Prostaglandin E synthase (PGES) is an enzyme that acts downstream of COX-2 and catalyzes the final step of PGE_2 biosynthesis (Fig. 1). To date, three isoforms of PGES, cytosolic PGES (cPGES), microsomal PGES (mPGES) -1 and mPGES-2, have been cloned and characterized. Among the PGES isozymes, mPGES-1 was originally known as microsomal glutathione S-transferase 1-like 1 (MGST1-L1), and it is a glutathione-dependent enzyme. Most importantly, mPGES-1 shows coordinated induction with COX-2 under inflammatory conditions in various cells and tissues [7, 8]. Although cPGES is also a glutathione dependent enzyme, it is expressed in most tissues under basal conditions and is not upregulated under inflammatory conditions [9]. In contrast, mPGES-2 is not dependent on glutathione for its activity and is coupled to both COX-1 and COX-2 for PGE_2 production. Similar to cPGES, it is constitutively expressed and its activity is not significantly increased during tissue inflammation [10]. Thus, mPGES-1 could be considered to be the most promising PGES isozyme that might be utilized as a therapeutic target to treat inflammatory diseases. The present review focuses on the recent advances in understanding the roles of mPGES-1 in the pathophysiology of RA.

Induction of mPGES-1 in joint tissues and cells of RA patients

We have previously demonstrated that mPGES-1 and COX-2 are coordinately induced in cultured synovial fibroblasts obtained from RA patients, upon stimulation by pro-inflammatory cytokines such as IL-1β and TNF-α [11, 12]. Interestingly, the low levels of mPGES-1 and COX-2 expression are observed in the untreated multi-passaged cells from RA patients, but fleshly isolated cells highly express both enzymes without stimulation with exogenous proinflammatory cytokines. In cytokine stimulated cells, mPGES-1 and COX-2 are co-localized in the perinuclear region and cooperate to increase PGE_2 production [12]. In contrast to mPGES-1, cPGES and mPGES-2 are expressed constitutively in RA synovial fibroblasts and their expressions are not affected by proinflammatory cytokine stimulation [13]. Furthermore, these studies also indicated that mPGES-1 expression can be inhibited by glucocorticoids. Similar regulation of mPGES-1 expression has also been observed in chondrocytes [14].

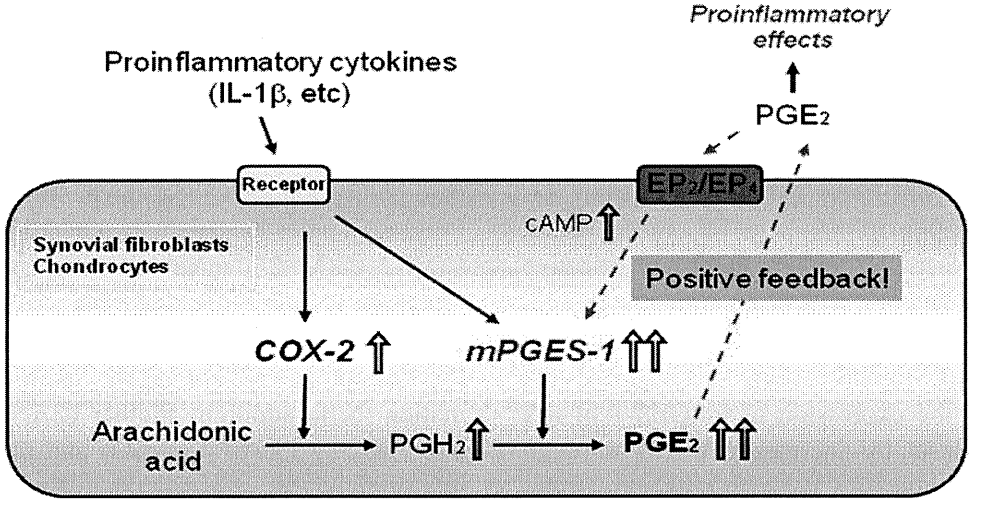

Fig. 2 Regulation of mPGES-1 expression in rheumatoid synovial fibroblasts and chondrocytes

COX-2 and mPGES-1 are coordinately induced in cultured synovial fibroblasts and chondrocytes by stimulation of pro-inflammatory cytokines such as interleukin1β (IL-1β). Abundant PGE_2 production at the inflammation sites of rheumatoid arthritis (RA) is caused by the coordinated up-regulation of mPGES-1 and COX-2. PGE_2 further enhances mPGES-1 expression associated with increase of cyclic AMP (cAMP) *via* the EP_2 and EP_4 receptors. The positive feedback regulation of mPGES-1 expression by PGE_2 may play an important role in the vicious circle of inflammation associated with RA [11-14].

In the synovium of RA patients, expression of mPGES-1 has been found to be localized in synovial lining cells, mononuclear and fibroblast-like cells present in the sublining, infiltrating synovial macrophages and vascular endothelial cells. Moreover, mPGES-1 expression was upregulated in synovium selectively in active RA and was minimally expressed in synovial tissue during inactive RA [10, 15]. However, during both active and inactive RA, mPGES-2 expression was unchanged in the synovial tissue. In addition to synovium and infiltrating cells, mPGES-1 expression has also been detected in the chondrocytes obtained from articular cartilage [16]. Although cPGES is also expressed in the synovial lining, sublining, and vascular endothelium, the number of cPGES-positive cells is significantly lower than that of mPGES-1-positive cells [15]. These findings suggest that induction of mPGES-1 during active RA plays a major role in the production of PGE_2, while mPGES-2 and/or cPGES may play a minor role in the production of PGE_2 in RA.

We unexpectedly found that NSAIDs, including selective COX-2 inhibitors, downregulate mPGES-1 expression in IL-1β-stimulated RA synovial fibroblasts [13]. This inhibition was relieved by the addition of PGE_2. It has been well known that PGE_2 exerts its role by binding different G-protein coupled receptors, EP_1, EP_2, EP_3 and EP_4. However, stimulation of only EP_2 and EP_4 receptors has been demonstrated to increase cyclic AMP *via* activation of adenylate cyclase [17]. We demonstrated expression of both EP_2 and EP_4 receptors in synovial fibroblasts obtained from RA patients. Stimulation of EP_2 and EP_4 receptors on synovial fibroblasts by either PGE_2 or selective agonists for the EP_2 and EP_4 or an activator of adenylate cyclase, forskolin, results in significant upregulation of mPGES-1 expression. This suggests that PGE_2 enhances the expression of mPGES-1 in RA synovial fibroblasts by increasing cAMP through activation of EP_2 and EP_4. We also obtained similar results using chondrocytes. Hence, PGE_2 may be a strong enhancer of mPGES-1 expression in synovial fibroblasts and chondrocytes. This positive feedback between mPGES-1 expression and PGE_2 may play an important role in amplifying inflammation associated with RA. Fig. 2 demonstrates the positive feedback mechanism regulating mPGES-1 upregulation and PGE_2 production.

Interestingly, Kusunoki *et al* demonstrated that adiponectin, an important adipokine, also induces the expression of mPGES-1 as well as COX-2, resulting in the increase of PGE_2 production by RA synovial fibroblasts [18]. This indicates that adiponectin may play a role in the pathogenesis of synovitis in RA patients by regulating PGE_2 biosynthesis. The expression of mPGES-1 has been also reported to be regulated by several intracellar factors. Naraba *et al* demonstrated that the induction of mPGES-1 expression requires the presence of tandem GC boxes adjacent to the transcription initiation site. The group further demonstrated that the binding of the transcription factor Egr-1 to the proximal GC box is essential for transcriptional regulation of the human and murine mPGES-1 gene [19, 20]. The induction of mPGES-1 is also positively regulated by a MAP kinases such as ERK-1/2 and p38 [21]. The induction of mPGES-1 may therefore be regulated by multiple signaling factors in RA.

Effect of genetic deletion of mPGES-1 on experimental autoimmune inflammatory arthritis in mice

Mice lacking mPGES-1 (mPGES-$1^{-/-}$ mice) have been generated and have provided novel insights into the role of mPGES-1 in eicosanoid biology [22, 23]. We and other groups have independently shown that mPGES-1 deficiency results in a reduction in the severity of arthritis, along with decreased synovial hyperplasia, tissue destruction and infiltration of inflammatory cells in type II collagen (CII) induced arthritis (CIA), a representative animal model of RA [22, 24]. We have also shown a significant reduction in pain perception after mechanical stimulation in CIA. Previous studies have demonstrated that mice lacking COX-2 (COX-$2^{-/-}$ mice) also display a significant reduction in both clinical and histological arthritis in CIA [25]. Likewise, administration of a COX-2 inhibitor, but not a COX-1 inhibitor, reduces paw inflammation [26]. In addition, another CIA study, utilizing mice lacking the EP_2 receptor and pharmacological inhibition of EP_4, supports the essential role of PGE_2 mediated by EP_2/EP_4 signaling in the development of autoimmune arthritis [27]. Taken together, the data indicates that COX-2/mPGES-1-derived PGE_2 may play a role in the development of RA, predominantly *via* EP_2/EP_4 signaling.

Role of mPGES-1 in T-cell dependent humoral immune response

RA is strongly dependent on the immunologic events associated with autoimmunity. Generation of a humoral immune response is a critical event in the

development of autoimmune diseases, including RA. Humoral immune response can be divided into T-cell dependent (TD) and T-cell independent (TI) response, based on the requirement for T-cell activation (Fig. 3). TD response requires T-cell/B-cell interaction *via* interaction between cell surface receptors and ligands to initiate antibody production. In contrast, TI response does not require T-cell interaction and antibody production occurs after B-cell activation, following binding of antigens to B-cell receptors (BCR).

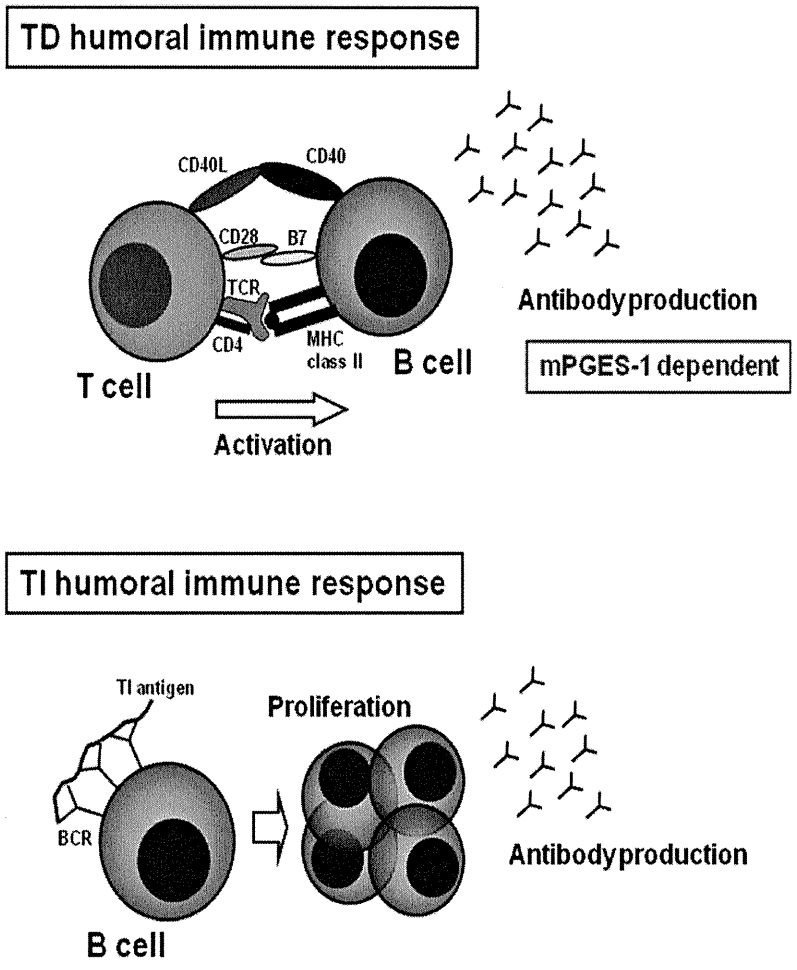

Fig. 3 mPGES-1 mediates T-cell dependent humoral immune response

The humoral immune responses, a critical event for autoimmune diseases, are divided into T-cell dependent (TD) and T-cell independent (TI) response, according to their requirement of T-cells. TD response requires T-cell/B-cell contact *via* interaction of cell surface molecules and ligands to develop antibody production by B-cells. In contrast, TI response does not require T-cells and the antibody production directly occurs after B-cell activation followed by binding of antigens to B-cell receptor (BCR) on B-cells. mPGES-1 and PGE_2-dependent action plays a key role in evocation of TD humoral response.

PGE_2 has been reported to modulate immunologic events including dendritic cell maturation, macrophage activation, B-cell and T-cell function [28]. However, the immunomodulatory role of mPGES-1 was unclear. We have shown that the levels of type II collagen (CII) -specific IgG subclasses (total IgG, IgG_1, IgG_{2a}, $IgG2_b$, IgG_{2c} and IgG_3) are significantly reduced in mPGES-$1^{-/-}$ mice during the development of CIA, when compared to WT mice [24]. The reduction of CII-specific antibody production correlates with the reduction in the incidence and severity of arthritis, suggesting an important role of mPGES-1 and its derived PGE_2 in the development of acquired immune response in CIA. The changes observed in

the immunological responses of mPGES-1$^{-/-}$ mice in our study are consistent with previous observations in COX-2$^{-/-}$ mice during CIA [25], suggesting the pivotal role of COX-2/mPGES-1/PGE_2 axis in the development of an antibody response to CII, a classic TD antigen that induces humoral and cellular immunity. These findings provide novel insights relevant to the therapeutic potential for pharmacologic inhibition of mPGES-1 in autoimmune inflammatory diseases, including RA.

Role of mPGES-1 in altering the cytokine profile

Recent studies have demonstrated the key role of Th17, a major subset of T-cells producing IL-17, in the pathogenesis of arthritis [29]. PGE_2 has been shown to facilitate expansion of the Th17 subset of T helper cells *via* its specific receptor subtypes [30, 31]. Hence, we examined the effect of mPGES-1 gene deletion on the production of IL-17 in cultured splenocytes obtained on day 10 following immunization with CII in the CIA model. Levels of IL-17 were significantly increased after CII stimulation in splenocytes of WT mice. However, significantly lower levels of IL-17 were observed in mPGES-1$^{-/-}$ splenocytes compared to WT on exposure to CII (Fig. 4). Likewise, the genetic deletion of mPGES-1 resulted in a failure to increase PGE_2 production in mPGES-1$^{-/-}$ splenocytes compared with WT after stimulation with CII, demonstrating the essential role of mPGES-1 in producing PGE_2 in this model [32]. The relative decrease in production of IL-17 associated with the reduction of PGE_2 production from CII-stimulated splenocytes in mPGES-1$^{-/-}$ mice *vs.* WT mice *in vitro* suggests that the mPGES-1 and its derivative PGE_2 may alter the differentiation and/or maintenance of IL-17-producing Th17 subset of T-cells in CIA. This altered cytokine environment may be one of the mechanisms by which immunoglobulin levels and arthritis severity are reduced in mPGES-1$^{-/-}$ mice *in vivo* in the CIA model [24].

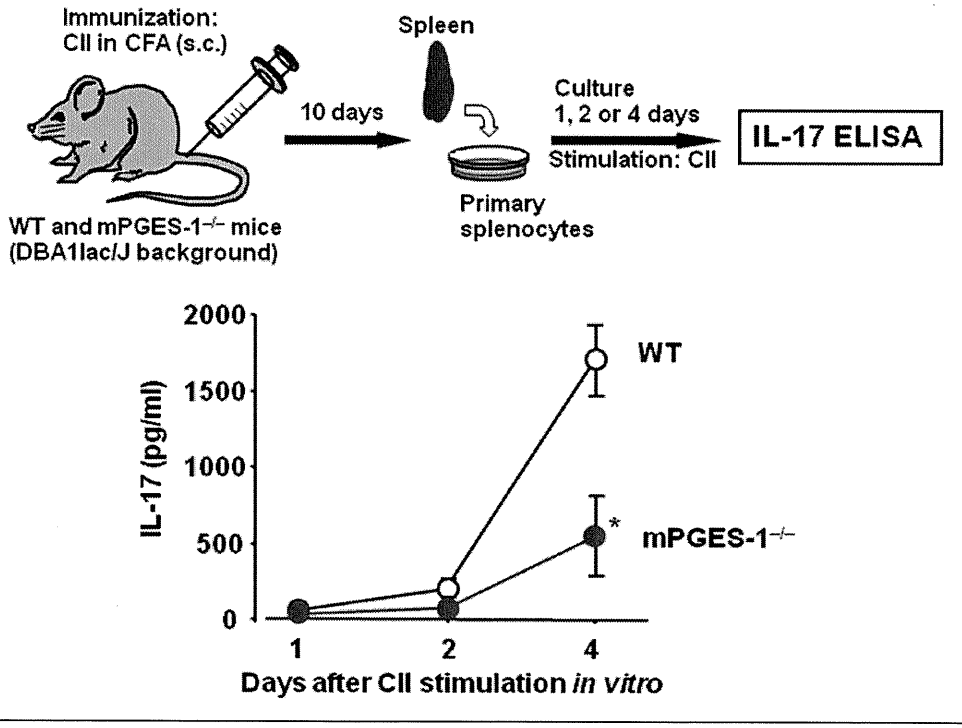

Fig. 4 mPGES-1-PGE_2 mediates Th17 responses in CIA

Splenocytes were isolated from WT and mPGES-1$^{-/-}$ mice at day 10 after immunization with bovine type II collagen (CII) in complete Freund's adjuvant (CFA). The cells were then cultured with CII (100 μg/ml) for 1, 2 or 4 days *in vitro*. The levels of interleukin-17 (IL-17) in culture medium were measured by ELISA. The production of IL-17 in CII-stimulated mPGES-1$^{-/-}$ splenocytes was significantly lower than that in WT cells (n=3; **P*<0.05). mPGES-1-PGE_2 system may alter the differentiation and/or maintenance of Th17 lymphocytes in collagen-induced arthritis (CIA) [32].

The effects of PGE_2 on dendritic cell phenotype and function have also been the subject of investigation. Our recent study with mPGES-$1^{-/-}$ mice showed that mPGES-1 is required for PGE_2 production after lipopolysaccharide (LPS) stimulation in dendritic cells derived from bone marrow *in vitro* [33]. Although mPGES-$1^{-/-}$ dendritic cells exhibit normal maturation and migration, the level of IL-12, a critical Th1 polarizing cytokine, after LPS stimulation is significantly lower when compared to WT cells. Interestingly, we observed that mPGES-1 deletion results in diversion of prostanoid production from PGE_2 to PGD_2, suggesting shunting of prostanoid biosynthesis in dendritic cells associated with mPGES-1 deficiency. In addition, the increase in PGD_2, and not decreased PGE_2, may be the likely cause of diminished IL-12 production in LPS-stimulated mPGES-$1^{-/-}$ dendritic cells. Taken together, mPGES-1 may be an attractive target for the treatment of autoimmune inflammatory diseases such as RA by altering cytokine production of immune effector cells.

Inhibition of mPGES-1 and its future prospect

Selective COX-2 inhibitors are extensively used for the treatment of RA. However, several clinical trials demonstrated that some of selective COX-2 inhibitors increase the risk of cardiovascular events in RA patients [34]. One of the possible mechanisms to explain the cardiovascular side effects due to COX-2 inhibition may be the concomitant decrease in production of PGE_2 and anti-thrombotic PGI_2 [35]. In order to minimize the side effect of selective COX-2 inhibitors, selective suppression of mPGES-1-derived PGE_2 production will be an attractive therapeutic alternative. In fact, loss of mPGES-1 leads to significant reduction in PGE_2 production and increase in PGI_2 production with no alterations in blood pressure or thrombosis in mice, when fed with normal or high salt diet [36, 37]. In addition, the deletion of mPGES-1 delays the development of atherosclerosis in parallel with reduction in PGE_2 synthesis and increased PGI_2 production [38].

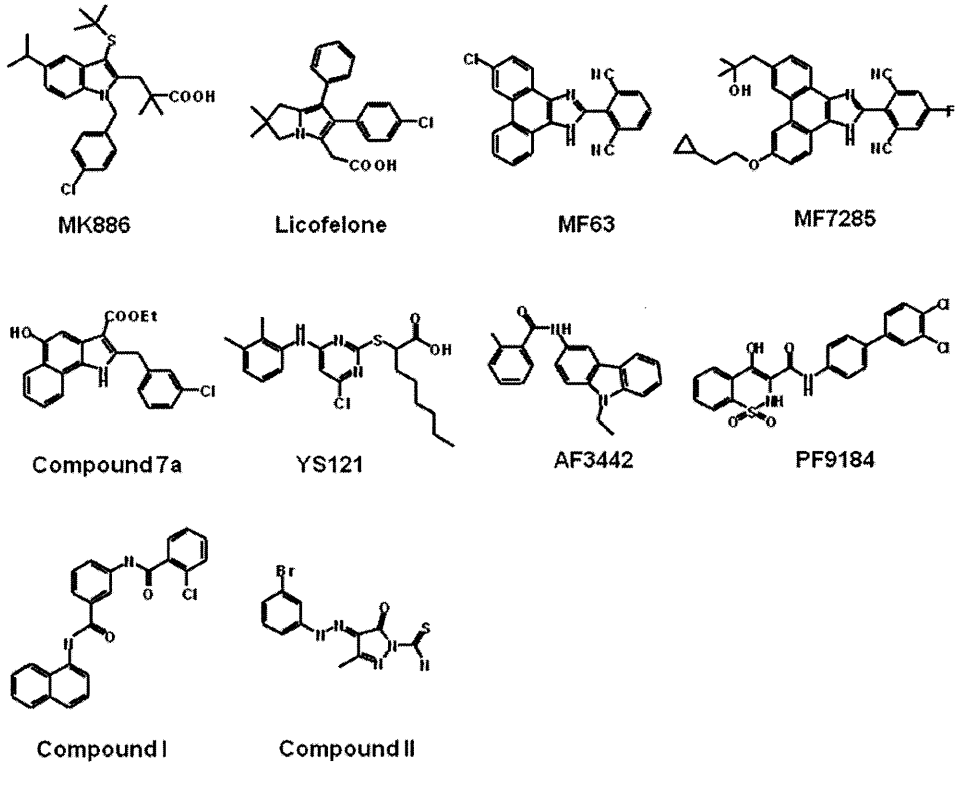

Fig. 5 Synthetic inhibitors targeting mPGES-1 activity

Synthetic chemicals that have been reported their specific and/or non-specific inhibitory effects on mPGES-1 activity to produce PGE_2 are indicated in this figure. The name and chemical structure of each inhibitor are from references [39-46].

To date, selective mPGES-1 inhibitors are not widely available, but some synthetic and natural chemicals that inhibit mPGES-1 have been investigated *in vivo* and *in vitro* by several groups. The chemicals that have been reported for their specific and/or non-specific inhibitory effects on mPGES-1 activity to produce PGE_2 are shown in Fig. 5 [39-46]. Notably, several studies have shown that some of these compounds are potent inhibitors of human mPGES-1, but do not inhibit murine mPGES-1. Hence, human-mPGES-1 knock-in mice have been generated and are being utilized to determine the efficacy of these compounds *in vivo*. It will be very important to assess if mPGES-1 inhibitors can mimic the results observed in mPGES-$1^{-/-}$ mice. In addition, therapeutic efficacy and safety of mPGES-1 inhibitors need to be critically studied in humanized animal models.

Acknowledgements

We would like to thank Dr. Jerold G. Woodward (University of Kentucky), Dr. Mohit Kapoor (University of Montreal), Dr. Lorenzo Polenzani (Angelini Research Center) and Dr. Natsuko Kusunoki (Toho University School of Medicine) for their suggestions. This work was supported by the NIH/NIAMS R01 AR049010, Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and grant from the Hokkaido Heart Association.

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Mod Rheumatol
DOI 10.1007/s10165-012-0623-z

ORIGINAL ARTICLE

Resistin is associated with the inflammation process in patients with systemic autoimmune diseases undergoing glucocorticoid therapy: comparison with leptin and adiponectin

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Received: 9 November 2011 / Accepted: 14 February 2012

Abstract
Objectives We investigated the role of adipokines in patients with systemic autoimmune diseases who received glucocorticoid therapy.
Methods Fifty-two patients with systemic autoimmune diseases who had started glucocorticoid therapy were prospectively enrolled. One hundred forty healthy persons were also studied as controls. Serum levels of 3 adipokines [resistin, leptin, and high molecular weight (HMW)-adiponectin] were measured with enzyme-linked immunosorbent assay kits before and at weekly intervals for 4 weeks during glucocorticoid therapy. The effects of lipopolysaccharide and dexamethasone on adipokine expression in human peripheral blood mononuclear cells (PBMCs) were also examined.
Results The serum resistin level was significantly higher in patients than in controls before glucocorticoid therapy, and it decreased after glucocorticoid therapy. Consistent with these results, dexamethasone inhibited lipopolysaccharide-induced upregulation of resistin expression in PBMCs in vitro. Serum leptin and HMW-adiponectin levels were lower in the patients than in the controls at baseline, and both adipokine levels were increased after glucocorticoid therapy. There was a significant correlation between serum resistin and high-sensitivity C-reactive protein. However, there was no association between serum adipokines and intima-media thickness.
Conclusion Resistin may be associated with the inflammatory process but not atherosclerosis in patients with systemic autoimmune diseases.

Keywords Systemic autoimmune diseases · Resistin · Leptin · High molecular weight adiponectin · Glucocorticoids

Introduction

Adipose tissue synthesizes and releases physiologically active molecules that are known as adipokines or adipocytokines, including resistin, leptin, and adiponectin, as well as interleukin (IL)-1, IL-1 receptor antagonist, IL-6, IL-10, and tumor necrosis factor (TNF)-α [1]. Adipocytes are known to play an important role in regulating the energy balance and glucose homeostasis [2], while adipokines have more recently also been implicated as mediators of immune and inflammatory processes. Systemic autoimmune diseases are associated with chronic intractable inflammation. Although the etiology of these diseases is still unknown, investigations into their pathogenesis have confirmed the involvement of various proinflammatory cytokines. Some studies have suggested that adipokines may also play a role in the pathogenesis or inflammatory processes of systemic autoimmune diseases [1], but these were mainly cross-sectional investigations that could be influenced by many factors. Accordingly, a detailed longitudinal study of the changes in serum adipokine levels during the courses of various systemic autoimmune diseases is needed.

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Published online: 21 March 2012

In the present study, the association of 3 adipokines [resistin, leptin, and high molecular weight (HMW)-adiponectin] with inflammation and atherosclerosis-related measures was investigated in patients with new-onset active autoimmune diseases. The influence of glucocorticoid therapy on the serum levels of these adipokines was also investigated. Furthermore, we studied the effects of inflammatory mediators and glucocorticoids on adipokine expression in vitro using human peripheral blood mononuclear cells (PBMCs).

Subjects and methods

Patients and healthy controls

This study was approved by the Ethics Committees of Toho University and Kitasato University. The patients with systemic autoimmune diseases and the healthy control subjects all gave their written informed consent for participation in the study, and they were studied at Toho University Omori Hospital or at the Research Center for Clinical Pharmacology of Kitasato University, respectively.

This was a prospective study that involved 52 patients with systemic autoimmune diseases, including systemic lupus erythematosus (SLE, $n = 18$), vasculitis syndrome ($n = 16$), polymyositis/dermatomyositis (PM/DM, $n = 14$), and adult onset Still's disease (AOSD, $n = 4$). All subjects were older than 20 years, and they started glucocorticoid therapy (prednisolone at 30 mg or more daily) as inpatients of Toho University Omori Hospital. Patients who had previously taken glucocorticoids or other immunosuppressive drugs were excluded. One hundred forty healthy persons were recruited at Kitasato University as controls. They did not meet any of the criteria for systemic autoimmune diseases and did not have any other diseases at the time of investigation.

Clinical and laboratory measurements

Clinical information and laboratory data were obtained from a structured interview, self-reported questionnaires, physical examination, and blood tests. Body mass index (BMI) was calculated from the height and weight measurements. Blood pressure was determined as the average of two measurements at baseline. In the morning after an overnight fast, blood was taken to measure the baseline serum levels of high-sensitivity C-reactive protein (Hs-CRP) by latex nephelometry (Siemens Health Care Diagnostics Inc., Deerfield, IL, USA), total cholesterol (T-chol) by the cholesterol dehydrogenase/ultraviolet method (Sysmex Corporation, Kobe, Japan), high-density lipoprotein cholesterol (HDL-chol) by the homogeneous method (Sekisui Medical Co., Ltd., Tokyo, Japan), and triglycerides (TG) by enzymatic assay (Sekisui Medical Co., Ltd.). Low-density lipoprotein cholesterol (LDL-chol) was calculated by the formula of Friedewald et al. [3]. We assessed the smoking status, the presence or absence of hypertension (defined as a blood pressure $\geq$140/90 mmHg or the use of antihypertensive medications), and the presence or absence of diabetes mellitus (defined according to Committee of Japan Diabetes Society criteria [4] or as the use of antidiabetic medications) as traditional risk factors for atherosclerosis.

Serum levels of adipokines

Fasting serum samples were collected before (baseline) and weekly for 4 weeks after starting glucocorticoid therapy, and were stored at -80 °C. Serum levels of adipokines were measured with enzyme-linked immunosorbent assay (ELISA) kits (resistin and leptin, B-Bridge International, Inc., Sunnyvale, CA, USA; HMW-adiponectin, Fujirebio Inc., Tokyo, Japan).

Carotid ultrasonography

The carotid arteries were examined by ultrasonography to detect premature atherosclerosis, as previously described by Kumeda et al. [5] with some modifications, before glucocorticoid therapy or within 1 month after starting the therapy at the latest. In brief, the right and left proximal common carotid, distal common carotid, and internal carotid arteries as well as the carotid bulb were examined with a Xario (SSA-660A) ultrasound diagnostic system (Toshiba Medical Systems Corporation, Ohtawara, Japan). Plaque was defined as a focal protrusion >1.1 mm in thickness on the wall of any of the abovementioned arteries. The intima-media thickness (IMT) was measured in the right and left proximal and distal common carotid arteries. The maximum IMT of each artery was then obtained by averaging the maximum measurements for the right and left sides.

Culture of human PBMCs

Reagents were purchased from the following sources: *Eschericha coli* lipopolysaccharides (LPS) was from Sigma–Aldrich (St. Louis, MO, USA), and dexamethasone was from Wako Pure Chemical Industries (Osaka, Japan). LPS was dissolved in sterile phosphate-buffered saline (PBS) containing 0.1 % bovine serum albumin (BSA) to prepare a stock solution, while dexamethasone was dissolved in dimethyl sulfoxide (DMSO). The final concentration of BSA or DMSO was always <0.1 %, and control

dishes contained an equivalent concentration of the vehicle. RPMI 1640 medium, penicillin/streptomycin, fetal bovine serum (FBS), and 0.25 % trypsin/EDTA were obtained from Invitrogen Corporation (Carlsbad, CA, USA). PBS was purchased from Takara Shuzo Co., Ltd. (Otsu, Japan). All other chemicals were purchased from Wako Pure Chemical Industries.

Human PBMCs were isolated from heparinized whole blood samples of healthy volunteers by density gradient centrifugation. Briefly, heparinized whole blood was layered over Polymorphprep (AXIS-SHIELD PoC AS, Oslo, Norway), and centrifugated for 30 min at $500 \times g$. The interface containing the mononuclear cells was collected, washed three times in PBS, and subsequently used for experiments. About 1×10^6 cells/mL were resuspended in 3 mL of RPMI 1640 medium supplemented with 1 % (v/v) FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, followed by seeding into 35 mm dishes and culture in a 5 % CO_2 humidified atmosphere at 37 °C.

To evaluate the influence of dexamethasone on adipokine production after LPS stimulation, PBMCs were incubated with or without 10 μg/mL LPS for 24 h, and then dexamethasone (0, 10, 100, or 1,000 nmol/L) was added overnight. The culture supernatants were collected, centrifuged and stored at −80 °C for subsequent analysis of adipokines by ELISA according to the instructions of the manufacturers.

Adipokine gene expression

Expression of resistin, leptin, and adiponectin mRNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) analysis. Cells were cultured under various conditions in medium containing 1 % (v/v) FBS, and total RNA was extracted using an RNeasy mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription was performed with a SuperScript first-strand synthesis system for RT-PCR (Invitrogen Corporation) according to the manufacturer's instructions, with 1 μg of total RNA from the cells as a template. Equal amounts of each reverse-transcribed product were amplified by PCR with HotStarTaq polymerase (Qiagen GmbH). The sequences of the primers were as follows: resistin, sense 5′-TCTAGCAAGACCC TGTGC and antisense 5′-CAGGTTTATTTCCAGCTCC; leptin, sense 5′-CCATCCTGACCTTATCCAAG and antisense 5′-TCCCTTAACGTAGTCCTTGC; adiponectin, sense 5′-TTTCCGGGAATCCAAGGCAG and antisense 5′-TCCATTACGCTCTCCTTCCC; β-actin (endogenous control), sense 5′-CCTCGCCTTTGCCGATCC and antisense 5′-GGATCTTCATGAGGTAGTCAGTC. After initial denaturation at 95 °C for 15 min, PCR involved amplification for a variable number of cycles of 35 s at 94 °C, 30 s at 56 °C, and 45 s at 72 °C, followed by elongation for 5 min at 72 °C. The amplified cDNA fragments were resolved by electrophoresis on 2 % (w/v) agarose gel, and were detected under ultraviolet light by an LAS-3000 (Fujifilm Corp., Tokyo, Japan) after the gel had been stained with ethidium bromide.

Real-time PCR of resistin mRNA

To evaluate the expression of resistin mRNA in detail, real-time PCR was performed using the real-time TaqMan system with a Sequence Detection System model 7000 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Cells were cultured under various conditions in medium containing 1 % (v/v) FBS, after which extraction of total RNA and synthesis of cDNA were performed as described above. Specific probes for resistin and β-actin were obtained from the TaqMan Gene Expression Assay (Applied Biosystems), with the ID numbers of the products being Hs00220767_m1 for the resistin probe and 4326315 for the β-actin probe. The threshold number of cycles was calculated from a standard curve, and the expression of resistin mRNA was normalized to that of β-actin mRNA.

Statistical analysis

Results are expressed as the mean ± SD or as the median with interquartile range (IQR). Statistical analysis was performed using StatFlex software (ver. 6; ARTEC Co., Ltd., Osaka, Japan). Significant differences in background data at baseline for the patients and healthy control subjects were evaluated by Student's t test for normally distributed continuous variables and by the Mann–Whitney U test for continuous variables without a normal distribution. Categorical variables were compared by the chi-square test or Fisher's exact test. Significant differences in serum adipokine levels between patients and healthy controls or among the 4 diseases were evaluated by Kruskal–Wallis one-way analysis of variance (ANOVA), while the significance of changes in serum adipokine levels was investigated by Friedman's ANOVA, followed by Dunn's multiple comparison test when the main effect of ANOVA was significant. Simple linear regression was used to assess correlations between serum adipokine levels and patient characteristics. Stepwise forward multiple regression analysis was employed for multivariate analysis. Non-numerical variables were analyzed as categorical variables in the regression model. ANOVA was used to determine differences in adipokine mRNA expression or protein levels among groups in the in vitro study, with Bonferroni's post hoc analysis being applied for pairwise

comparison when the main effect of ANOVA was significant. In all analyses, two-sided probability values of less than 0.05 were taken to indicate statistical significance.

Results

Profile of the patients and healthy controls

The baseline characteristics of the 52 patients (19 men and 33 women) with systemic autoimmune diseases and the 140 healthy control subjects (22 men and 118 women) are shown in Table 1. There were no significant demographic differences between the patients and the healthy control subjects, except for the age of the male subjects and the sex ratios of the two groups.

Clinical and laboratory data of the patients with systemic autoimmune diseases were shown in Table 2. All patients had active disease and no history of immunosuppressive therapy, including glucocorticoids. The mean initial daily dose of prednisolone was 46.7 ± 9.4 mg (males 50.0 ± 9.4 mg; females 44.9 ± 9.1 mg). Risk factors for atherosclerosis (hypertension, diabetes mellitus, smoking, serum levels of cholesterol, TG and Hs-CRP, and BMI) were similar between the male and female patients, except for current smoking.

Serum adipokine levels

The serum adipokine levels of the patients and healthy controls are shown in Fig. 1. Serum resistin levels were significantly higher in both male and female patients at baseline [males: median 10.0 (5.0–27.4) ng/mL, $p < 0.05$; females: median 7.4 (1.3–16.9) ng/mL, $p < 0.001$] compared with the controls [males: median 3.7 (3.3–5.1) ng/mL; females: median 3.5 (3.0–4.4) ng/mL] (Fig. 1a). Baseline serum leptin and HMW-adiponectin levels were significantly lower in the female patients [median 3.1 (1.9–10.0) ng/mL, $p < 0.01$, and 8.1 (2.4–11.9) μg/mL, $p < 0.001$, respectively] compared with female controls [median 7.0 (3.8–12.0) ng/mL, and 15.2 (9.7–21.6) μg/mL, respectively]. However, no significant differences in leptin and HMW-adiponectin levels were observed between the male patients and controls (Fig. 1b, c). As indicated above, the mean age of the patients was significantly elevated when compared with that of male healthy controls. We then examined the independent influence of age on the serum level of each adipokine by performing multiple regression analyses adjusted for gender, BMI, the presence of autoimmune diseases, serum levels of other two adipokines, and Hs-CRP in all subjects. The multiple regression analyses showed that only serum HMW-adiponectin level was significantly associated with age ($\beta = 0.018$, $p = 0.003$, R^2, coefficient of determination = 0.162). There was no association between serum levels of resistin or leptin and age (resistin: $\beta = 0.0005$, $p = 0.857$, R^2, coefficient of determination = 0.381; leptin: $\beta = -0.002$, $p = 0.589$, R^2, coefficient of determination = 0.476).

The adipokine and Hs-CRP levels at baseline in the patients with each systemic autoimmune disease are shown in Table 3. Serum Hs-CRP levels in patients with these 4 diseases were significantly different ($p < 0.001$ by Kruskal–Wallis test). The serum Hs-CRP level in SLE patients was significantly lower than those in patients with vasculitis syndrome ($p < 0.001$ by Dunn's multiple comparison test) and AOSD ($p < 0.05$), respectively. The serum Hs-CRP level in patients with PM/DM was significantly lower ($p < 0.001$) than that in patients with vasculitis syndrome, but other comparisons were not statistically significant. Similarly, serum resistin levels in patients with these 4 diseases were significantly different ($p < 0.01$ by Kruskal–Wallis test). Although serum resistin levels in patients with SLE and PM/DM tended to be lower than those in patients with vasculitis syndrome and AOSD, there was no significant difference except in the comparison between patients with PM/DM and vasculitis syndrome ($p < 0.05$ by Dunn's multiple comparison test). Differences in serum leptin and

Table 1 Demographic characteristics of the patients and controls

	Gender	Patients ($n = 52$)	Controls ($n = 140$)	p value
Sex, male:female (% female)		19:33 (63.5 %)	22:118 (84.3 %)	0.0028
Age (years)	Male	58.3 ± 14.7	45.6 ± 13.8	0.0072
	Female	51.2 ± 17.7	56.8 ± 16.7	0.0897
Body height (m)	Male	1.68 ± 0.08	1.70 ± 0.06	0.4192
	Female	1.54 ± 0.06	1.55 ± 0.06	0.9932
Body weight (kg)	Male	62.3 ± 13.6	64.5 ± 9.9	0.5648
	Female	53.2 ± 10.3	53.0 ± 7.0	0.8618
BMI (kg/m^2)	Male	21.8 ± 3.3	22.3 ± 2.7	0.6477
	Female	22.4 ± 4.3	22.3 ± 3.0	0.9165

Data are shown as the mean ± SD. Significant differences ($p < 0.05$) are underlined

BMI Body mass index

Table 2 Clinical and laboratory data of the patients with systemic autoimmune diseases

	Total ($n = 52$)	Males ($n = 19$)	Females ($n = 33$)	p value (M vs F)
Systemic autoimmune disease				
SLE	18 (34.6 %)	4 (21.1 %)	14 (42.4 %)	
Vasculitis syndrome	16 (30.8 %)	8 (42.1 %)	8 (24.2 %)	
PM/DM	14 (26.9 %)	6 (31.6 %)	8 (24.2 %)	
AOSD	4 (7.7 %)	1 (5.3 %)	3 (9.1 %)	
Disease duration (weeks)	4 (3–10)	5 (3–12)	4 (3–8)	0.383
Comorbidities				
Systolic blood pressure (mmHg)	121.6 ± 15.1	123.7 ± 14.9	120.4 ± 15.4	0.452
Diastolic blood pressure (mmHg)	72.6 ± 10.6	74.0 ± 11.8	71.8 ± 10.0	0.480
Hypertention (%)	18 (34.6 %)	8 (42.1 %)	10 (30.3 %)	0.546
Diabetes mellitus (%)	5 (9.6 %)	3 (15.8 %)	2 (6.2 %)	0.342
Current smoking (%)	10 (19.2 %)	7 (36.8 %)	3 (9.1 %)	0.026
Ever smoked (%)	19 (36.5 %)	13 (68.4 %)	6 (18.2 %)	0.261
Carotid artery plaque (%)[a]	30/44 (68.2 %)	13/16 (81.3 %)	17/28 (60.7 %)	0.195
Maximum IMT (mm)[a]	0.70 (0.50–0.85)	0.75 (0.61–1.01)	0.68 (0.50–0.79)	0.166
Laboratory data				
Total cholesterol (mmol/L)	3.94 ± 1.06	3.88 ± 1.15	3.97 ± 1.02	0.775
HDL cholesterol (mmol/L)	0.80 ± 0.28	0.83 ± 0.32	0.79 ± 0.27	0.670
LDL cholesterol (mmol/L)	2.47 ± 0.90	2.36 ± 0.94	2.53 ± 0.88	0.515
Tryglycerides (mmol/L)	1.30 (0.88–1.86)	1.32 (0.88–2.27)	1.21 (0.87–1.83)	0.488
Hs-CRP (mg/L)[b]	19.7 (3.2–64.4)	20.6 (3.4–98.1)	18.7 (1.5–66.0)	0.732
Medications				
Initial daily prednisolone dose (mg)	46.7 ± 9.4	50.0 ± 9.4	44.9 ± 9.1	0.057
Immunosuppressive agents (%)[c]	24 (46.2 %)	9 (50.0 %)	15 (45.5 %)	1.000
Antihypertensive agents (%)	14 (26.9 %)	7 (36.8 %)	7 (21.2 %)	0.331
Antidiabetic agents (%)	3 (5.8 %)	2 (10.5 %)	1 (3.0 %)	0.546
Statins (%)	6 (11.5 %)	1 (5.3 %)	5 (15.1 %)	0.397

Data are shown as the number (%), the mean ± SD, or the median (interquartile range). Significant differences between male and female patients ($p < 0.05$) are underlined

M male, *F* female, *SLE* systemic lupus erythematosus, *PM/DM* polymyositis/dermatomyositis, *AOSD* adult onset Still's disease, *IMT* intima-media thickness, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *Hs-CRP* high-sensitivity C-reactive protein

[a] Only 44 patients (16 men and 28 women) had this examination

[b] Hs-CRP level of healthy control subjects was 0.278 (0.146–0.509) mg/L [males 0.294 (0.175–0.393); females 0.266 (0.140–0.533)]

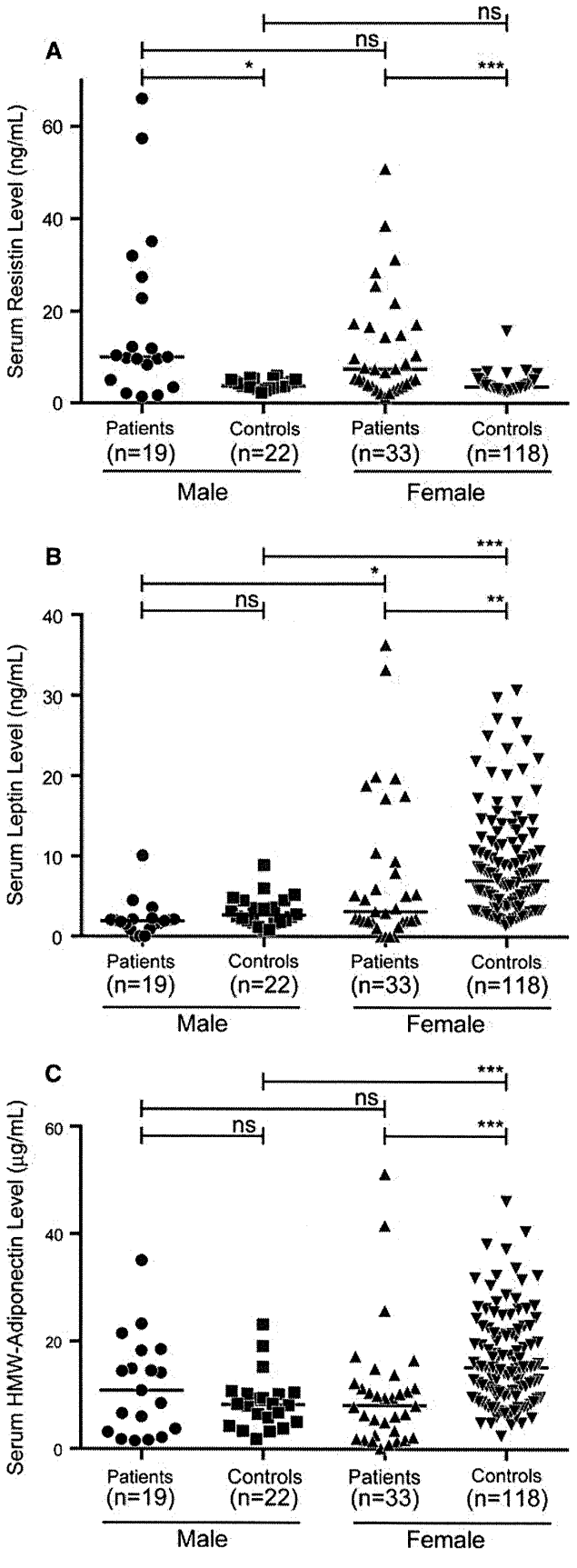
[c] Azathioprine ($n = 1$), cyclophosphamide ($n = 14$), cyclosporin A ($n = 3$), methotrexate ($n = 4$), or tacrolimus ($n = 2$) was administered 2–4 weeks after glucocorticoid therapy

HMW-adiponectin levels among patients with these diseases were not statistically significant according to the Kruskal–Wallis test.

In the 18 patients with SLE, baseline serum resistin levels were significantly higher for both male and female patients [males: median 16.4 (6.2–55.2) ng/mL, $p < 0.01$; females: median 5.2 (3.5–15.1) ng/mL, $p < 0.05$, respectively] than for male and female controls [data shown above], while baseline serum leptin and HMW-adiponectin levels were significantly lower in female patients [median 2.5 (1.4–7.1) ng/mL, $p < 0.01$, and 8.0 (3.3–14.7) μg/mL, $p < 0.01$, respectively] than in female controls.

Association between adipokines and carotid atherosclerosis

Carotid ultrasonography was conducted in 44 patients with systemic autoimmune diseases. Thirty of the 44 patients (68.2 %) had carotid artery plaques, and the median value of the maximum IMT was 0.70 (0.50–0.85) mm for the patients (Table 2). Among the SLE patients, 47 % had carotid artery plaques, and the median value of their maximum IMT was 0.60 mm (SLE patients were aged 43.2 ± 14.0 years old), while the corresponding values were 92.9 % and 0.81 mm for vasculitis syndrome patients



◀ **Fig. 1** Serum levels of resistin (**a**), leptin (**b**), and high molecular weight (HMW)-adiponectin (**c**) in patients with systemic autoimmune diseases and healthy control subjects. *Horizontal bars* indicate median values. Statistical significance was determined by Kruskal–Wallis one-way analysis of variance (ANOVA), followed by Dunn's multiple comparison test when the main effect of ANOVA was significant. *ns* no significant difference. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

(64.9 ± 16.3 years old), 81.8 % and 0.37 mm for PM/DM patients (55.8 ± 14.3 years old), and 66.7 % and 0.50 mm for AOSD patients (49.8 ± 12.4 years old). We employed the serum levels of adipokines (resistin, leptin, and HMW-adiponectin) in addition to patient characteristics (gender, age, and BMI) and traditional risk factors (hypertension, diabetes mellitus, smoking status, and serum levels of T-chol, HDL-chol, LDL-chol, TG and Hs-CRP) in a model predicting the maximum carotid IMT of the patients with autoimmune diseases. Multivariate analysis showed that the significant determinants were age and the presence of hypertension (age: β, regression coefficient = 0.013, $p = 0.034$; hypertension: $\beta = 0.248$, $p = 0.019$, R^2, coefficient of determination = 0.282). There was no association between any of the adipokines and carotid premature atherosclerosis (maximum IMT) in the patients with autoimmune diseases.

Multivariate analysis of factors associated with serum Hs-CRP

We employed the presence of systemic autoimmune disease, subject characteristics (gender, age, and BMI), and serum levels of adipokines in models predicting the serum level of Hs-CRP in all subjects. Significant univariate predictors of the Hs-CRP level included the presence of autoimmune disease, female gender, age, resistin, leptin, and HMW-adiponectin (Table 4, univariate model). Inclusion of these univariate predictors in a multivariate model resulted in a final model with 3 significant predictors: autoimmune disease, age, and resistin (Table 4, multivariate model).

Changes in adipokine levels with glucocorticoid therapy

The influence of glucocorticoid therapy on serum adipokine levels is shown in Table 5. We examined whether treatment with a glucoorticoid for 4 weeks affected serum adipokine levels in our patients with systemic autoimmune diseases. We found that resistin showed a significant decrease to the normal range, while leptin and HMW-adiponectin increased significantly after 4 weeks of glucocorticoid therapy.

Table 3 Adipokine and Hs-CRP levels at baseline in patients with various systemic autoimmune diseases

	SLE ($n = 18$)	Vasculitis syndrome ($n = 16$)	PM/DM ($n = 14$)	AOSD ($n = 4$)	p value
Adipokines					
Resistin (ng/mL)	6.3 (3.8–18.3)	12.0 (9.7–28.2)	4.2 (2.1–8.9)	23.1 (7.5–52.7)	<0.008
Leptin (ng/mL)	2.2 (1.4–5.5)	2.0 (1.0–5.2)	2.1 (1.6–8.5)	6.2 (1.9–16.7)	0.651
HMW-adiponectin (μg/mL)	8.0 (3.3–18.3)	10.2 (6.1–14.4)	2.0 (1.6–12.1)	13.2 (10.1–23.1)	0.081
Hs-CRP (mg/L)	5.8 (0.6–18.0)	100.9 (53.9–147.5)	4.9 (1.3–23.5)	72.3 (34.3–103.1)	<0.001

Data are shown as the median (interquartile range). Significant differences among subgroups of systemic autoimmune diseases ($p < 0.05$) as estimated via the Kruskal–Wallis test are underlined

SLE systemic lupus erythematosus, *PM/DM* polymyositis/dermatomyositis, *AOSD* adult onset Still's disease, *Hs-CRP* high-sensitivity C-reactive protein

Table 4 Crude and adjusted associations of subject characteristics with serum Hs-CRP

	Hs-CRP[a]				
	Univariate model		R^2	Multivariate model	
	β	p value		β	p value
Systemic autoimmune diseases	1.978	<0.001	0.635	1.729	<0.001
Female sex	−0.495	0.011	0.034	−0.082	0.507
Age	0.023	0.014	0.031	0.022	<0.001
BMI	0.071	0.152	0.011	0.039	0.233
Resistin[a]	1.987	<0.001	0.373	0.742	<0.001
Leptin[a]	−0.502	0.013	0.032	0.211	0.169
HMW-adiponectin[a]	−0.320	0.005	0.041	0.006	0.929
R^2				0.711	

Significant correlations ($p < 0.05$) are underlined

β regression coefficient, R^2 coefficient of determination, *BMI* body mass index, *Hs-CRP* high-sensitivity C-reactive protein, *HMW* high molecular weight

[a] Logarithmic transformation was used for highly skewed variables when required for multivariate analysis

Table 5 Changes in serum adipokine levels in patients who received glucocorticoid therapy for 4 weeks

	Baseline	1 week	2 weeks	3 weeks	4 weeks
Resistin (ng/mL)	9.1 (4.1–13.7)	5.7 (3.6–13.5)	8.0 (3.6–14.1)	5.6 (2.8–14.9)*	4.1 (2.6–8.6)*
Leptin (ng/mL)	2.1 (1.6–5.3)	4.6 (2.2–13.1)*	3.3 (1.9–11.6) *	4.8 (2.1–12.6)*	4.6 (2.1–17.5)*
HMW-adiponectin (μg/mL)	9.0 (2.8–14.5)	16.2 (4.7–24.5)*	13.6 (4.0–21.8)*	16.5 (3.9–26.8)*	17.8 (5.8–28.5)*

Data are presented as the median (interquartile range). Assessment of changes in serum adipokines levels was performed by Friedman's ANOVA followed by Dunn's multiple comparison procedure when the main effect of ANOVA was significant. Resistin, leptin, and HMW-adiponectin levels of healthy control subjects ($n = 140$) were 3.5 (3.1–4.5) ng/mL, 5.8 (3.3–10.7) ng/mL, and 13.4 (8.4–20.1) μg/mL, respectively

HMW high molecular weight

* $p < 0.001$ compared with baseline

Effects of LPS and dexamethasone on adipokine expression in human PBMCs

We also investigated the effects of LPS and dexamethasone on adipokine mRNA expression and secretion in vitro, especially resistin (which had a strong association with the inflammatory marker Hs-CRP). As shown in Fig. 2a, resistin mRNA expression was only detectable at very low levels in unstimulated human PBMCs. Resistin mRNA expression was increased by stimulation with LPS, and LPS-induced upregulation of resistin mRNA expression was reversed by dexamethasone in a dose-dependent manner. Stimulation with dexamethasone alone had no effect on resistin mRNA expression. Leptin and

adiponectin mRNA were not detectable in human PBMCs.

Real-time PCR was performed to quantitatively evaluate resistin mRNA expression. This showed that stimulation by LPS increased resistin mRNA expression 17-fold ($p < 0.05$) compared with that in unstimulated human PBMCs, and this upregulation of resistin mRNA expression was reversed by dexamethasone in a dose-dependent manner (Fig. 2b).

Furthermore, we examined resistin protein levels in the culture supernatants by ELISA. Resistin protein was detected in the supernatants of unstimulated human PBMCs (0.17 ± 0.01 ng/mL), and its level was increased significantly by LPS stimulation (0.34 ± 0.02 ng/mL, $p < 0.01$). The increase in resistin secretion induced by LPS was inhibited by the addition of dexamethasone to cultures (Fig. 2c).

Discussion

To our knowledge, this is the first study to prospectively investigate the serum levels of 3 adipokines (resistin, leptin, and HMW-adiponectin) before and after glucocorticoid therapy in new patients with systemic autoimmune diseases in the active phase. Serum resistin levels were initially high in these patients and decreased towards normal after glucocorticoid therapy, while serum leptin and HMW-adiponectin levels were initially low and then increased after glucocorticoid therapy. We also found a significant association between the serum levels of resistin and Hs-CRP (an inflammatory marker) in these patients. Furthermore, our in vitro study revealed that dexamethasone inhibited LPS-induced upregulation of resistin mRNA and protein expression by human PBMCs in a concentration-dependent manner.

To evaluate the association of adipokines with inflammation, we performed multivariate analysis, which showed that the serum resistin level was significantly associated with serum Hs-CRP, along with the presence of autoimmune disease and older age. Serum CRP levels in patients with SLE [6] and PM/DM [7] are known to be low regardless of disease activity. In this study, same trends in the serum Hs-CRP levels in these diseases were noted. In addition, serum resistin levels among patients with SLE, vasculitis syndrome, PM/DM, and AOSD were significantly different, and those in patients with SLE and PM/DM tended to be lower than those in patients with vasculitis syndrome and AOSD. These data suggest that an increase in resistin might contribute to inflammation in patients with systemic autoimmune diseases.

Assessment of carotid plaque and IMT by ultrasonography provides a noninvasive and reliable measurement of

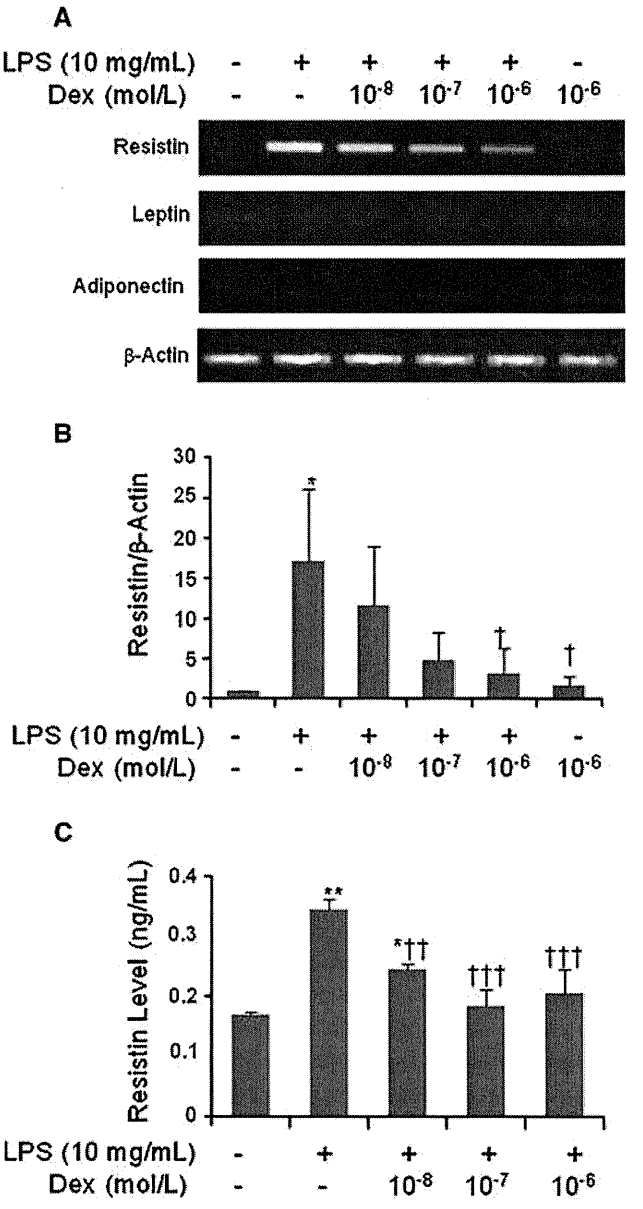


Fig. 2 Effects of lipopolysaccharides (LPS) and dexamethasone (Dex) on adipokine gene expression and secretion in human peripheral blood mononuclear cells (PBMCs). **a** Effects of LPS and Dex on adipokine mRNA gene expression in human PBMCs detected by reverse transcription-polymerase chain reaction (PCR) analysis. **b** Real-time-PCR study of resistin mRNA expression in human PBMCs. LPS-induced upregulation of resistin mRNA expression is inhibited by Dex in PBMCs. Resistin mRNA expression was normalized to that of β-actin mRNA. Fold induction was measured relative to mRNA expression by control PBMCs incubated without LPS or Dex. Data are presented as the mean ± SD ($n = 3$). **c** Resistin levels in culture supernatants of human PBMCs. LPS-induced upregulation of resistin secretion into culture supernatants is inhibited by Dex. Resistin was measured by enzyme-linked immunosorbent assay. Data are presented as the mean ± SD ($n = 3$). Significance was evaluated by ANOVA, followed by Bonferroni's post hoc analysis for pairwise comparison when the main effect of ANOVA was significant. $^{*}p < 0.05$; $^{**}p < 0.001$ compared with unstimulated cells; $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$ compared with LPS-stimulated cells

the systemic burden of atherosclerosis [8]. In our patients (mean age 58.3 years), there was a high prevalence of carotid artery plaque (68.2 %), a finding compatible with previous reports of a similar prevalence of carotid plaque in patients with SLE (mean age 52 years) [9] and patients with giant cell arteritis (mean age 79 years) [10]. The median IMT of our patients was also consistent with that for patients with SLE [9] and patients with giant cell arteritis [10].

Ross [11] suggested that chronic inflammation contributes to accelerated atherogenesis and plays a role in all stages of atherosclerosis. Cho et al. [12] demonstrated that resistin can accelerate the progression of atherosclerotic plaque by aggravating inflammation of the vessel wall through stimulating monocyte infiltration, and by activating of endothelial cells and smooth muscle cells. In the present study, we found a significant association between IMT and age or hypertension, well-known atherosclerotic risk factors, but we could not find any significant relationship between premature atherosclerosis and serum adipokine levels.

Resistin was originally identified as a 12.5 kDa polypeptide expressed and secreted by white adipose tissue [13]. Human adipocytes express resistin at very low levels, if at all, whereas high levels are expressed by PBMCs (especially monocytes and T lymphocytes), macrophages, neutrophils, and bone marrow cells that take part in the inflammatory response [14–17]. We found high serum resistin levels in our patients with active disease, suggesting an association of resistin with the inflammatory processes related to autoimmune diseases. Previous cross-sectional studies suggested that serum resistin levels were not increased in SLE patients compared with those in control subjects [18–20]. In this study, however, the serum resistin level of 18 patients with SLE was significantly higher than that of healthy controls, and the high resistin level of the patients declined after glucocorticoid therapy. We recently reported that serum resistin levels were elevated in patients with Kawasaki disease. They were decreased by the intravenous administration of immune globulin [21]. Therefore, the discrepancy between our findings and previous reports might be due to differences in disease activity or treatment, since most of the subjects enrolled in the previous studies were outpatients who were already on immunosuppressive therapy, including glucocorticoids.

We also investigated the association of resistin with inflammation by performing an in vitro experiment using human PBMCs, since they contain monocytes and T lymphocytes, which are reported to produce resistin [17]. Our study showed that LPS upregulated the expression of resistin mRNA and protein in human PBMCs. Such findings may help to explain hyperresistinemia in our patients with acute inflammation. Induction of resistin by LPS may be partly mediated by the activation of nuclear factor-κB (NF-κB), activator protein 1 (AP-1), or mitogen-activated protein kinase (MAPK) [22, 23]. Although the significance of hyperresistinemia in patients with autoimmune diseases has not yet been clarified, Tarkowski et al. [24] reported that resistin induced the production of proinflammatory cytokines such as IL-6 and IL-8 by interacting with Toll-like receptor 4 (TLR4). Thus, it is possible that resistin plays an important role in inflammation associated with systemic autoimmune diseases.

In the present study, we demonstrated that resistin expression was downregulated by glucocorticoids both in vivo and in vitro. The promoter region of the human resistin gene contains binding sites for proinflammatory transcription factors such as cRel (one of 5 NF-κB subunits) and AP-1, but has no glucocorticoid response elements (GREs) [25]. Accordingly, glucocorticoid therapy might inhibit resistin expression by repressing the activation of these transcription factors through the glucocorticoid receptor [26].

Previous cross-sectional studies of serum leptin levels in patients with SLE have not obtained definitive results. Some studies have shown that leptin levels were higher in patients than in healthy control subjects [18, 20, 27–29], while others have found that leptin levels did not differ between patients and healthy controls [30]. On the other hand, our prospective study demonstrated that serum leptin levels were lower in patients with active autoimmune diseases than in healthy controls, and that the leptin levels of the patients increased after glucocorticoid therapy. Kümpers et al. [31] performed a prospective study of serum leptin levels in patients with antineutrophilic cytoplasmic antibody (ANCA)-associated vasculitis receiving glucocorticoid therapy. They found lower leptin levels in the ANCA-associated vasculitis patients than in healthy controls, and there was an increase in leptin after glucocorticoid therapy, consistent with our results.

In this study, we found low leptin levels in patients with active autoimmune diseases. It has been reported that long-term exposure to proinflammatory cytokines such as IL-1β or TNFα cause suppression of leptin protein and gene expression in adipose tissue [32–34], while acute stimulation by proinflammatory cytokines increases leptin release in vitro [32, 33]. Serum leptin levels were high in some of our patients but low in most of them. Thus, it is possible that these variations in serum leptin levels were caused by different inflammatory states.

Previous studies have found that glucocoricoids increase the serum leptin level in healthy humans [35, 36]. It was also reported that glucocorticoids upregulate leptin expression by cultured human adipose tissue [37]. These results support our finding that serum leptin levels

increased after glucocorticoid therapy in patients with systemic autoimmune diseases. It has been demonstrated that the human *obese* (*ob*) gene, the product of which is leptin, contains GREs in the promoter region [38], suggesting that glucocorticoids may activate *ob* gene transcription by interacting with these GREs.

In this study, the baseline serum HMW-adiponectin level (before glucocorticoid therapy) was lower in our patients with autoimmune diseases than in the healthy control subjects. However, previous cross-sectional studies of the serum total adiponectin level in SLE patients have yielded conflicting results, and some studies have indicated that serum adiponectin levels were higher in SLE patients than in healthy controls [18, 19, 28]. In the present study, the serum HMW-adiponectin level in the 18 patients with SLE was significantly lower than that in the healthy controls. The discrepancy between this finding and previous reports might be explained by differences in disease activity or therapy, as was discussed for resistin. Furthermore, total adiponectin (containing different isoforms) was measured in previous studies [18, 19, 28], whereas we measured the HMW fraction of adiponectin. It is known that HMW-adiponectin is the more active and clinically relevant form of this protein [39, 40]. The serum HMW-adiponectin level in male patients tended to be higher than that in healthy controls. Since multiple regression analysis in this study showed that the serum HMW-adiponectin level was positively associated with age, this trend might be explained by the increased age of the male patients.

Bruun et al. [41] have reported the reciprocal suppression of adiponectin and proinflammatory cytokines (such as TNFα and IL-6) in cultured human adipose tissue, and they also noted the same effect for serum levels in vivo. Therefore, an increase in proinflammatory cytokines might have suppressed adiponectin production in our patients with active autoimmune diseases. Adiponectin may have a protective effect against atherosclerotic vascular changes [42]. Systemic autoimmune diseases are known to be associated with an increase in premature atherosclerosis and with increased mortality due to cardiovascular diseases [8, 43, 44]. Thus, hypoadiponectinemia might be an important cardiovascular risk factor in patients with systemic autoimmune diseases.

In this study, serum HMW-adiponectin levels were found to be increased after glucocorticoid therapy. Both adiponectin and HMW-adiponectin levels have previously been reported to increase with oral glucocorticoid therapy in healthy subjects [45] and patients with type 2 diabetes [46]. Adiponectin and proinflammatory cytokines suppress each other [41], while glucocorticoids inhibit the production of proinflammatory cytokines such as TNFα [47]. Thus, the reduction in proinflammatory cytokine levels resulting from glucocorticoid therapy may have led to an increase in HMW-adiponectin in our patients with systemic autoimmune diseases.

Acknowledgments This work was partly supported by Heisei 20 and 23 Strategic Research Foundation grants from the Japanese Ministry of Education, Culture, Sports, Science & Technology to Toho University, grants from the Japan Society for the Promotion of Science (JSPS 23591449) to S.K., and grants from the Nakatomi Foundation and the Takeda Science Foundation to N.K. We wish to thank Ms. Sonoko Sakurai for secretarial assistance.

Conflict of interest None.

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The Pharmacogenomics Journal (2011), 1–10

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ORIGINAL ARTICLE

A whole-genome association study of major determinants for allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients

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Received 12 January 2011; revised 10 August 2011; accepted 11 August 2011

Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are severe, cutaneous adverse drug reactions that are rare but life threatening. Genetic biomarkers for allopurinol-related SJS/TEN in Japanese were examined in a genome-wide association study in which Japanese patients ($n=14$) were compared with ethnically matched healthy controls ($n=991$). Associations between 890 321 single nucleotide polymorphisms and allopurinol-related SJS/TEN were analyzed by the Fisher's exact test (dominant genotype mode). A total of 21 polymorphisms on chromosome 6 were significantly associated with allopurinol-related SJS/TEN. The strongest association was found at rs2734583 in *BAT1*, rs3094011 in *HCP5* and GA005234 in *MICC* ($P=2.44\times10^{-8}$; odds ratio = 66.8; 95% confidence interval, 19.8–225.0). rs9263726 in *PSORS1C1*, also significantly associated with allopurinol-related SJS/TEN, is in absolute linkage disequilibrium with *human leukocyte antigen-B*5801*, which is in strong association with allopurinol-induced SJS/TEN. The ease of typing rs9263726 makes it a useful biomarker for allopurinol-related SJS/TEN in Japanese.
The Pharmacogenomics Journal advance online publication, 13 September 2011; doi:10.1038/tpj.2011.41

Keywords: allopurinol; Stevens–Johnson syndrome; toxic epidermal necrolysis; human lymphocyte antigen; single nucleotide polymorphism; genome-wide association study

Introduction

Allopurinol is a xanthine oxidase inhibitor that prevents the production of uric acid to reduce plasma uric acid levels to a normal range. It is the most frequently used anti-hyperuricemic agent in the world due to its long-term pharmacological effect.[1] However, allopurinol is also one of the most frequent causes of a variety of delayed severe cutaneous adverse drug reactions (SCARs).[2] According to spontaneous reports of severe adverse drug reactions to the Ministry of Health, Labor, and Welfare of Japan, allopurinol-related SCARs accounted for about 11% of all reported SCAR cases in Japan in 2008.[3] Allopurinol-related SCARs include the drug-induced hypersensitivity syndrome, Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).[4] SJS/TEN are characterized by high fever, malaise and rapid development of blistering exanthema, with macules and target-like lesions, accompanied by mucosal involvement.[5] Even though the incidence of SJS/TEN is extremely low, the mortality rate of TEN can be as high as 26%.[5] Therefore, SJS/TEN is a serious problem in allopurinol therapy, in spite of the ideal anti-hyperuricemic effect of allopurinol.