Case Report

Systemic lupus erythematosus complicated by recurrent pneumothorax: Case report and literature review

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summary

Pneumothorax is a rare pleuropulmonary manifestation of systemic lupus erythematosus. We encountered a 37-year-old Japanese woman who had systemic lupus erythematosus complicated by recurrent pneumothorax during treatment for recurrent serositis with glucocorticoid therapy. She was admitted for the third episode of lupus peritonitis in December 2005. Intravenous cyclophoshamide and increased dose of oral prednisolone were administered. In early January 2006, hemoptysis was observed and bronchofiberscopy revealed hemorrhage from the left lower lobe. After intravenous methylprednisolone pulse therapy and oral cyclosporine therapy were added, pleurisy and pulmonary hemorrhage improved. On February 22nd, she suddenly developed pneumothorax on the right side, followed by pneumothorax on the left side after 2 days. This pneumothorax on the left side did not improve despite chest tube drainage for over one month. She underwent thoracoscopic partial lobectomy of lower lobe of the left lung, and her symptoms improved.

Review of the literature identified 10 case reports of systemic lupus erythematosus complicated by pneumothorax. All of the patients including our case had underlying pulmonary lesions, and 9/11 patients had pleurisy. Besides 10/11 patients received glucocorticoid therapy before the occurrence of pneumothorax. Tissue fragility caused by these factors might contribute to the complication of pneumothorax in patients with systemic lupus erythematosus.

Key words systemic lupus erythematosus, recurrent pneumothorax, pleurisy, peritonitis

Introduction

The most frequent pleuropulmonary manifestation of systemic lupus erythematosus is pleurisy, while less common lesions include acute lupus pneumonitis, pulmonary hemorrhage, diffuse interstitial lung disease, pulmonary embolism, pulmonary hypertension, and shrinking lung[1]. However, pneumothorax is rarely found in patients with systemic lupus erythematosus. On the other hand, pneumothorax is not a rare complication in patients with dermatomyosis[2,3], rheumatoid arthritis[4,5], and scleroderma[6~11]. Spontaneous pneumomediastinum is also a relatively common complication of dermatomyositis[12,13]. Here we report a patient who had systemic lupus erythematosus complicated by pulmonary hemorrhage and recurrent pneumothorax during treatment of intractable serositis. We also provide a review of the relevant literature.

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Case report

In August 2004, a 37-year-old Japanese woman was admitted to Toho University Medical Center Omori Hospital with abdominal pain (Fig. 1). On admission, she was diagnosed as having systemic lupus erythematosus according to the 1982 revised American College of Rheumatology criteria (updated in 1997), because of the presence of photosensitivity, serositis (pleurisy and peritonitis), leukopenia, proteinuria (>0.5 g/day), positive antinuclear antibody, and positive anti-Sm antibody. To treat severe pleurisy and peritonitis, intravenous methylprednisolone pulse therapy was started at a dose of 1 g daily for 3 days. After pulse therapy, oral prednisolone was commenced at a dose of 50 mg daily. As a result, her symptoms improved and the dose of prednisolone was gradually tapered to 10 mg/day.

In March 2005, she presented with abdominal pain and vomiting, and was admitted again for the treat-

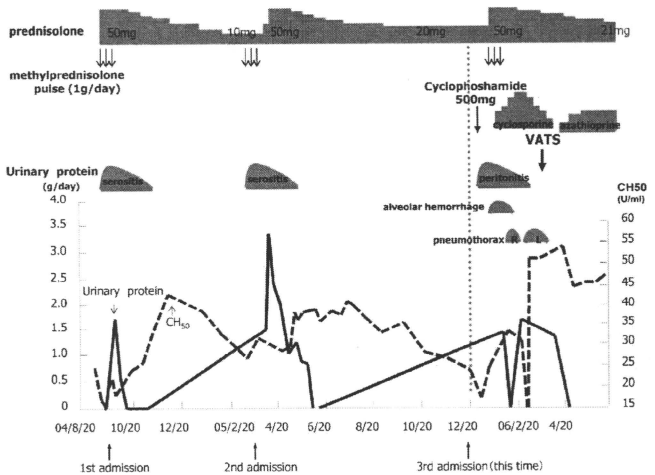

Fig. 1. Clinical course of the patient.

Table 1. Laboratory tests on admission

Urinalysis		Serological test	
Protein	(2+)	C-reactive protein	0.0 mg/dl
Glucose	(−)	IgG	922 mg/dl
Red blood cell	(+)	IgA	240 mg/dl
Sediment		IgM	104 mg/dl
Red blood cell	6-10/HPE	C3	46 mg/dl
Hyaline cast	(+)	C4	5 mg/dl
Protein	1.4 g/24 hr	CH_{50}	17.3 U/ml
Erythrocyte sedimentation rate	8 mm/hr	Antinuclear antibody	160×
Complete blood counts		Speckled pattern	
White blood cell	5300/μl	Anti-DNA antibody	<2.0 IU/ml
Lymphocyte	1632/μl	Anti-dsDNA-IgG antibody	≦5
Red blood cell	513×10^4/μl	Anti-RNP antibody	16×
Hemoglobin	13.8 g/dl	Anti-Sm antibody	Negative
Platelets	20.5×10^4/μl	Anti-cardiolipin β2GP I antibodies	<0.7 U/ml
Blood Chemistry		Anti-cardiolipin-IgG antibodies	≦8
Total protein	6.1 g/dl	Lupus anticoagulant	33.0
Albumin	3.2 g/dl	Anti-Jo-1 antibody	Negative
Total bilirubin	0.8 mg/dl	Anti-Scl-70 antibody	Negative
AST	15 IU/l	Anti-centromere antibody	Negative
ALT	13 IU/l	P-ANCA	<10 EU
LDH	261 IU/l	C-ANCA	<10 EU
BUN	11 mg/dl	KL-6	299 U/ml
Cr	0.46 mg/dl	Coagulation test	
Na	141 mEq/l	Prothrombin time	12.7 s
K	3.3 mEq/l	Activated partial thromboplastin time	24.9 s
Cl	109 mEq/l		

ment of lupus peritonitis. Methylprednisolone intravenous pulse therapy was also effective for this exacerbation. While she was in hospital, renal biopsy was performed and histopathological examination revealed minimal change disease.

In December 2005, she was admitted again for the third episode of lupus peritonitis. Examination of the chest revealed no abnormalities, including no pericardial or pleural rub. There was mild tenderness of her abdomen. There was no muscular weakness or active synovitis. Laboratory tests on admission were summarized in Table 1.

Her chest X-ray film showed no signs of pneumonia or pleurisy. Chest computed tomography only revealed old pneumonia in the right lower lobe. Abdominal computed tomography revealed duodenitis with edema of the duodenal wall, but no ascites.

Clinical course

Intravenous administration of cyclophoshamide (500 mg) and oral administration of prednisolone (50 mg/day) were started. In early January 2006, a small amount of hemoptysis was observed, and her symptoms worsened on January 16th. Chest computed tomography showed consolidation of the left lower lobe and bilateral massive pleural effusions (Fig. 2), while bronchofiberscopy revealed hemorrhage from the left lower lobe. To treat her lupus pleurisy, peritonitis, and pulmonary hemorrhage, intravenous methylprednisolone pulse therapy was given at a dose of 1 g daily for 3 days, and cyclosporine (50 mg/day) was added.

Pleurisy and pulmonary hemorrhage improved, but cavities appeared in the bilateral lower lobes. Chest

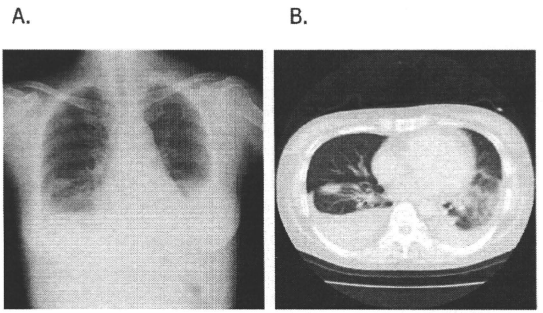

Fig. 2. A) Chest X-ray film shows hypolucence in the bilateral lower lung fields on January 16th, 2006.
B) Chest computed tomography. There is consolidation in the lower lobe of the left lung and massive bilateral pleural effusions on January 16th, 2006.

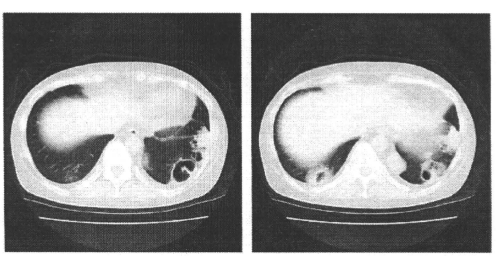

Fig. 3. Chest computed tomography on February 20th, 2006. There is no pleurisy or pulmonary hemorrhage, but cavities can be observed in the bilateral lower lobes on February 20th, 2006.

computed tomography scans obtained on February 20th are shown in Fig. 3. On February 22nd, she suddenly developed dyspnea and a chest X-ray film revealed pneumothorax on the right side (Fig. 4. A). She was treated with chest tube drainage and the lung soon re-expanded. However, pneumothorax also occurred on the left side after 2 days (Fig. 4. B. C). This pneumothorax did not improve despite chest tube drainage for over one month, so she underwent thoracoscopic partial lobectomy of lower lobe of the left lung on March 24th. Histological examination of lower lobe of the left lung was shown in Fig. 5. Her symptoms improved postoperatively and she was discharged from hospital on May 3rd, 2006. As of October 2009, she was in a stable condition.

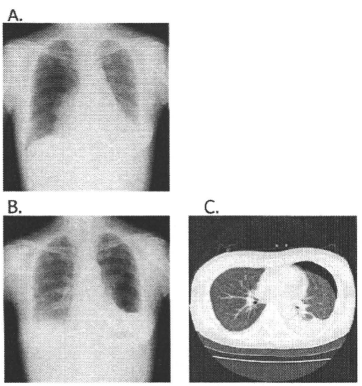

Fig. 4. A) Chest X-ray film shows pneumothorax on the right side on February 22nd, 2006.
B) Chest X-ray film shows pneumothorax with pleural effusion on the left side on Feburuary 24th, 2006.
C) Chest computed tomography. There is pneumothorax with pleural effusion on the left lung on Feburuary 24th, 2006.

Discussion

In this patient, pulmonary hemorrhage and recurrent pneumothorax occurred during treatment for her 3rd episode of serositis. The microphotographs show extensive coagulation necrosis (lung infarction) in the subpleural lung parenchyma. Pulmonary fibrosis, granuloma formation, and vasculitis are not observed. Pneumothorax was thought to be due to rupture of subpleural cavities in the lower lobes triggered by lung infarction. Because our patient suffered from recurrent serositis, pneumonia, and pulmonary hemorrhage, the cavities could have been formed by rupture of degenerating alveolar walls. In general, glucocorticoids have an antagonistic effect on growth factors and on collagen deposition during wound healing, resulting in tissue fragility[14,15]. On the other hand, it has been reported that a few cases of cyclophosphamide-induced late-onset lung disease could develop pneumothorax followed by pulmonary fibrosis and pleural thickening in the passage of several years in patients with glomerulonephritis, Wegener's granulomatosis, lymphoma, Hodgkin's

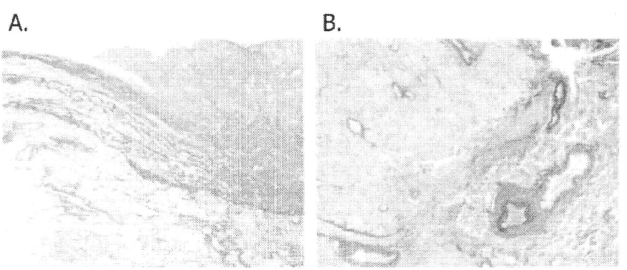

Fig. 5. A) The microphotograph shows granulation tissue formation with fibrin deposition on the pleura. The underlying lung parenchyma shows extensive coagulation necrosis with hemorrhage (Elastica von Gieson's stain, ×49).
B) The microphotograph shows a border between coagulation necrosis and unaltered lung, which contains a pulmonary artery with organized thrombotic occlusion (Elastica von Gieson's stain, ×49). The existence of pulumonary fibrosis, granuloma formation, and vasculitis is not observed.

disease, leukemia, multiple myeloma, and breast cancer[16]. Our patient with systemic lupus erythematosus had only once administration of cyclophoshamide one and a half months before the pneumothorax appearance. She did not have pulmonary fibrosis. Therefore, it does not seem that the administration of cyclophosphamide contributed to development of pneumothorax in this case. In our patient, it seems likely that several factors, including lung cavitation, subpleural lung infarction and the administration of glucocorticoids, contributed to the occurrence of pneumothorax.

Table 2 summarizes the 10 previously reported cases[17~25] and our case of systemic lupus erythematosus complicated by pneumothorax. Literature searches of PubMed were conducted for pneumothorax and systemic lupus erythematosus. Four of the 11 patients were men (36%). All of the patients had underlying pulmonary lesions, such as pneumonia, pulmonary suppuration, pyothorax, interstitial lung disease, pulmonary hemorrhage, pulmonary embolism, or cyst formation. It has been reported that pneumothorax can be caused by rupture of the subpleural cyst or honeycomb lung developed by pulumonary fibrosis[20]. Some reports showed that microthromboembolism or coagulation necrosis in pleura area might cause pneumothorax in patients with systemic lupus erythematosus as well as our case[22,23]. The incidence of pleurisy and pleural effusions is generally 30–50%[4,26] in patients with systemic lupus erythematosus. However, among the 11 lupus patients with pneumothorax who we summarized in Table 2, 9 had pleurisy and pleural effusion (81%). High frequency underlying pulmonary lesions, especially pleurisy suggests that the tissue fragility caused by these lesions may contribute to pneumothorax in patients with systemic lupus erythematosus. On the other hand, pneumothorax in patients with systemic lupus erythematousus is not likely associated with other features listed in Table 2, such as nephritis, anti-dsDNA-IgG, anti-Sm and anti-U1-RNP antibodies. The incidences of these characteristics were almost the same as those in all the patients with systemic lupus erythematosus[26].

Ten of the 11 patients (91%) were treated with glucocorticoids for at least 3 weeks before the occurrence of pneumothorax. However, there was 1 patient in whom pneumothorax developed despite the absence of glucocorticoid therapy. This implies that not only glucocorticoids but also the disease itself might influence the occurrence of pneumothorax in patients with systemic lupus erythematosus.

Four of the 11 patients died (36%), with the causes of death being respiratory failure (n=2), pulmonary edema (n=1), and renal failure (n=1). Five of the 7 alive patients including our patient had undergone surgical procedure (pleurectomy, pleural abration, or partial lobectomy), while none of dead patients had

Table 2. Cases of systemic lupus erythematosus with pneumothorax.

Case no.	First author	Age (years)	Sex	Pulmonary lesions	Pleurisy /PE	Nephritis	Anti-dsDNA Ab	Anti-Sm Ab	Anti-RNP Ab	GC	Surgery	Outcome	Ref. no.
1	Sawkar	27	F	pneumonia, cyst	+	−	ND	ND	ND	−	+	alive	17
2	Richards	34	F	pneumonia, IP	+	−	ND	ND	ND	+	−	dead[a]	18
3	Passero	35	M	+	+	ND	ND	ND	ND	+	+	alive	19
4	Passero	27	M	IP, alveolar hemorrhage, pulmonary infarction	+	+	ND	ND	ND	+	−	dead[b]	19
5	Masuda	41	F	IP, cyst	−	+	+	−	−	+	−	dead[c]	20
6	Paira	36	M	IP	+	ND	+	ND	ND	+	−	dead[c]	21
7	Nishitsuzi	23	M	alveolar hemorrhage, pulmonary infarction	+	+	+	−	ND	+	−	alive	22
8	Yen	17	F	IP, alveolar hemorrhage	+	+	−	−	+	+	−	alive	23
9	Wilhelm	17	F	abscess	+	+	−	ND	ND	+	+	alive	24
10	Maeda	53	F	cyst	−	ND	ND	ND	ND	+	+	alive	25
11	Our case	37	F	pneumonia, alveolar hemorrhage, cyst, pulmonary infarction	+	+	−	+	+	+	+	alive	
			M/F 4/7	100%	81%	75%	50%	25%	67%	91%	45%	dead/alive 4/7	

IP, interstitional pneumonia; PE, pleural effusion; Ab, antibody; GC, glucocorticoid treatment before pneumothorax; ND, not described; Ref, Reference; a, pulmonary edema; b, renal failure; c, respiratory failure

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.

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Adiponectin Stimulates Prostaglandin E_2 Production in Rheumatoid Arthritis Synovial Fibroblasts

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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.

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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-

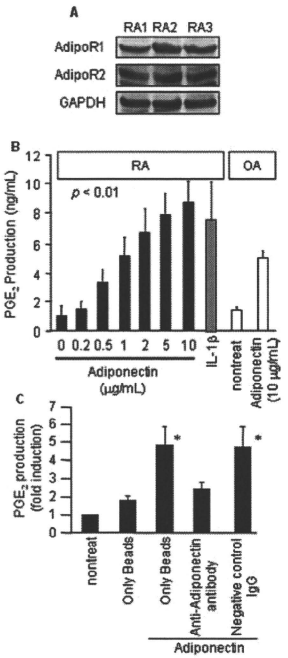

Figure 1. A, Western blot analysis of lysates of rheumatoid arthritis synovial fibroblasts (RASFs) for adiponectin receptor 1 (AdipoR1), AdipoR2, and GAPDH. RA1 = RA patient 1. **B,** Prostaglandin E_2 (PGE_2) production by RASFs and osteoarthritis synovial fibroblasts (OASFs) treated with various concentrations 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.

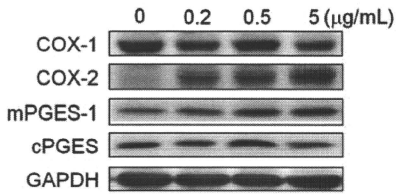

Figure 2. Effect of adiponectin on expression of cyclooxygenase 1 (COX-1), COX-2, membrane-associated prostaglandin E synthase 1 (mPGES-1), and cytosolic PGES (cPGES). Rheumatoid arthritis synovial fibroblasts were incubated for 18 hours with adiponectin at the indicated concentrations. Protein from 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).

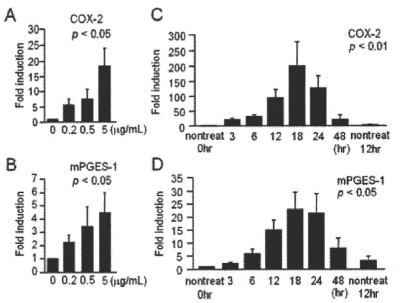

Figure 3. **A** and **B**, Fold induction of COX-2 (**A**) and mPGES-1 (**B**) in rheumatoid arthritis synovial fibroblasts (RASFs) incubated with adiponectin at the indicated concentrations for 18 hours. **C** and **D**, Fold induction of COX-2 (**C**) and mPGES-1 (**D**) in RASFs incubated with or 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

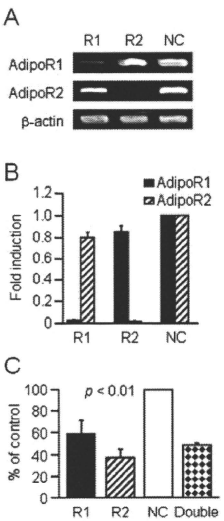

Figure 4. Effect of RNA interference on PGE_2 production by RASFs. RASFs were transfected with small interfering RNA (siRNA) for AdipoR1 (R1), siRNA for AdipoR2 (R2), negative control siRNA (NC), or with siRNA for both receptors (double). **A,** Reverse transcription–polymerase chain reaction (RT-PCR) 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.

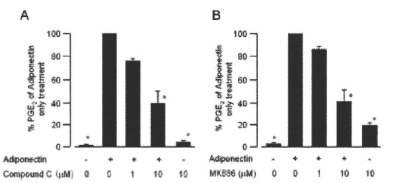

Figure 5. A, Concentration of PGE_2 in RASFs incubated for 18 hours without adiponectin, with adiponectin (10 μg/ml) alone, with adiponectin and compound C, or with compound C alone. **B,** Concentration of PGE_2 in RASFs incubated for 18 hours without adiponectin, with adiponectin (10 μg/ml) alone, 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.

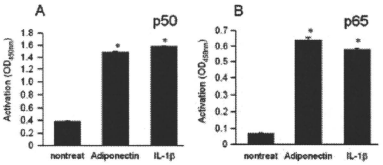

Figure 6. Activation of nuclear translocation of NF-κB in RASFs incubated without serum for 18 hours and then left untreated or incubated with adiponectin (2 μg/ml) or IL-1β (1 ng/ml) for 3 hours. The cell lysate was diluted and applied to the ELISA plate. Transcription factor activation was assessed 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.

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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.

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

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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
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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