES (cPGES). Rheumatoid arthritis synovial fibroblasts were incubated for 18 hours with adiponectin at the indicated concentrations. Protein from the cells was subjected to Western blot analysis for COX-1, COX-2, mPGES-1, cPGES, and GAPDH. Representative results from 3 patients are shown.

pressed in RASFs from each of 3 RA patients (data not shown), as previously demonstrated (10,19). RASFs also expressed adiponectin receptor proteins (Figure 1A).

Effect of adiponectin on PGE_2 production by RASFs. To determine whether adiponectin increased the production of PGE_2 from arachidonic acid by RASFs, we measured PGE_2 concentrations in the culture medium of RASFs incubated with adiponectin (Figure 1B). We found that adiponectin significantly increased PGE_2 production by RASFs in a concentration-dependent manner. The effect of 5 μg/ml of adiponectin was equal to the effect of 1 ng/ml of IL-1β. In OASFs, adiponectin also stimulated PGE_2 production, but its effect was weaker. Production of 2,3-dinor-6-keto-$PGF_{1\alpha}$ (a metabolic product of PGI_2), PGD_2, $PGF_{2\alpha}$, and thromboxane B_2 (a metabolic product of thromboxane A_2) by RASFs was not enhanced after adiponectin treatment (data not shown). Adiponectin-induced PGE_2 production was inhibited by the presence of antiadiponectin antibody (Figure 1C).

Effect of adiponectin on protein levels and expression of mRNA for enzymes related to PGE_2 synthesis. To determine whether adiponectin increased the expression of enzymes related to PGE_2 synthesis, we performed Western blotting with selective antibodies for COX-1, COX-2, mPGES-1, and cPGES. As shown in Figure 2, adiponectin increased the expression of COX-2 protein in a concentration-dependent manner. The expression of mPGES-1 protein was also increased by adiponectin, whereas COX-1 and cPGES protein levels were unchanged, as measured by densitometry analyses of the enzyme:GAPDH expression ratio (data not shown).

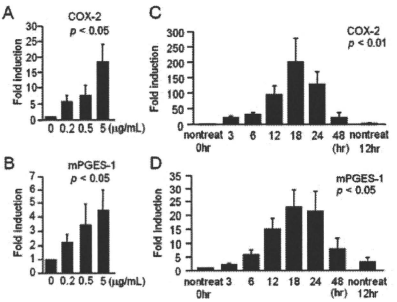

Figure 3. **A** and **B**, Fold induction of COX-2 (**A**) and mPGES-1 (**B**) in rheumatoid arthritis synovial fibroblasts (RASFs) incubated with adiponectin at the indicated concentrations for 18 hours. **C** and **D**, Fold induction of COX-2 (**C**) and mPGES-1 (**D**) in RASFs incubated with or without adiponectin (2 μg/ml) for the indicated times. First-strand cDNA was synthesized from total cellular RNA and was subjected to real-time polymerase chain reaction for COX-2 and mPGES-1, as described in Materials and Methods. The threshold cycle was calculated from a standard curve, which was drawn using data from interleukin-1β–stimulated cells. Expression of the target mRNA was normalized to the expression of β-actin mRNA. Fold induction was measured relative to mRNA expression by cells incubated without adiponectin in **A** and **B** and relative to mRNA expression by cells incubated with adiponectin for 0 hours in **C** and **D**. Bars show the mean and SEM (n = 3). Significance across groups was evaluated by Kruskal-Wallis test. nontreat = untreated (see Figure 2 for other definitions).

Figures 3A and B show that adiponectin caused a concentration-dependent increase in the expression of COX-2 and mPGES-1 mRNA, as detected by real-time PCR. As shown in Figures 3C and D, COX-2 and mPGES-1 mRNA expression were both increased by adiponectin treatment in a time-dependent manner. COX-2 mRNA expression was detected after 3 hours of incubation with adiponectin and was maximal at 18 hours; mPGES-1 mRNA expression also peaked after 18 hours of treatment.

Decrease in PGE_2 production by RASFs after RNAi with adiponectin receptors. To determine whether the induction of PGE_2 production by adiponectin occurred via adiponectin receptors, we examined the effect of RNAi with the 2 adiponectin receptors (AdipoR1 and AdipoR2). RASFs were transfected with siRNA for AdipoR1 or AdipoR2, or with negative control siRNA, and then expression of AdipoR1 or AdipoR2 mRNA was detected by RT-PCR (Figure 4A) and real-time PCR (Figure 4B). When cells were seeded in 96-well plates and incubated with adiponectin for 18 hours, PGE_2 production by RASFs transfected with the siRNA

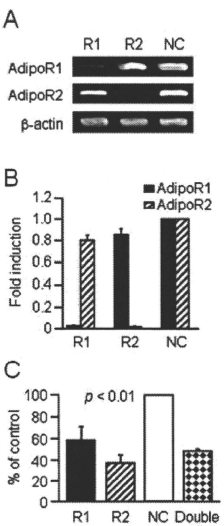

Figure 4. Effect of RNA interference on PGE_2 production by RASFs. RASFs were transfected with small interfering RNA (siRNA) for AdipoR1 (R1), siRNA for AdipoR2 (R2), negative control siRNA (NC), or with siRNA for both receptors (double). **A**, Reverse transcription–polymerase chain reaction (RT-PCR) for AdipoR1, AdipoR2, and β-actin. Total RNA was isolated from cells and was subjected to RT-PCR as described in Materials and Methods. Representative results from fibroblasts obtained from 3 patients are shown. **B**, Fold induction of mRNA for AdipoR1, AdipoR2, and negative control in RASFs transfected with siRNA for AdipoR1, AdipoR2, or negative control. First-strand cDNA was synthesized from total cellular RNA and was subjected to real-time PCR for AdipoR1 or AdipoR2 as described in Materials and Methods. The threshold cycle was calculated from a standard curve, which was drawn using data from nontransfected cells. Expression of the target mRNA was normalized to the expression of β-actin mRNA. Fold induction was measured relative to mRNA expression by negative control cells. Bars show the mean and SEM (n = 3). **C**, Concentration of PGE_2 in the culture medium of cells transfected with siRNA for AdipoR1, AdipoR2, negative control, or both receptors and incubated with adiponectin (10 μg/ml) for 18 hours. The concentration of PGE_2 in the culture medium was measured by ELISA. Bars show the mean and SEM (n = 3). Significance across groups was evaluated by Kruskal-Wallis test. See Figure 1 for other definitions.

for AdipoR1 or AdipoR2 was significantly reduced compared with that by RASFs transfected with control siRNA (Figure 4C). The mRNA knockdown of both of the adiponectin receptor genes also decreased adiponectin-induced PGE_2 production (Figure 4C).

AdipoR1 and AdipoR2 protein expression in RASFs transfected with siRNA for each receptor were measured using cell-based ELISA. The siRNA for AdipoR1 down-regulated mean ± SEM AdipoR1 protein expression by 34.6 ± 18.8% (n = 3 patients) compared with the negative control, whereas the siRNA for AdipoR2 down-regulated mean ± SEM AdipoR2 protein expression by 8.3 ± 11.3% (n = 3 patients) compared with the negative control. However, these differences were not statistically significant.

Effects of compound C and MK886 on adiponectin-induced PGE_2 production by RASFs. The data shown in Figure 4 suggest that both AdipoR1 and AdipoR2 participate in PGE_2 production by RASFs exposed to adiponectin. Previous studies have shown that phosphorylation and activation of AMP-activated protein kinase (AMPK) are stimulated by adiponectin via AdipoR1 (20,21). In the present study, we found that compound C, an inhibitor of AMPK, decreased adiponectin-induced PGE_2 production (Figure 5A). Adiponectin has also been shown to enhance peroxisome proliferator–activated receptor α (PPARα) signaling via AdipoR2 (22). We examined the effect of MK886, an inhibitor of PPARα, on adiponectin-induced PGE_2 production in RASFs. As shown in Figure 5B, MK886 significantly inhibited adiponectin-induced PGE_2 production.

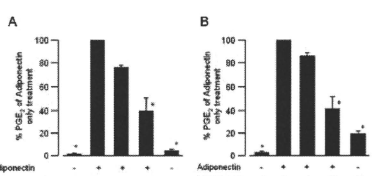

Figure 5. A, Concentration of PGE_2 in RASFs incubated for 18 hours without adiponectin, with adiponectin (10 μg/ml) alone, with adiponectin and compound C, or with compound C alone. **B,** Concentration of PGE_2 in RASFs incubated for 18 hours without adiponectin, with adiponectin (10 μg/ml) alone, with adiponectin and MK886, or with MK886 alone. The concentration of PGE_2 in the culture medium was measured by ELISA. Bars show the mean and SEM (n = 3). * = $P < 0.01$ versus cells treated with adiponectin only, by Tukey's multiple comparison test. See Figure 1 for definitions.

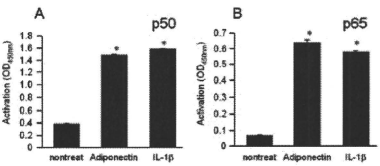

Figure 6. Activation of nuclear translocation of NF-κB in RASFs incubated without serum for 18 hours and then left untreated or incubated with adiponectin (2 μg/ml) or IL-1β (1 ng/ml) for 3 hours. The cell lysate was diluted and applied to the ELISA plate. Transcription factor activation was assessed using antibodies to p50 (**A**) or p65 (**B**), subtypes of NF-κB. Bars show the mean and SEM of triplicate cultures. Representative results from 2 independent experiments are shown. * = $P < 0.01$ versus untreated cells, by Tukey's multiple comparison test. OD = optical density (see Figure 1 for other definitions).

Effect of adiponectin on nuclear translocation of NF-κB. NF-κB is an essential transcription factor involved in the up-regulation of COX-2 (23) and mPGES-1 (24). To determine the association of NF-κB with adiponectin-induced PGE_2 production in RASFs, we examined whether adiponectin activated the nuclear translocation of NF-κB. As shown in Figures 6A and B, adiponectin induced nuclear translocation of the p50 and p65 subunits of NF-κB, similar to the effects of IL-1β. However, translocation of the c-Rel, p52, and RelB subunits was not altered by adiponectin treatment (data not shown).

DISCUSSION

In the present study, we demonstrated that exposure to adiponectin induced the expression of mRNA and protein for COX-2 and mPGES-1, resulting in PGE_2 overproduction by RASFs. Addition of antiadiponectin antibody or siRNA for adiponectin receptor gene decreased adiponectin-induced PGE_2 production. Recently, we demonstrated that adiponectin stimulates IL-8 production by RASFs and that the culture supernatant of RASFs treated with adiponectin induces chemotaxis (18). These results may help to explain the contribution of adiponectin to inflammation in patients with RA.

With regard to its role in inflammation, physiologic concentrations of adiponectin have been shown to inhibit TNFα–induced adhesion of human monocytic THP-1 cells in a dose-dependent manner. Adiponectin also decreases TNFα-induced expression of vascular cell

adhesion molecule 1, endothelial leukocyte adhesion molecule 1 (E-selectin), and intracellular adhesion molecule 1 by human aortic endothelial cells (25). In contrast, adiponectin activates NF-κB, an essential transcription factor for the expression of inflammatory proteins, in a time- and dose-dependent manner in U937 cells (26). These findings suggest that adiponectin might have antiinflammatory and/or proinflammatory properties under different experimental conditions.

Turner et al (27) reported that commercial recombinant adiponectin (Biovendor Laboratory Medicine) contained endotoxin at concentrations of 30 pg/μg of adiponectin. The endotoxin contamination of the adiponectin concentrations used in our study (1–10 μg/ml) can be estimated as 30–300 pg/ml. Picogram levels of lipopolysaccharide did not induce PGE_2 production in previous studies using monocytes (28) or RASFs (29). To confirm that the induction of PGE_2 by adiponectin is due to adiponectin itself, we conducted an experiment neutralizing adiponectin using antiadiponectin antibody. As shown in Figure 1C, antiadiponectin antibody significantly reduced adiponectin-induced PGE_2 production, whereas negative control IgG did not decrease PGE_2 production. Therefore, we confirmed that the induction of PGE_2 production by recombinant adiponectin was caused by adiponectin itself and not by endotoxin or other contaminants.

The plasma concentration of adiponectin in RA patients and healthy controls has been shown to be ~10 μg/ml (9). In our experiments, adiponectin (0.5–10 μg/ml) increased PGE_2 production from RASFs by enhancing the expression of COX-2 and mPGES-1. However, leptin and resistin (2 other adipokines) did not increase PGE_2 production by RASFs at levels up to 100-fold higher (1 μg/ml) (data not shown) than their serum concentrations in RA patients (9,30). The potency of adiponectin for inducing these enzymes in RASFs was almost equal to that of IL-1β (1 ng/ml). Therefore, adiponectin may have a proinflammatory influence on RASFs in RA patients through induction of PGE_2 production.

In our study, adiponectin also induced PGE_2 production from OASFs. However, the PGE_2 production seemed to be weaker than that from RASFs. Tan et al (11) reported that expression of mRNA for AdipoR1, but not AdipoR2, in RASFs was significantly higher than that in OASFs. This might explain the difference between RASFs and OASFs with regard to the degree of the effect of adiponectin on PGE_2 production.

Shibata et al (31) demonstrated that adiponectin induced COX-2–dependent synthesis of PGE_2, resulting in the protection of cardiomyocytes against ischemia-reperfusion injury. Yokota et al (32) suggested that adiponectin prevents preadipocyte differentiation via induction of COX-2 expression and the release of PGE_2 by stromal preadipocytes. In this study, we showed that treatment of RASFs with adiponectin induced 2 key enzymes related to PGE_2 production, which were COX-2 and mPGES-1. Contributions of the PGE_2 biosynthesis pathway, including cytosolic phospholipase A_2 (33), COX-2 (34), mPGES-1 (35,36), and EP4 receptor of PGE_2 (37), to arthritis in mouse models have been reported, and mice with knockdown of each molecule show amelioration of arthritis compared with wild-type mice. Therefore, adiponectin-induced PGE_2 production might be a factor that promotes aggravation of inflammation in RA patients.

Adiponectin has been shown to stimulate RANKL and to inhibit osteoprotegerin expression in human osteoblasts via the MAPK signaling pathway (38). Adiponectin also induces the expression of nitric oxide synthase and matrix metalloproteinases in chondrocytes (39). It has been suggested that adiponectin might play an important role not only as a proinflammatory molecule (such as in its effect on PGE_2 production), but also in regulating bone metabolism.

Previous studies have demonstrated that the concentration of adiponectin in the synovial fluid of patients with RA is significantly higher than that in the synovial fluid of patients with OA (6–8) and that serum and plasma concentrations of adiponectin are higher in RA patients than in healthy controls (7,9). These findings may indicate that adiponectin plays a role as a proinflammatory cytokine in RA. However, some studies have shown that the serum concentration of adiponectin in RA patients increases by ~20% during anti-TNFα therapy (40–43). The mean adiponectin concentration detected before anti-TNFα therapy in these studies was higher than that in healthy controls in observational studies (7,9). The reason the already high serum adiponectin concentration in RA patients increased further during anti-TNFα therapy cannot be explained at present. It is possible that adiponectin is not directly related to inflammation caused by TNFα.

In this study, we detected expression of protein and mRNA for 2 adiponectin receptors (AdipoR1 and AdipoR2) in RASFs, as has previously been shown in RASFs (10,19) and in various other tissues (20). In addition, adiponectin-induced PGE_2 synthesis was reduced by siRNA targeting of both adiponectin receptor genes. Reduction of PGE_2 production by double knockdown of AdipoR1 and AdipoR2 genes showed almost

the same results as knockdown of the individual receptor genes. These results demonstrate that adiponectin-induced PGE_2 production was mediated, at least in part, by these adiponectin receptors in RASFs. Pathways other than AdipoR1 and AdipoR2 might exist in adiponectin-induced PGE_2 production in RASFs. Although mRNA expression was reduced almost completely by transfection of siRNA for the target gene, the inhibitory effect of each receptor on protein expression was not significant in our experimental condition. Additional studies of receptor proteins are needed.

After adiponectin combines with AdipoR1, activation of AMPK occurs (20,21). Therefore, we investigated the effect of compound C, an inhibitor of AMPK, on PGE_2 production by RASFs stimulated with adiponectin. Adiponectin-induced PGE_2 production was significantly decreased by compound C, suggesting that this PGE_2 production at least involved signal transduction via AdipoR1. Yamauchi et al (22) demonstrated that the PPARα signaling pathway existed downstream of AdipoR2. In our study, MK886, an antagonist of the PPARα pathway, reduced the PGE_2 production that was induced by adiponectin treatment in RASFs.

NF-κB is known to play a central role in the regulation of inflammatory reactions in various cells (44). With regard to PGE_2 production by RASFs, NF-κB is an important factor in the transcriptional regulation of COX-2 (23). In the present study, adiponectin activated the translocation of NF-κB in RASFs. This suggests that adiponectin induced COX-2 expression in RASFs via activation of NF-κB translocation. Since the mPGES-1 promoter does not contain an NF-κB–responsive element, expression of mPGES-1 might be induced indirectly after activation of NF-κB (45), unlike COX-2. An increase in PGE_2 production by COX-2 activation after adiponectin treatment could lead to autocrine enhancement (14) of mPGES-1 expression.

ACKNOWLEDGMENT

We thank Sonoko Sakurai for secretarial assistance.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Kawai had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Kusunoki, Kawai.

Acquisition of data. Kusunoki, Kitahara, Tanaka, Kaneko, Suguro.

Analysis and interpretation of data. Kusunoki, Kojima, Endo, Kawai.

REFERENCES

1. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. Trends Endocrinol Metab 2000;11:327–32.
2. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004;50:1511–25.
3. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6:772–83.
4. Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. Diabetes Obes Metab 2007;9:282–9.
5. Brennan FM, McInnes IB. Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest 2008;118:3537–45.
6. Schaffler A, Ehling A, Neumann E, Herfarth H, Tarner I, Scholmerich J, et al. Adipocytokines in synovial fluid [letter] [published erratum appears in JAMA 2004;291:563]. JAMA 2003;290:1709–10.
7. Senolt L, Pavelka K, Housa D, Haluzik M. Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. Cytokine 2006;35:247–52.
8. Chen TH, Chen L, Hsieh MS, Chang CP, Chou DT, Tsai SH. Evidence for a protective role for adiponectin in osteoarthritis. Biochim Biophys Acta 2006;1762:711–8.
9. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis 2006;65:1198–201.
10. Ehling A, Schaffler A, Herfarth H, Tarner IH, Anders S, Distler O, et al. The potential of adiponectin in driving arthritis. J Immunol 2006;176:4468–78.
11. Tan W, Wang F, Zhang M, Guo D, Zhang Q, He S. High adiponectin and adiponectin receptor 1 expression in synovial fluids and synovial tissues of patients with rheumatoid arthritis. Semin Arthritis Rheum 2009;38:420–7.
12. Giles JT, Allison M, Bingham CO III, Scott WM Jr, Bathon JM. Adiponectin is a mediator of the inverse association of adiposity with radiographic damage in rheumatoid arthritis. Arthritis Rheum 2009;61:1248–56.
13. Kojima F, Naraba H, Sasaki Y, Okamoto R, Koshino T, Kawai S. Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. J Rheumatol 2002;29:1836–42.
14. Kojima F, Naraba H, Sasaki Y, Beppu M, Aoki H, Kawai S. Prostaglandin E_2 is an enhancer of interleukin-1β–induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. Arthritis Rheum 2003;48:2819–28.
15. Kusunoki N, Yamazaki R, Kawai S. Induction of apoptosis in rheumatoid synovial fibroblasts by celecoxib, but not by other selective cyclooxygenase 2 inhibitors. Arthritis Rheum 2002;46:3159–67.
16. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
17. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039–49.
18. Kitahara K, Kusunoki N, Kakiuchi T, Suguro T, Kawai S. Adiponectin stimulates IL-8 production by rheumatoid synovial fibroblasts. Biochem Biophys Res Commun 2009;378:218–23.
19. Tang CH, Chiu YC, Tan TW, Yang RS, Fu WM. Adiponectin enhances IL-6 production in human synovial fibroblast via an AdipoR1 receptor, AMPK, p38, and NF-κB pathway. J Immunol 2007;179:5483–92.

20. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002; 8:1288–95.
21. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762–9.
22. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007;13:332–9.
23. Crofford LJ, Tan B, McCarthy CJ, Hla T. Involvement of nuclear factor κB in the regulation of cyclooxygenase-2 expression by interleukin-1 in rheumatoid synoviocytes. Arthritis Rheum 1997; 40:226–36.
24. Catley MC, Chivers JE, Cambridge LM, Holden N, Slater DM, Staples KJ, et al. IL-1β-dependent activation of NF-κB mediates PGE2 release via the expression of cyclooxygenase-2 and microsomal prostaglandin E synthase. FEBS Lett 2003;547:75–9.
25. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100: 2473–6.
26. Haugen F, Drevon CA. Activation of nuclear factor-κB by high molecular weight and globular adiponectin. Endocrinology 2007; 148:5478–86.
27. Turner JJ, Smolinska MJ, Sacre SM, Foxwell BM. Induction of TLR tolerance in human macrophages by adiponectin: does LPS play a role? Scand J Immunol 2009;69:329–36.
28. Kato M, Nishida S, Kitasato H, Sakata N, Kawai S. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: investigation using human peripheral monocytes. J Pharm Pharmacol 2001;53:1679–85.
29. Sugiyama E, Taki H, Kuroda A, Mino T, Yamashita N, Kobayashi M. Interleukin-4 inhibits prostaglandin E_2 production by freshly prepared adherent rheumatoid synovial cells via inhibition of biosynthesis and gene expression of cyclo-oxygenase II but not of cyclo-oxygenase I. Ann Rheum Dis 1996;55:375–82.
30. Toussirot E, Streit G, Wendling D. The contribution of adipose tissue and adipokines to inflammation in joint diseases. Curr Med Chem 2007;14:1095–100.
31. Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, et al. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2–dependent mechanisms. Nat Med 2005;11:1096–103.
32. Yokota T, Meka CS, Medina KL, Igarashi H, Comp PC, Takahashi M, et al. Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins. J Clin Invest 2002;109:1303–10.
33. Hegen M, Sun L, Uozumi N, Kume K, Goad ME, Nickerson-Nutter CL, et al. Cytosolic phospholipase A2α-deficient mice are resistant to collagen-induced arthritis. J Exp Med 2003;197: 1297–302.
34. Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, et al. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum 2000;43:2687–93.
35. Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci U S A 2003;100:9044–9.
36. Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, et al. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. J Biol Chem 2004;279:33684–95.
37. McCoy JM, Wicks JR, Audoly LP. The role of prostaglandin E_2 receptors in the pathogenesis of rheumatoid arthritis. J Clin Invest 2002;110:651–8.
38. Luo XH, Guo LJ, Xie H, Yuan LQ, Wu XP, Zhou HD, et al. Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. J Bone Miner Res 2006;21:1648–56.
39. Lago R, Gomez R, Otero M, Lago F, Gallego R, Dieguez C, et al. A new player in cartilage homeostasis: adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes. Osteoarthritis Cartilage 2008;16:1101–9.
40. Komai N, Morita Y, Sakuta T, Kuwabara A, Kashihara N. Anti-tumor necrosis factor therapy increases serum adiponectin levels with the improvement of endothelial dysfunction in patients with rheumatoid arthritis. Mod Rheumatol 2007;17:385–90.
41. Nishida K, Okada Y, Nawata M, Saito K, Tanaka Y. Induction of hyperadiponectinemia following long-term treatment of patients with rheumatoid arthritis with infliximab (IFX), an anti-TNF-α antibody. Endocr J 2008;55:213–6.
42. Serelis J, Kontogianni MD, Katsiougiannis S, Bletsa M, Tektonidou MG, Skopouli FN. Effect of anti-TNF treatment on body composition and serum adiponectin levels of women with rheumatoid arthritis. Clin Rheumatol 2008;27:795–7.
43. Nagashima T, Okubo-Fornbacher H, Aoki Y, Kamata Y, Kimura H, Kamimura T, et al. Increase in plasma levels of adiponectin after administration of anti-tumor necrosis factor agents in patients with rheumatoid arthritis. J Rheumatol 2008;35:936–8.
44. Barnes PJ, Karin M. Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997;336: 1066–71.
45. Fahmi H. mPGES-1 as a novel target for arthritis. Curr Opin Rheumatol 2004;16:623–7.

Clinical Activity After 12 Weeks of Treatment with Nonbiologics in Early Rheumatoid Arthritis May Predict Articular Destruction 2 Years Later

YOICHI ICHIKAWA, TERUNOBU SAITO, HISASI YAMANAKA, MASASHI AKIZUKI, HIROBUMI KONDO, SHIGETO KOBAYASHI, HISAJI OSHIMA, SHINICHI KAWAI, NOBUAKI HAMA, HIDEHIRO YAMADA, TSUNEYO MIMORI, KOICHI AMANO, YASUSHI TANAKA, YASUO MATSUOKA, SUMIKI YAMAMOTO, TSUKASA MATSUBARA, NORIKAZU MURATA, TOMIAKI ASAI, YASUO SUZUKI; the Study Group for the Japanese Ministry of Health, Labor and Welfare, Research for Establishment of Therapeutic Guidelines in Early Rheumatoid Arthritis Program

ABSTRACT. ***Objective.*** To investigate earlier prediction of future articular destruction in patients with early rheumatoid arthritis (RA).

Methods. We randomly allocated patients with RA with disease duration < 2 years to different nonbiologic disease modifying antirheumatic drug (DMARD) therapies in a double-blind trial. Progression of articular destruction over the 96-week treatment period was assessed using the modified Sharp method.

Results. Progression of articular destruction correlated more strongly with the American College of Rheumatology (ACR) core set measures after 12 weeks of treatment than with pretreatment values. Multiple regression analysis of data after 12 weeks yielded a correlation coefficient of 0.711. The sensitivity and specificity to predict articular destruction over the 75th percentile of the cohort were 78.6% and 84.6%, respectively. Patients who showed articular destruction over the 75th percentile of the cohort had low response to treatment at 12 weeks, and continued to have high clinical disease activity thereafter. Contrasting data were found in patients with slow progression of articular destruction.

Conclusion. In patients with early RA, ACR core set measures after 12 weeks of nonbiologic DMARD treatment may predict articular destruction 2 years later. Low response to treatment at 12 weeks and continuing high disease activity thereafter were found in patients with rapid radiological progression. These data can be used to determine the appropriateness of treatment at 12 weeks and aid the decision to introduce biologic DMARD. (First Release March 1 2010; J Rheumatol 2010;37:723–9; doi:10.3899/jrheum.090776)

Key Indexing Terms:

RHEUMATOID ARTHRITIS PROGNOSIS DISEASE ACTIVITY
DISEASE MODIFYING ANTIRHEUMATIC DRUGS JOINT EROSIONS

From the Department of Rheumatic, Collagen and Allergic Diseases, St. Marianna University School of Medicine, Kawasaki; Institute of Rheumatology, Tokyo Woman's Medical University, Tokyo; Department of Internal Medicine, Yokohama Municipal Citizen's Hospital, Yokohama; Department of Internal Medicine, Kitazato University School of Medicine, Sagamihara; Department of Internal Medicine, Juntendo University School of Medicine, Tokyo; Department of Internal Medicine, Fujita Health University, Toyoake; Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki; Department of Internal Medicine, School of Medicine, Keio University, Tokyo; Department of Rheumatology and Clinical Immunology, Saitama Medical Center, Kawagoe; Department of Internal Medicine, Konan Kakogawa Hospital, Kakogawa; Department of Internal Medicine, Kawasaki Municipal Hospital, Kawasaki; The Centre for Rheumatic Diseases, Matsuyama Red Cross Hospital, Matsuyama; Department of Orthopedics, Matsubara Mayflower Hospital, Kato; Department of Rheumatology, National Hospital Organization, Osaka Minami Medical Center, Osaka; and Department of Orthopedics, National Hospital Organization, Nagoya Medical Center, Nagoya, Japan.

Supported by a "Research for Establishment of Therapeutic Guidelines in Early Rheumatoid Arthritis" grant from the Japanese Ministry of Health, Labor and Welfare. Santen Pharmaceutical Co., Ltd. and Wyeth Co., Ltd. kindly supplied bucillamine and methotrexate, respectively, as well as the corresponding placebos.

Y. Ichikawa, MD, Professor of Medicine, Department of Rheumatic, Collagen and Allergic Diseases, St. Marianna University School of Medicine; T. Saito, MD, Professor of Medicine; H. Yamanaka, MD, Professor of Medicine, Institute of Rheumatology, Tokyo Woman's Medical University; M. Akizuki, MD, Department of Internal Medicine, Yokohama Municipal Citizen's Hospital; H. Kondo, MD, Professor of Medicine, Department of Internal Medicine, Kitazato University School of Medicine; S. Kobayashi, MD, Assistant Professor, Department of Internal Medicine, Juntendo University School of Medicine; H. Oshima, MD, Professor of Medicine, Department of Internal Medicine, Fujita Health University; S. Kawai, MD, Professor of Medicine, Institute of Medical Science, St. Marianna University School of Medicine (current affiliation: Professor of Medicine, Division of Rheumatology, Department of Internal Medicine, School of Medicine, Faculty of Medicine, Toho University); N. Hama, MD, Assistant Professor; H. Yamada, MD, Associate Professor, Department of Rheumatic, Collagen and Allergic Diseases, St. Marianna University School of Medicine; T. Mimori, MD, Department of Internal Medicine, School of Medicine, Keio University (current affiliation: Professor of Medicine, Department of Clinical Immunology, University of Kyoto); K. Amano, MD, Associate Professor, Second Department of Internal Medicine, Saitama Medical Center; Y. Tanaka, MD, Department of Internal Medicine, Konan Kakogawa Hospital; Y. Matsuoka, MD, Department of Internal Medicine, Kawasaki Municipal Hospital; S. Yamamoto, MD, The Centre for Rheumatic Diseases, Matsuyama Red

Cross Hospital; T. Matsubara, MD, Department of Orthopedics, Matsubara Mayflower Hospital; N. Murata, MD, Department of Rheumatology, National Hospital Organization, Osaka Minami Medical Center; T. Asai, MD, Department of Orthopedics, National Hospital Organization, Nagoya Medical Center; Y. Suzuki, MD, Department of Rheumatic, Collagen and Allergic Diseases, St. Marianna University School of Medicine (current affiliation, Professor of Medicine, Department of Rheumatic Diseases, Tokai University).

Address correspondence to Dr. Y. Ichikawa, Department of Internal Medicine, St. Joseph's Hospital, 28 Midorigaoka, City of Yokosuka, Kanagawa-ken, Japan 238-8502. E-mail: j-incho@aqua.ocn.ne.jp

Accepted for publication November 25, 2009.

The usefulness of biologic disease-modifying antirheumatic drug (DMARD) therapy is well known in the treatment of rheumatoid arthritis (RA), and in particular the effects in suppressing articular destruction are revolutionary[1-3]. These therapies are expensive, however, and sometimes cause severe adverse reactions. It is necessary to select those patients who will benefit most from the treatment.

In general, treatment commences with nonbiologic DMARD, and biologic DMARD are introduced when disease activity cannot be fully controlled, progression of articular destruction is rapid, or prognosis is otherwise poor[4,5]. Conversely, patients with a rather benign disease course would prefer treatment without biologic DMARD in order to avoid the potential adverse reactions and added expense.

It has been reported that rheumatoid factor (RF) positivity[1,6-12], anticyclic citrullinated peptide (CCP) antibody positivity[10-14], presence of HLA-DRB1 genes for shared epitope[7,9,12,14,15], and female sex[16] are poor prognostic factors for articular destructions in patients with early RA. Other prognostic factors include indicators of disease activity, such as swollen joint count[12], serum C-reactive protein (CRP)[13], and erythrocyte sedimentation rate (ESR)[7,12]. The averaged values of clinical activities over an observation period correlated significantly with the progression of articular destruction[17,18]. However, it is important to be able to anticipate bone destruction at an early stage, rather than depending on mean values over a longer period.

We conducted a randomized double-blind controlled study evaluating prognostic factors, including pretreatment of clinical disease activity and treatment at 12-week intervals thereafter, with the aim of determining the measures that better and earlier predict the progression of articular destruction over 96 weeks of treatment.

MATERIALS AND METHODS

We conducted a double-blind controlled trial of the efficacy and safety of methotrexate (MTX) monotherapy 8 mg/week, bucillamine monotherapy 200 mg/day (BUC; with molecular structure similar to that of D-penicillamine[19]), and MTX and BUC combination therapy for 96 weeks[20]. At the same time, we investigated prognostic factors for the progression of articular destruction. Because the dosage of MTX, 8 mg per week at most, is set by official regulation in Japan, the initial dosage was determined accordingly.

We enrolled 55 patients who fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA[21], with symptoms for < 2 years. The Institutional Review Board of St. Marianna Medical College approved the study protocol, and all participants provided informed consent at the time of enrollment. All patients had a tender joint count of at least 6 out of 48 joints and a swollen joint count of at least 3 of 46 joints, and either serum CRP ≥ 1.0 mg/dl or ESR ≥ 30 mm/h. All subjects had taken no DMARD previously, and were receiving a corticosteroid dosage ≤ 7.5 mg/day prednisolone equivalent.

The study was conducted at 15 participating institutions, using a double-dummy double-blind method. The following factors were assessed at 12-week intervals: tender joint count, swollen joint count, patient's pain estimation using a visual analog scale (VAS), patient's global assessment of disease activity using a VAS, physician's overall assessment of disease activity by VAS, the modified Health Assessment Questionnaire (MHAQ)[22], ESR using the Westergren method, and serum CRP.

HLA-DRB1 typing was done using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (SRL Inc., Tokyo, Japan). Anti-CCP antibody was assayed by MBL Co., Ltd. (Nagoya, Japan).

The initially allocated DMARD could be changed after 24 weeks if an ACR20 response was not achieved, and DMARD could be changed if adverse reactions did not permit continuation. Subsequent treatment was at the discretion of the treating physician, including the dose of MTX being increased more than 8 mg per week.

Articular destruction was evaluated using Sharp's method modified by van der Heijde[23], scoring plain radiographs of both hands taken at commencement of treatment and after 96 weeks' treatment simultaneously, with the dates concealed. The total Sharp score, the erosion score, and the joint space narrowing score were the mean of scores determined independently by 3 rheumatologists (YI, NH, and HY).

We examined the relationships between the ACR core criteria measures[24] and the increase in the total Sharp score during 96 weeks using simple and multiple linear regression analyses. We used stepwise methods to determine a multivariate model. We used the StatView statistical analysis software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Findings at the start of the study in the 55 patients are shown in Table 1. The mean duration of disease was 9.2 months. The mean serum CRP was 4.09 mg/dl and the mean DAS28 4.78. The mean increase in total Sharp score during the 96-week study period was 24.2 ± SD 26.4; median and 25th and 75th percentiles were 16.0, 6.3, and 30.1, respectively.

The mean increase in total Sharp score was more than twice as rapid in patients positive for HLA-DRB1*0405 or with shared epitope than in patients who were negative ($p = 0.034$, $p = 0.037$, respectively; Table 2). Progression of articular destruction in patients positive for RF and positive for anti-CCP antibody was also more than twice as rapid as in the corresponding negative patients, although the differences were not statistically significant.

Simple linear regression analysis of laboratory data and radiographic findings, other than ACR core set measures, at enrollment and the progression of articular destruction during 96 weeks were studied. The initial total Sharp score (correlation coefficient $R = 0.382$, $p = 0.0004$), erosion score ($R = 0.363$, $p = 0.007$), joint space narrowing score ($R = 0.327$, $p = 0.015$), and serum matrix metalloproteinase (MMP-3) levels ($R = 0.327$, $p = 0.022$) correlated significantly with the progression of articular destruction, but no significant correlation was seen with the RF titer ($R =$

Table 1. Characteristics of study patients at enrollment.

Characteristics	Mean ± SD or %
Age, yrs	51.2 ± 12.0
Female, %	78.2
Duration of joint symptoms before study, mo	9.2 ± 5.1
No. of tender joints (0–48)*	14.4 ± 8.8
No. of swollen joints (0–46)*	10.0 ± 6.1
MHAQ (0–3)*	0.76 ± 0.40
Pain estimation by patients (0–100)*	66.4 ± 24.2
Global assessment of disease activity by patient (0–100)*	67.0 ± 24.3
Global assessment of disease activity by physician (0–100)*	66.4 ± 18.4
ESR, mm/h	68.7 ± 32.2
CRP, mg/dl	4.09 ± 3.84
MMP-3, ng/ml	280 ± 297
DAS28-4 (CRP)	4.78 ± 0.91
Total Sharp score, mean ± SD, median, 25th, 75th percentile	18.7 ± 14.8 13.3, 6.9, 27.9
Positive rheumatoid factor, % (> 20 IU/ml)**	90.9
Positive anti-CCP antibody, % (≥ 4.5 U/ml)**	89.6
Positive antinuclear antibody, % (≥ 40)**	60.0
HLA-DRB1*0405+, %	65.5
HLA shared epitope, %†	74.5
Corticosteroid therapy, %	23.6
Dose (prednisolone equivalent), mg/day	4.7 ± 1.7
Treatment: MTX/BUC/combination	19/20/16

* Ranges of possible values, ** Values that are considered positive. † includes HLA-DRB1*0405+, 0101+, and 0401+.

Table 2. Patients' characteristics and increase in total Sharp score over 96 week study period.

Characteristic	+/–	Increase in Total Sharp Score, mean ± SD +	–	p
HLA-DRB1*0405	36/19	29.7 ± 30.3	14.0 ± 11.4	0.034
HLA-DRB1 shared-epitope*	41/14	28.6 ± 28.7	11.7 ± 10.4	0.037
Rheumatoid factor-positive	50/5	25.7 ± 27.1	10.3 ± 9.6	0.216
Anti-CCP antibody-positive	43/5	23.9 ± 27.2	10.9 ± 13.5	0.304
Female/male	43/12	27.1 ± 28.8	14.2 ± 9.1	0.136
Age > 52 yrs	28/27	26.3 ± 31.2	27.3 ± 25.6	0.570

* Includes HLA-DRB1*0405+, 0101+, 0401+.

0.060, p = 0.661) or anti-CCP antibody titer (R = 0.069, p = 0.641).

Table 3 shows the correlation coefficients between the ACR core set measures, at pretreatment and at 12 and 24-week intervals, and the increase in the total Sharp score over 96 weeks' treatment. Of the core set measures evaluated at baseline, only CRP levels and the swollen joint count showed significant correlation. However, high correlation coefficients around 0.5 were seen for many core set measures and for Disease Activity Score 28 [DAS28-4(CRP); http://www.das-score.nl][25] after 12 weeks of treatment. The mean values of many measures over the 96-week period yielded high correlation coefficients > 0.5.

As shown in the upper part of Table 4, "Articular destruction A," the initial total Sharp score (b1), swollen joint count at 12 weeks treatment (b2), CRP at 12 weeks (b3), and pain estimation by patients at 12 weeks (b4) were all significantly and independently involved in the multiple linear regression model. The predicted value, $y = -13.097 + 0.590 \times b1 + 1.365 \times b2 + 1.761 \times b3 + 0.308 \times b4$, correlated well with the actual progression of articular destruction ($R = 0.711$, $p < 0.0001$). With $R^2 = 0.505$, this regression model was able to explain more than 50% of the progression of articular destruction. Multivariate logistic regression analysis with the core set measures at 12 weeks of treatment and the dichotomous variables, such as positivity of HLA shared-epitope alleles, RF positivity, and anti-CCP antibody positivity, failed to yield higher correlation coefficients than linear regression analysis (data not shown). The results of multiple linear regression analysis with the initial total Sharp score and the mean values of measures over 96 weeks as independent variables are shown in the lower part of Table 4, "Articular destruction B." The predicted values correlated well with the progression of articular destruction ($R = 0.728$, $p < 0.0001$).

The sensitivity and specificity of the prediction of articular destruction greater than the 75th percentile of the cohort were calculated by receiver-operating characteristic (ROC) curve analysis, where the predicted values of the multiple regression model at 12 weeks were used as cutoff points. The sensitivity and specificity with a cutoff of 32.06 were 78.6% and 84.6%, respectively. The sensitivity and specificity for the prediction of articular destruction less than the 25th percentile of the cohort were 78.6% and 76.9%, respectively, where the cutoff was 17.68.

In Table 5, patients are divided into 3 groups, whose progression of articular destruction over 96 weeks was greater than 75th percentile, between 75th and 25th percentiles, and less than 25th percentile of the cohort. The mean swollen joint count, serum CRP level, and pain estimation by patients, which were selected as independent variables in the multiple regression analysis, in the 3 patient groups at baseline and after 12 weeks treatment are given in Table 5. The percentage decrease from the mean of initial values to the mean of 12-weeks values ranged from 8.8% to 21.6%, 28.2% to 50.6%, and 51.7% to 62.6%, respectively.

Differences of distribution of initial DMARD treatments among the 3 groups were not statistically significant. Patients whose DMARD regimens were changed because of insufficient effectiveness as defined above were 57.1%, 23.1%, and 6.7% of patients in the respective groups. DMARD regimens were changed between Weeks 24 and 60 (mean 34.4 ± 15.0 weeks) to MTX with dosage up to 12.5 mg per week in 6 cases, to MTX + BUC combination therapy in 5, to sulfasalazine in 2, and others. Total Sharp score at start and HLA-DRB1*0408 positivity tended to be higher in the group above the 75th percentile.

Table 3. Correlation coefficients between ACR core set measures and DAS28 determined at 12 to 24 week intervals and means of these variables over the 96 week period, and the increase in total Sharp score over 96 weeks.

	Initial	12 Weeks	24 Weeks	48 Weeks	72 Weeks	96 Weeks	Mean#
CRP	0.292*	0.477***	0.562***	0.521***	0.479***	0.227	0.573***
ESR	0.235	0.491***	0.402**	0.350*	0.055	0.028	0.380**
MHAQ	0.138	0.183	0.210	0.250	0.246	0.005	0.272
Patients' pain estimation†	0.163	0.521***	0.428**	0.405**	0.472**	0.025	0.531***
Patients' global assessment††	0.152	0.500***	0.470***	0.382**	0.563***	0.049	0.554***
Swollen joint count	0.279*	0.434**	0.411**	0.518***	0.266	0.214	0.523***
Tender joint count	0.085	0.257	0.149	0.202	0.240	0.031	0.275*
Physicians' global assessment†††	0.253	0.449***	0.478***	0.453***	0.419**	0.101	0.524***
DAS28-(CRP)	0.384**	0.592***	0.610***	0.538***	0.447**	0.293*	0.618***

† Patients' estimation of pain on visual analog scale (VAS). †† Patients' global assessment of disease activity on VAS. ††† Physicians' global assessment of disease activity on VAS. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. # Mean of values determined every 12 weeks over 96 week treatment period.

Table 4. Multiple linear regression analysis of prognostic factors for articular destruction.

Dependent Variable	Independent Variable	Regression Coefficient	Standardized Regression Coefficient	p
Articular destruction A*			0.711***	< 0.0001
	Constant	−13.097		0.0455
	Initial total Sharp score	0.590	0.332	0.0032
	Swollen joint count after 12 wks	1.365	0.278	0.0213
	CRP after 12 wks	1.761	0.228	0.0491
	Patients' pain after 12 wks†	0.308	0.283	0.0229
Articular destruction B**			0.728***	< 0.0001
	Constant	−11.902		0.0383
	Initial total Sharp score	0.477	0.272	0.0120
	Mean swollen joint count††	3.521	0.407	0.0002
	Mean CRP††	4.837	0.354	0.0021

* Determined by multiple linear regression analysis of relationship between initial total Sharp score and the ACR core set measure after 12 weeks' treatment and the progression of articular destruction. ** Determined by multiple linear regression analysis with the initial total Sharp score and the mean values of measures over the 96 week study period as independent variables. *** Multiple regression coefficient. † Patients' pain estimation after 12 weeks. †† Mean of values determined every 12 weeks for 96 weeks.

As shown in Table 6, the means of both serum CRP levels and DAS28 of the group above the 75th percentile showed definitely higher values than those of patients in the other groups at 12 weeks, and continued at higher values thereafter to 72 weeks. Contrary results were observed in CRP and DAS28 of the group under the 25th percentile.

DISCUSSION

If RA is considered to be an aggregation of different disease types, then RF positivity and anti-CCP antibody positivity denote a patient group with typical disease. A patient group possessing a genetic predisposition in the HLA shared-epitope alleles can also be considered a representative group. The degree of articular destruction seen on plain radiographs at the commencement of observation has been reported to correlate well with the degree of articular destruction one or several years later[7-10,12]. This may indicate the presence of a patient group with rapid articular destruction, or another core group of RA. Other proposed factors include female sex[16] and advanced age[9,10].

In addition to these prognostic factors that do not change during the course of treatment, the connection between various inflammatory markers and articular destruction is well known. Initial levels of inflammatory markers that correlate significantly with the progression of bone destruction are ESR[7,12,16], CRP[13], MMP-3[26], swollen joint count[1,12], patient's global health assessment[8], and grip strength[12]. However, it has also been reported that initial level of CRP[27] or ESR[9] did not correlate with articular destruction.

The time-averaged DAS and CRP over 1 to 5 years were also reported to correlate significantly with changes in the Sharp score[2,17,18]. In our study, mean values over the 96 weeks' study period of all ACR core set measures, apart

Table 5. Improvement of inflammatory indices after 12 weeks of treatment and the observed characteristics in 3 groups of different radiological progression.

	Radiological Progression		
	≥ 75th Percentile†	75–25th Percentile	≤ 25th Percentile
Improvement of core set at 12 weeks			
No. cases	14	26	15
Swollen joint count			
Basal	12.2 ± 6.9	9.4 ± 6.6	8.9 ± 3.9
At 12 weeks	9.6 ± 6.5**	4.7 ± 4.3	4.3 ± 3.3
% decrease††	21.6	50.6	51.7
CRP			
Basal	6.0 ± 2.9*	4.4 ± 4.5	1.8 ± 2.1**
At 12 weeks	5.5 ± 3.4**	3.1 ± 3.5	0.7 ± 0.9**
% decrease††	8.8	28.2	62.6
Patient's estimation of pain			
Basal	70.4 ± 22.4	69.5 ± 25.1	57.2 ± 23.1#
At 12 weeks	59.0 ± 26.2**	42.3 ± 21.5	26.0 ± 16.5**
% decrease††	16.1	39.2	54.5
Characteristics of 3 groups			
Initial treatment (MTX, BUC, MTX+BUC, respectively)	5, 7, 2	11, 6, 9	3, 7, 5
No. cases, initial DMARD regimens changed (%)†††	8 (57.1)**	6 (23.1)	1 (6.7)*
Total Sharp score at start	24.9 ± 15.0#	19.8 ± 16.0	10.9 ± 8.49*
Increase of total Sharp score during 96 weeks	60.0 ± 28.1***	17.3 ± 7.4	3.12 ± 3.12***
HLA-DRB1*0405-positive, %	85.7#	61.5	53.3
RF-positive, %	100.0	92.3	80.0
Anti-CCP antibody-positive, %	90.0	95.7	80.0

† Progression of articular destruction during 96 weeks is greater than the 75th percentile of the cohort. †† Percentage decrease from mean of initial values to mean of 12 week values. ††† Regimen was changed when ACR20 was not achieved after 24 weeks. # $p < 0.1$ vs cases other than this group; * $p < 0.05$ vs cases other than this group; ** $p < 0.01$ vs cases other than this group; *** $p < 0.0001$ vs cases other than this group.

Table 6. Time courses of CRP levels and DAS28 in 3 groups of different radiological progression.

	No. Cases	0 Week	12 Weeks	24 Weeks	48 Weeks	72 Weeks	96 Weeks
Serum CRP							
≥ 75 percentile group †	14	5.99 ± 2.93*	5.46 ± 3.37**	5.24 ± 3.65***	3.75 ± 2.74***	2.03 ± 2.21*	1.00 ± 0.85
75–25 percentile group	26	4.36 ± 4.45	3.13 ± 3.53	1.28 ± 1.21	1.40 ± 1.58	1.21 ± 1.34	0.72 ± 0.83
≤ 25 percentile group††	15	1.84 ± 2.09**	0.69 ± 0.92**	0.55 ± 1.16**	0.81 ± 1.53*	0.43 ± 0.61*	0.50 ± 0.84*
DAS28-4 (CRP)							
≥ 75 percentile group†	14	5.30 ± 0.95*	4.72 ± 0.91#	4.00 ± 1.06***	3.75 ± 0.97***	3.10 ± 1.45*	2.31 ± 0.61
75–25 percentile group	26	4.72 ± 0.86	3.66 ± 0.92	2.92 ± 0.86	2.73 ± 1.08	2.47 ± 1.18	2.26 ± 1.08
≤ 25 percentile group††	15	4.41 ± 0.80	2.96 ± 0.85**	2.35 ± 0.94**	2.30 ± 0.90*	1.62 ± 0.84**	1.43 ± 0.56**

† Cases showed radiological progression greater than 75 percentile of the cohort. †† Cases showed radiological progression less than 25 percentile of the cohort. * $p < 0.05$ vs cases of the other 2 groups; ** $p < 0.01$ vs cases of the other 2 groups; *** $p < 0.001$ vs cases of the other 2 groups; # $p < 0.0001$ vs cases of the other 2 groups.

from the MHAQ, correlated strongly with the progression of articular destruction.

It is important, however, to be able to anticipate bone destruction at an early stage. Patients with higher DAS28 scores at Week 14 showed greater progression of joint damage from baseline to Week 54 than those with lower DAS28 scores[1]. In this study, the initial values of a few measures correlated significantly with the progression of articular destruction, whereas most measures correlated strongly after 12 weeks of treatment. The levels of inflammatory markers measured after 12 weeks of treatment would be influenced by the therapeutic effect of DMARD administered and patients' responsiveness to DMARD.

The correlation coefficients between the ACR core set measures and the DAS28 at 12 weeks' treatment and the progress of articular destruction were similar to those of the corresponding mean values over 96 weeks. Multiple linear regression analysis of initial values yielded a correlation coefficient of 0.548 for the progression of articular destruction (data not shown), whereas values after 12 weeks of

treatment yielded a higher correlation coefficient of 0.711, about the same as that obtained from the mean values over the 96 weeks' study period. These results indicate that measures assessed after 12 weeks of DMARD therapy can predict the progression of articular destruction 2 years later as well as mean values over the entire 2-year period.

The predicted value of 32.06 for articular destruction obtained by multiple regression analysis of the ACR core set measures at 12 weeks' treatment was used as the cutoff point of ROC analysis that could select patients whose articular destruction would be greater than the 75th percentile of the cohort with a sensitivity and specificity around 80%. This patient group may be considered candidates for a change of nonbiologic DMARD therapy, or for treatment with biologic DMARD. On the other hand, ROC analysis with a cutoff point of 17.68 could select patients with minimal articular destruction, less than the 25th percentile of the cohort, with sensitivity and specificity of nearly 80%. These patients would not require any changes in their DMARD therapy.

The decision to change initial RA treatment is usually 3 months after start of treatment[4,9]. Our findings support the clinical status quo that one considers changes in DMARD therapy 3 months after initiation of therapy from the viewpoint of articular destruction 2 years later.

In this study, 3 kinds of treatment were randomly allocated for patients studied, who were divided into 3 groups according to radiological progression during 96 weeks (Tables 5 and 6). In patients with radiological progression greater than the 75th percentile of the cohort, clinical activity was not definitely high at commencement, but responses to treatment were small at 12 weeks. Moreover, a relatively high level of clinical activity continued thereafter in these patients, although most of the patients changed their initial DMARD because of not achieving ACR20. In contrast, patients whose radiological progression was less than the 25th percentile of the cohort showed good response at 12 weeks, and continued with low clinical activity thereafter, while DMARD regimens were rarely changed because of insufficient effectiveness.

Although 3 DMARD regimens were allocated randomly and the distribution of initial DMARD regimens was not significantly different between the 3 groups, clinical activity at 12 weeks and responses to treatment at 12 weeks showed definite differences between the groups. The different clinical activities observed at 12 weeks in the 3 groups continued thereafter.

The question is, what caused differences in responsiveness to DMARD treatment at 12 weeks and in different continuing activity thereafter in the 3 groups of patients. HLA shared-epitope, RF, and anti-CCP antibody positivity may be involved. High total Sharp score at commencement may also be important, although it is not clear what factors influence this phenomenon. There may still be other unknown prognostic factors that result in the difference in treatment responses and clinical activities thereafter.

HLA-DRB1*0405 positivity was found to be common in Japanese patients with RA[28,29]. Wakitani, *et al* reported that the HLA-DRB1*0405 genotype was more common in patients in the more erosive subset and the most erosive subset with mutilating disease than in the least erosive subset[28]. In our study, the progression of articular destruction, determined by Sharp's method modified by van der Heijde, was more rapid in HLA-DRB1*0405-positive or HLA shared-epitope-positive than in the respectively negative patients.

REFERENCES

1. Smolen JS, van der Heijde DM, St. Clair EW, Emery P, Bathon JM, Keystone E, et al. Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis of Early Onset (ASPIRE) Study Group. Predictors of joint damage in patients with early rheumatoid arthritis treated with high-dose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. Arthritis Rheum 2006;54:702-10.
2. Landewe R, van der Heijde D, Klareskog L, van Vollenhoven R, Fatenejad S. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. Arthritis Rheum 2006;54:3119-25.
3. Lipsky PE, van der Heijde DM, St. Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000;343:1594-602.
4. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis: 2002 update. Arthritis Rheum 2002;46:328-46.
5. Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Care Res 2008;59:762-84.
6. Brennan P, Harrison B, Barrett E, Chakravarty K, Scott D, Silman A, et al. A simple algorithm to predict the development of radiological erosions in patients with early rheumatoid arthritis: prospective cohort study. BMJ 1996;313:471-6.
7. Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J, et al. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. Arthritis Rheum 2001;44:1736-43.
8. Guillemin F, Gerard N, van Leeuwen M, Smedstad LM, Kvien TK, van den Heuvel W; EURIDISS Group. Prognostic factors for joint destruction in rheumatoid arthritis: a prospective longitudinal study of 318 patients. J Rheumatol 2003;30:2585-9.
9. Goronzy JJ, Matteson EL, Fulbright JW, Warrington KJ, Chang-Miller A, Hunder GG, et al. Prognostic markers of radiographic progression in early rheumatoid arthritis. Arthritis Rheum 2004;50:43-54.
10. Forslind K, Ahlmen M, Eberhardt K, Hafstrom I, Svensson B; BARFOT Study Group. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004;63:1090-5.
11. de Vries-Bouwstra JK, Goekoop-Ruiterman YP, Verpoort KN, Schreuder GM, Ewals JA, Terwiel JP, et al. Progression of joint damage in early rheumatoid arthritis: association with HLA-DRB1,

rheumatoid factor, and anti-citrullinated protein antibodies in relation to different treatment strategies. Arthritis Rheum 2008;58:1293-8.
12. Dixey J, Solymossy C, Young A; Early RA Study. Is it possible to predict radiological damage in early rheumatoid arthritis (RA)? A report on the occurrence, progression, and prognostic factors of radiological erosions over the first 3 years in 866 patients from the Early RA Study (ERAS). J Rheumatol 2004;31 Suppl 69:48-54.
13. Lindqvist E, Eberhardt K, Bendtzen K, Heinegard D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. Ann Rheum Dis 2005;64:196-201.
14. Quinn MA, Gough AK, Green MJ, Devlin J, Hensor EM, Greenstein A, et al. Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis and predict radiological and functional outcome. Rheumatology 2006;45:478-80.
15. Listing J, Rau R, Muller B, Alten R, Gromnica-Ihle E, Hagemann D, Zink A. HLA-DRB1 genes, rheumatoid factor, and elevated C-reactive protein: independent risk factors of radiographic progression in early rheumatoid arthritis. Berlin Collaborating Rheumatological Study Group. J Rheumatol 2000;27:2100-9.
16. Fex E, Jonsson K, Johnson U, Eberhardt K. Development of radiologic damage during the first 5-6 yr of rheumatoid arthritis. A prospective follow-up study of a Swedish cohort. Br J Rheumatol 1996;35:1106-15.
17. Plant MJ, Williams AL, O'Sullivan MM, Lewis PA, Coles EC, Jessop JD. Relationship between time-integrated C-reactive protein levels and radiologic progression in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:1473-7.
18. Welsing PM, Landewe RB, van Riel PL, Boers M, van Gestel AM, van der Linden S, et al. The relationship between disease activity and radiologic progression in patients with rheumatoid arthritis: a longitudinal analysis. Arthritis Rheum 2004;50:2082-93.
19. Chatham WW, Blackburn WD Jr. Gold and D-penicillamine. In: Coopman WJ, editor. Arthritis and allied conditions. 14th ed. Philadelphia: Lippincott Williams and Wilkins; 2001:717-33.
20. Ichikawa Y, Saito T, Yamanaka H, Akizuki M, Kondo H, Kobayashi S, et al, and MTX–BUC Combination Study Group. Therapeutic effects of the combination of methotrexate and bucillamine in early rheumatoid arthritis: a multicenter, double-blind, randomized controlled study. Mod Rheumatol 2005;15:323-8.
21. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
22. Pincus T, Summey JA, Soraci SA Jr, Wallston KA, Hummon NP. Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983;26:1346-53.
23. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol 1999;26:743-5.
24. Felson DT, Anderson JJ, Boers M, Bombardier C, Chernoff M, Fried B, et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. Arthritis Rheum 1993;36:729-40.
25. van Riel PL, van Gestel AM, van de Putte LBA. Development and validation of response criteria in rheumatoid arthritis: steps towards an international consensus on prognostic markers. Br J Rheumatol 1996;35 Suppl 2:4-7.
26. Yamanaka H, Matsuda Y, Tanaka M, Sendo W, Nakajima H, Taniguchi A, et al. Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early rheumatoid arthritis. Arthritis Rheum 2000;43:852-8.
27. Aman S, Paimela L, Leirisalo-Repo M, Risteli J, Kautiainen H, Helve T, et al. Prediction of disease progression in early rheumatoid arthritis by ICTP, RF and CRP. A comparative 3-year follow-up study. J Rheumatol 2000;39:1009-13.
28. Wakitani S, Murata N, Toda Y, Ogawa R, Kaneshige T, Nishimura Y, et al. The relationship between HLA-DRB1 alleles and disease subsets of rheumatoid arthritis in Japanese. Br J Rheumatol 1997;36:630-6.
29. Kochi Y, Yamada R, Kobayashi K, Takahashi A, Suzuki A, Sekine A, et al. Analysis of single-nucleotide polymorphisms in Japanese rheumatoid arthritis patients shows additional susceptibility markers besides the classic shared epitope susceptibility sequences. Arthritis Rheum 2004;50:63-71.