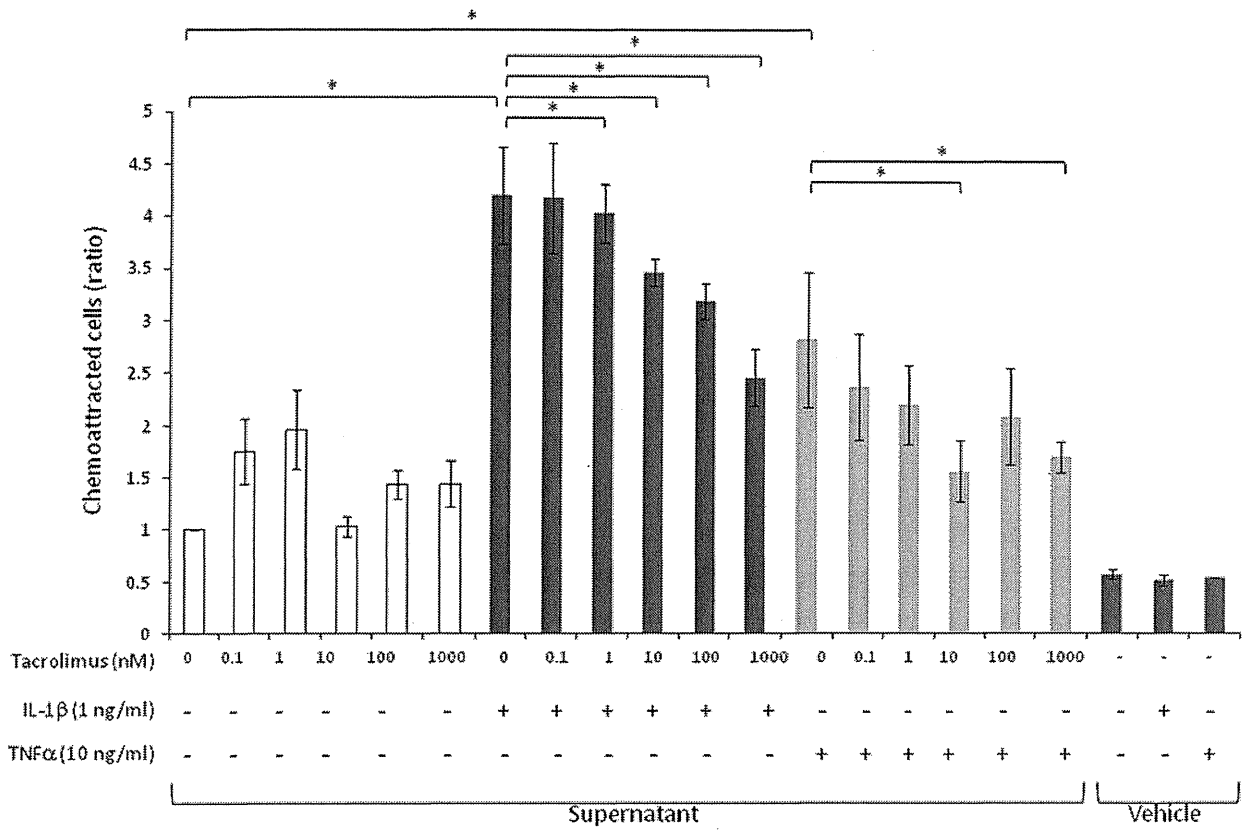


Fig. 5 IL-1β/TNFα-induced PMN migration is inhibited by tacrolimus. Human RSF were incubated with the vehicle, IL-1β, or TNFα with or without tacrolimus, and culture supernatants were collected after 24 h. For the chemotactic assay, PMN were prepared from healthy volunteers. Either culture supernatant or vehicle (29 µl) was added to the lower chamber, and PMN suspension (25 µl at a concentration of 1.0×10^6 PMN/ml) was placed onto a filter with 5-µm pores. After incubation for 1 h at 37 °C, the D/R ratio of cells that migrated into the lower chamber was assessed as described in "Methods". Experiments were performed in triplicate and all values are the mean ± SD. $^*P < 0.05$

was not regulated by IL-1β, TNFα, and/or tacrolimus (Fig. 7b).

Discussion

In this study, we investigated the direct effect of tacrolimus on synovial cells obtained from patients with RA. DNA microarray analysis showed that the expression of genes for various inflammatory cytokines was up-regulated by IL-1β, but the only genes down-regulated by tacrolimus were those for a few chemokines (CCL3, CCL4, and CXCL8). The results of microarray analysis were confirmed by ELISA and PCR studies using cultured synovial cells. Moreover, a therapeutic concentration of tacrolimus for RA (10 nM) inhibited the production of these chemokines. We also examined the biological effects of tacrolimus and demonstrated that IL-1β-induced chemotaxis, as well as TNFα-induced chemotaxis, of PMN was inhibited by tacrolimus in a dose-dependent manner. Therefore, we found that tacrolimus inhibited the production of chemokines induced by pro-inflammatory cytokines, which may be one of the mechanisms by which it is effective for the treatment of RA. We also demonstrated that RSF expressed FKBPs, calcineurin Aα, and calcineurin Aβ, which have been reported as targets of tacrolimus [13].

RA is characterized by chronic synovitis that affects multiple joints. Infiltration and activation of leukocytes results in the progressive destruction of cartilage and bone. Chemokines have an important role in the recruitment of leukocytes and angiogenesis. CCL3 is also known as macrophage inflammatory protein-1α (MIP-1α), and is a chemokine that recruits immune cells, such as lymphocytes, monocytes, neutrophils, basophils, and eosinophils [14, 15]. It has been reported that the CCL3 level in the synovial fluid of RA patients is higher than that in osteoarthritis patients [16], and CCL3 has been shown to have an essential role in collagen-induced arthritis [17]. CCL4, also known as macrophage inflammatory protein-1β (MIP-1β), is found in the synovial fluid of RA patients along with

MIP-1α [18]. CCL3 binds to CCR1 and CCR5, while CCL4 binds to CCR5, which are expressed on a variety of immune cells [19]. CCR1/CCR5 targeting therapy has been reported to be effective for experimental arthritis [8, 20, 21]. In patients with RA, CXCL8, better known as IL-8, is also an important chemokine [22–24], and high levels of CXCL8 have been detected in the synovial fluid, synovial tissue, and serum of RA patients [25–28]. An anti-CXCL8 antibody was shown to prevent neutrophil-dependent tissue damage as well as neutrophil infiltration in rabbits with lipopolysaccharide/IL-1-induced arthritis [29]. CXCL8 was reported to mediate angiogenesis [30], so inhibition of CXCL8 production could inhibit the chemotaxis of immune cells and angiogenesis.

In this study, we have chosen 24 h culture time because of the aim to have enough reaction time to correspond to the clinical settings, in which patients take tacrolimus every day and their synovial cells are exposed to tacrolimus continuously. However, further analysis would be needed for detailed evaluation of mRNA expression. Yoo et al. [31] showed that calcineurin activity in synovial cells was up-regulated by IL-1 or TNF. These results would be an

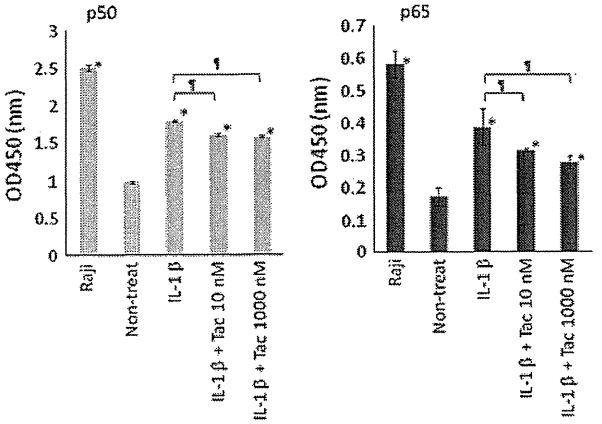


Fig. 6 Nuclear translocation of activated p50 (*left*) and p65 (*right*) NF-κB assessed by ELISA. RSF were incubated with IL-1β (1 ng/ml) with or without each dose of tacrolimus for 3 h, and nuclear extracts were obtained. NF-κB activation was assessed by using 2 μg of each nuclear extract per well. Raji nuclear extract was used as the positive control. Experiments were performed in triplicate and all values are the mean ± SD. $*P < 0.05$ versus untreated cells, $**P < 0.05$. *Tac* tacrolimus

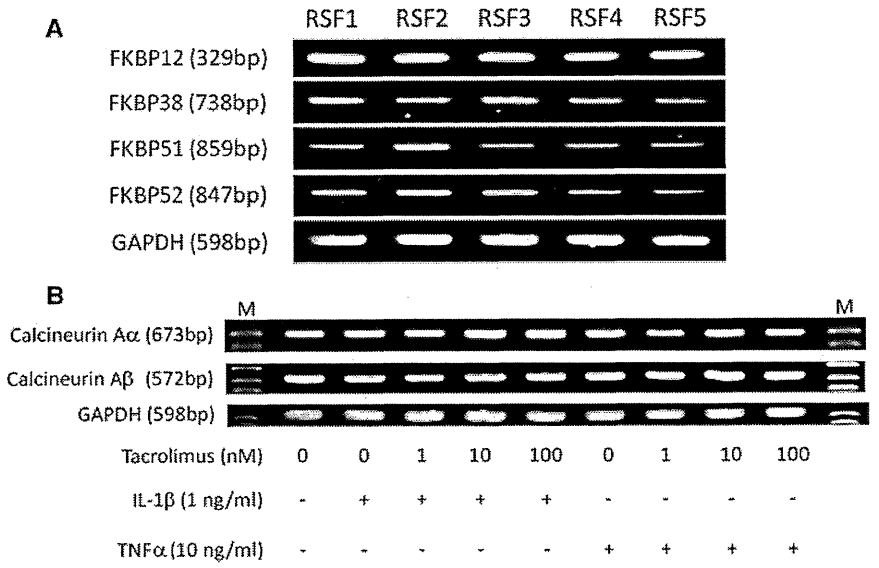


Fig. 7 Expression of **a** tacrolimus receptor and **b** calcineurin Aα and Aβ mRNA by RSF. **a** FKBPs mRNA expression in cultured human RSF obtained from 5 different patients was determined by RT-PCR. **b** Cells were incubated with PBS, IL-1β, or TNFα with or without each dose of tacrolimus for 24 h. mRNA expression was determined by RT-PCR

explanation why tacrolimus had effects on IL-1- or TNF-stimulated synovial cells. Okamoto et al. [32] demonstrated that tacrolimus inhibited IL-8 production via suppression of transcription through the AP-1 or κB-like sites in Jurkat cells. Tacrolimus may have effects on other cytokines or chemokines if AP-1 or κB-like sites are important for their expression in synovial cells. In our study, three chemokines were investigated because they were selected as candidates by a DNA microarray screening. Although we showed that nuclear translocation of NF-κB is inhibited by tacrolimus, further study is needed to reveal the detailed mechanism by which tacrolimus regulated these chemokine productions in synovial cells.

NF-κB plays a pivotal role in the expression of CCL3, CCL4, and CXCL8 [33]. Therefore, we examined the nuclear translocation of activated NF-κB. IL-1β induced NF-κB activation in RSF, and this effect was suppressed by tacrolimus. These findings were compatible with the results of DNA microarray analysis, ELISA, and real-time PCR. Tacrolimus exerts its effects through specific receptors (FKBPs), thus suppressing activation of the calcium-dependent phosphatase calcineurin. Calcineurin dephosphorylates NFAT, which undergoes translocation into the nucleus where it mainly binds to the IL-2 promoter. In addition to activation of NFAT, calcineurin has also been reported to synergistically promote NF-κB activation [13, 34]. Our data indicated that IL-1β-induced chemokine production by RSF was inhibited by tacrolimus via interference with p50 and p65 NF-κB activation. Not only expression of FKBP12 but also other FKBPs by RSF were detected in this study. FKBP12 is considered to be the main receptor for tacrolimus, but the functional differences of FKBPs need to be elucidated by further studies.

Rheumatoid synovial tissue is characterized by infiltration of inflammatory cells and increased vascularity. In the pathogenesis of RA, both immune cells and the synovial cells themselves participate in the inflammatory process by secreting a variety of mediators. In fact, synovial cells produced a number of inflammatory cytokines in response to stimulation with IL-1β in this study (data not shown). We demonstrated that rheumatoid synovial fibroblasts produced CCL3, CCL4, and CXCL8, while tacrolimus inhibited the production of these chemokines. Tacrolimus is a calcineurin inhibitor and its main mechanism of action is thought to involve the targeting of T cells. Clinical studies have shown that tacrolimus is effective for RA, but the mechanisms involved have not all been revealed. We performed a gene array analysis of RSF to identify cytokines regulated by tacrolimus. After candidate genes were selected by DNA microarray analysis, we confirmed that RSF secrete CCL3, CCL4, and CXCL8 in response to IL-1β stimulation and that production of these chemokines is inhibited by tacrolimus. Our results suggested one of the mechanisms by which tacrolimus acts as an anti-rheumatic drug.

Acknowledgments This work was supported in part by a Project Research Grant (20-12) and Dr. Takeshi Yanase's grant from Toho University School of Medicine to K.K.; Heisei 20 and 23 Strategic Research Foundation Grants from the Japanese Ministry of Education, Culture, Sports, Science & Technology to Toho University; Grants from Japan Society for the Promotion of Science (20591177, 23591449) to S.K.; and Grants from Nakatomi Foundation and Takeda Science Foundation to N.K. We wish to thank Ms. Sonoko Sakurai for secretarial assistance.

Conflict of interest S.K. has received honoraria for lectures and research grants from Astellas Pharma Inc. (Tokyo, Japan), the manufacturer of tacrolimus. The other authors declare no conflict of interest.

References

1. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. J Antibiot. 1987;40(9):1256–65.
2. Kelly PA, Burckart GJ, Venkataramanan R. Tacrolimus: a new immunosuppressive agent. Am J Health Syst Pharm. 1995; 52(14):1521–35.
3. Knoll G. Trends in kidney transplantation over the past decade. Drugs. 2008;68 (Suppl 1):3–10.
4. Kawai S, Hashimoto H, Kondo H, Murayama T, Kiuchi T, Abe T. Comparison of tacrolimus and mizoribine in a randomized, double-blind controlled study in patients with rheumatoid arthritis. J Rheumatol. 2006;33(11):2153–61.
5. Kondo H, Abe T, Hashimoto H, Uchida S, Irimajiri S, Hara M, et al. Efficacy and safety of tacrolimus (FK506) in treatment of rheumatoid arthritis: a randomized, double blind, placebo controlled dose-finding study. J Rheumatol. 2004;31(2):243–51.
6. Kitahara K, Kawai S. Cyclosporine and tacrolimus for the treatment of rheumatoid arthritis. Curr Opin Rheumatol. 2007; 19(3):238–45.
7. Kawai S. Current drug therapy for rheumatoid arthritis. J Orthop Sci. 2003;8:259–63.
8. Koch AE. Chemokines and their receptors in rheumatoid arthritis: future targets? Arthritis Rheum. 2005;52(3):710–21.
9. Kitahara K, Kusunoki N, Kakiuchi T, Suguro T, Kawai S. Adiponectin stimulates IL-8 production by rheumatoid synovial fibroblasts. Biochem Biophys Res Commun. 2009;378(2):218–23.
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31(3):315–24.
11. Frevert CW, Wong VA, Goodman RB, Goodwin R, Martin TR. Rapid fluorescence-based measurement of neutrophil migration in vitro. J Immunol Methods. 1998;213(1):41–52.
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1–2):55–63.
13. Baksh S, Burakoff SJ. The role of calcineurin in lymphocyte activation. Semin Immunol. 2000;12(4):405–15.
14. Wolpe SD, Davatelis G, Sherry B, Beutler B, Hesse DG, Nguyen HT, et al. Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J Exp Med. 1988;167(2):570–81.

15. Szekanecz Z, Strieter RM, Kunkel SL, Koch AE. Chemokines in rheumatoid arthritis. Springer Semin Immunopathol. 1998; 20(1–2):115–32.
16. Koch AE, Kunkel SL, Harlow LA, Mazarakis DD, Haines GK, Burdick MD, et al. Macrophage inflammatory protein-1 alpha. A novel chemotactic cytokine for macrophages in rheumatoid arthritis. J Clin Invest. 1994;93(3):921–8.
17. Chintalacharuvu SR, Wang JX, Giaconia JM, Venkataraman C. An essential role for CCL3 in the development of collagen antibody-induced arthritis. Immunol Lett. 2005;100(2):202–4.
18. Patel DD, Zachariah JP, Whichard LP. CXCR3 and CCR5 ligands in rheumatoid arthritis synovium. Clin Immunol 2001; 98(1):39–45.
19. Pokkali S, Das SD. Augmented chemokine levels and chemokine receptor expression on immune cells during pulmonary tuberculosis. Hum Immunol. 2009;70(2):110–5.
20. Ribeiro S, Horuk R. The clinical potential of chemokine receptor antagonists. Pharmacol Ther. 2005;107(1):44–58.
21. Vierboom MP, Zavodny PJ, Chou CC, Tagat JR, Pugliese-Sivo C, Strizki J, et al. Inhibition of the development of collagen-induced arthritis in rhesus monkeys by a small molecular weight antagonist of CCR5. Arthritis Rheum. 2005;52(2):627–36.
22. Szekanecz Z, Kim J, Koch AE. Chemokines and chemokine receptors in rheumatoid arthritis. Semin Immunol. 2003;15(1): 15–21.
23. Patterson AM, Schmutz C, Davis S, Gardner L, Ashton BA, Middleton J. Differential binding of chemokines to macrophages and neutrophils in the human inflamed synovium. Arthritis Res. 2002;4(3):209–14.
24. Hayashida K, Nanki T, Girschick H, Yavuz S, Ochi T, Lipsky PE. Synovial stromal cells from rheumatoid arthritis patients attract monocytes by producing MCP-1 and IL-8. Arthritis Res. 2001; 3(2):118–26.
25. Hogan M, Sherry B, Ritchlin C, Fabre M, Winchester R, Cerami A, et al. Differential expression of the small inducible cytokines GRO alpha and GRO beta by synovial fibroblasts in chronic arthritis: possible role in growth regulation. Cytokine. 1994;6(1): 61–9.
26. Seitz M, Dewald B, Ceska M, Gerber N, Baggiolini M. Interleukin-8 in inflammatory rheumatic diseases: synovial fluid levels, relation to rheumatoid factors, production by mononuclear cells, and effects of gold sodium thiomalate and methotrexate. Rheumatol Int. 1992;12(4):159–64.
27. Koch AE, Kunkel SL, Burrows JC, Evanoff HL, Haines GK, Pope RM, et al. Synovial tissue macrophage as a source of the chemotactic cytokine IL-8. J Immunol. 1991;147(7):2187–95.
28. Endo H, Akahoshi T, Takagishi K, Kashiwazaki S, Matsushima K. Elevation of interleukin-8 (IL-8) levels in joint fluids of patients with rheumatoid arthritis and the induction by IL-8 of leukocyte infiltration and synovitis in rabbit joints. Lymphokine Cytokine Res. 1991;10(4):245–52.
29. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K. Essential involvement of interleukin-8 (IL-8) in acute inflammation. J Leukoc Biol. 1994;56(5):559–64.
30. Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science. 1992;258(5089):1798–801.
31. Yoo SA, Park BH, Park GS, Koh HS, Lee MS, Ryu SH, et al. Calcineurin is expressed and plays a critical role in inflammatory arthritis. J Immunol. 2006;177(4):2681–90.
32. Okamoto S, Mukaida N, Yasumoto K, Rice N, Ishikawa Y, Horiguchi H, et al. The interleukin-8 AP-1 and kB-like sites are genetic end targets of FK506-sensitive pathway accompanied by calcium mobilization. J Biol Chem. 1994;269:8582–9.
33. Simmonds RE, Foxwell BM. Signalling, inflammation and arthritis: NF-kappaB and its relevance to arthritis and inflammation. Rheumatology. 2008;47(5):584–90.
34. Du S, Hiramatsu N, Hayakawa K, Kasai A, Okamura M, Huang T, et al. Suppression of NF-kappaB by cyclosporin a and tacrolimus (FK506) via induction of the C/EBP family: implication for unfolded protein response. J Immunol. 2009;182(11):7201–11.

Clin Rheumatol (2012) 31:1713–1721
DOI 10.1007/s10067-012-2082-5

ORIGINAL ARTICLE

Efficacy, pharmacokinetics, and safety of adalimumab in pediatric patients with juvenile idiopathic arthritis in Japan

Tomoyuki Imagawa · Syuji Takei · Hiroaki Umebayashi · Kenichi Yamaguchi · Yasuhiko Itoh · Toshinao Kawai · Naomi Iwata · Takuji Murata · Ikuo Okafuji · Mari Miyoshi · Yasuhiro Onoe · Yoshifumi Kawano · Noriko Kinjo · Masaaki Mori · Neelufar Mozaffarian · Hartmut Kupper · Sourav Santra · Gina Patel · Shinichi Kawai · Shumpei Yokota

Received: 12 April 2012 /Revised: 1 August 2012 /Accepted: 28 August 2012 /Published online: 2 October 2012

Abstract The objective of this study was to evaluate the efficacy, pharmacokinetics, and safety of adalimumab in patients with polyarticular juvenile idiopathic arthritis (JIA) in Japan. Patients aged 4 to 17 years were enrolled in a single-arm, open-label, multicentre study of adalimumab. Patients weighing <30 kg received 20 mg every other week (eow), and those ≥30 kg received 40 mg eow. Concomitant methotrexate (MTX) was allowed (≤10 mg/m^2 per week). The

T. Imagawa · M. Mori · S. Yokota
Yokohama City University,
Yokohama, Japan

S. Takei
Kagoshima University,
Kagoshima, Japan

H. Umebayashi
Miyagi Children's Hospital,
Miyagi, Japan

K. Yamaguchi
St. Luke's International Hospital,
Tokyo, Japan

Y. Itoh
Nippon Medical School Hospital,
Tokyo, Japan

T. Kawai
National Center for Child Health and Development,
Tokyo, Japan

N. Iwata
Aichi Children's Health and Medical Center,
Aichi, Japan

T. Murata
Osaka Medical College Hospital,
Takatsuki City, Japan

I. Okafuji
Kobe City Medical Center General Hospital,
Hyogo, Japan

M. Miyoshi
Hyogo Prefectural Kobe Children's Hospital,
Hyogo, Japan

Y. Onoe
National Hospital Organization Kokura Medical Center,
Kitakyushu, Japan

Y. Kawano
Kagoshima University Medical and Dental Hospital,
Kagoshima, Japan

N. Kinjo
Hospital, University of the Ryukyus,
Okinawa, Japan

N. Mozaffarian · G. Patel
Abbott Laboratories,
Abbott Park, IL, USA

H. Kupper
Abbott GmbH & Co. KG,
Ludwigshafen, Germany

S. Santra
GlaxoSmithKline Pharmaceuticals, Limited,
Bangalore, India

S. Kawai
Toho University School of Medicine,
Tokyo, Japan

T. Imagawa (✉)
Department of Pediatrics,
Yokohama City University School of Medicine,
3-9 Fukuura, Kanazawa-ku,
Yokohama, Kanagawa 236-0004, Japan
e-mail: timagawa@ca2.so-net.ne.jp

primary efficacy outcome was the percent of patients with American College of Rheumatology Pediatric 30 response (ACR Pedi 30) at week 16. JIA core variables, serum adalimumab concentrations, and anti-adalimumab antibodies (AAAs) were analysed. Patients were monitored for adverse events (AEs). Twenty-five patients (20 with concomitant MTX at baseline and 5 without) were enrolled: 24 patients completed 16 weeks of therapy and 22 patients completed 60 weeks. At week 16, 90 % of patients with MTX and 100 % without MTX achieved ACR Pedi 30; response rates were maintained through week 60 in 94 and 80 % of patients, respectively. Each JIA core variable improved over time. Six patients became AAA positive (two each at weeks 8, 16, and 60), some of which were transient. All six AAA-positive patients achieved ACR Pedi 30 at week 16, and four maintained that response at week 60. Six patients (all with MTX) experienced nine serious AEs (JIA, pyrexia, arthralgia, pneumonia, hepatitis B infection, pharyngitis, dehydration, pharyngeal pain, and pneumonia). In pediatric patients with polyarticular JIA in Japan, adalimumab was safe and effective for reducing disease activity for up to 60 weeks.

Keywords Adalimumab · Juvenile idiopathic arthritis · Methotrexate · Pharmacokinetics

Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children <16 years of age [1] and often causes significant pain, functional impairment, and diminished health-related quality of life [2–5]. The incidence of JIA in Japan is estimated to be 10 to 15 per 100,000 children [6]. The overall incidence in developed countries is estimated to range from 16 to 150 per 100,000 children [1]. Of the JIA subtypes, the incidence of polyarticular JIA, which affects five or more joints, has been reported to be relatively high among children in Japan, whereas pauciarticular JIA, which affects four or less joints, is more common in Europe and the USA [6].

The Pediatric Standing Committee of the Japan College of Rheumatology and the Pediatric Rheumatology Association of Japan collaborated to produce guidelines for the diagnosis and treatment of JIA in Japan, with an emphasis on the involvement of pediatric rheumatologists, early intervention, and individualised therapy [6]. For articular JIA, nonsteroidal anti-inflammatory drugs (NSAIDs) are the first-line therapy of choice, followed by disease-modifying antirheumatic drugs (DMARDs) such as methotrexate (MTX) and oral corticosteroids [6]. Patients with JIA often have persistent disease. Consequently, the use of these agents may be limited by long-term toxicity, including potential effects on growth, partial/inadequate responses, and inability to maintain disease remission [7–9]. Short-term toxicities and drug intolerance are also an issue in this population. Some patients with JIA may benefit from treatment with biologic agents. Further guidance on this topic was recently published by the American College of Rheumatology (ACR) and is under development elsewhere [10, 11]. Availability of biologic therapies, including the anti-tumour necrosis factor (anti-TNF) agents adalimumab, etanercept, and infliximab, the T-cell inhibitor abatacept, and the humanised anti-human interleukin 6 receptor monoclonal antibody tocilizumab, represents a major advancement in the treatment of rheumatic diseases. Of these agents, etanercept, tocilizumab, and adalimumab are approved for JIA in Japan [11–13].

Adalimumab is a fully human monoclonal antibody specific to human TNF-alpha that is approved in the USA (February 2008) and Europe (September 2008) for the treatment of polyarticular JIA and was recently approved for JIA in Japan [13–15]. TNF, involved in normal inflammatory and immune responses, is also elevated in the synovial fluid of JIA patients. Adalimumab binds specifically to TNF-alpha and blocks its interaction with cell surface TNF receptors. The relationship between the pharmacodynamic action of adalimumab and the mechanism(s) by which adalimumab exerts its clinical effects is unknown but has been hypothesized to involve significant inhibition of the pathologic inflammatory process. Adalimumab has been shown to be safe and effective in patients with JIA when dosed every other week (eow) for up to 3 years in Western populations [16]. Further, these patients maintained clinical responses and improvements in disease activity for up to 6 years, with no deaths or malignancies reported [17]. The primary objective of this study was to evaluate the efficacy, pharmacokinetics, and safety of adalimumab therapy in pediatric patients with polyarticular JIA in Japan.

Materials and methods

Patients

Main inclusion criteria

Pediatric patients aged 4 to 17 years with a diagnosis of polyarticular JIA by ACR criteria at screening, and active disease defined as five or more swollen joints (not due to deformity) and three or more joints with limitation of passive motion due to pain and/or pain by pressure (tenderness), were eligible. Patients could have had systemic, polyarticular, or pauciarticular JIA at disease onset but were required to be free of any systemic JIA manifestations

for at least 12 weeks before screening. All patients were to have had inadequate clinical response (or intolerance) to NSAIDs or MTX (8–10 mg per 1 m^2 of body surface area per week for 12 weeks or more), or other conventional therapies for JIA such as corticosteroids. Negative urine pregnancy tests at screening and baseline were required for all postpubertal females; use of adequate contraception during the study and for 150 days after last injection was required for all sexually active patients.

Main exclusion criteria

Patients with a history of or comorbid inflammatory joint disease other than JIA, including psoriatic arthritis, arthritis related to enthesopathy, and systematic lupus erythematosus, were excluded. Patients classified as functional class IV by ACR criteria, prior treatment with alkylating agents or biologic therapies for JIA, joint surgery within 8 weeks prior to screening, or articular corticosteroids or sodium hyaluronate within 28 days prior to baseline were also excluded. Other exclusion criteria included the following: significant cardiac disease; abnormal laboratory values; positive serology for anti-human immunodeficiency virus antibody, hepatitis B surface antigen, or hepatitis C antibody; history of a central nervous system (CNS) neoplasm; active CNS infection, demyelinating disease, degenerative neurological disease, or any progressive CNS disease; ongoing chronic or active infection or any major episode of infection requiring hospitalisation or treatment with intravenous antibiotics within 28 days or oral antibiotics within 14 days prior to screening; history of Listeria infection; tuberculosis confirmed by chest X-ray or chest computed tomography at screening or confirmed history of tuberculosis; history of cancer, lymphoma, leukemia, or lymphoproliferative disease other than a successfully treated nonmetastatic cutaneous squamous cell or basal cell carcinoma and/or localised carcinoma in situ of the cervix; and vaccination with a live vaccine within 12 weeks of study drug administration through 70 days of the last dose.

Approved/prohibited medications

MTX was either to be discontinued at least 14 days prior to baseline or, for patients receiving a stable dose (≤10 mg/m^2 per week) for ≥12 weeks prior to baseline, could be continued at the stable dose until week 16. After week 16, dose reduction or discontinuation of MTX was permitted based on investigator's judgement. If MTX was reduced or interrupted, it could subsequently be increased or restarted as long as the dose was ≤10 mg/m^2 per week.

DMARDs other than MTX were to be discontinued at least 28 days before baseline. NSAIDs or low-dose corticosteroids (≤0.2 mg/kg of prednisone per day, up to a maximum of 10 mg/day, including subcutaneous or intramuscular injection) were permitted at stable doses. Pain medications were not to be taken within 12 h prior to the study visit joint evaluation. Intraarticular injection of corticosteroids or sodium hyaluronate was prohibited until week 16, after which time, one intraarticular injection of corticosteroid or sodium hyaluronate was permitted; however, the joint was considered nonevaluable for 12 weeks after injection. Use of immunosuppressants or leukocytapheresis, alkylating agents such as cyclophosphamide, and analgesic opium alkaloid/synthetic narcotics was prohibited.

Study design and ethics

This single-arm, open-label, multicentre study evaluating the efficacy, pharmacokinetics, and safety of fixed dose adalimumab in pediatric patients with polyarticular JIA was conducted at 14 sites in Japan from May 2008 to August 2010 (NCT00690573). After a 2- to 4-week screening period, patients received subcutaneous adalimumab injections eow. Based on pharmacokinetic simulations performed using body surface area-dependent dosing data from a Western (USA and Europe) clinical JIA study (NCT00048542) [16], a fixed dose dosing regimen was established in which two different doses of adalimumab were administered based on body weight. Patients weighing <30 kg received adalimumab 20 mg eow and patients weighing ≥30 kg received adalimumab 40 mg eow. Adalimumab dosage was re-evaluated based on actual body weight at week 16 and every 12 weeks after week 24 through week 60.

The study was conducted in accordance with the Declaration of Helsinki. In accordance with harmonised good clinical practices, institutional review boards approved the study protocol and the informed consent form. The patient or parent or legal guardian of the patient reviewed and voluntarily signed the informed consent form prior to involvement in any study-related procedures.

Clinical assessments

Clinical measures were assessed at weeks 2, 4, 8, 16, and 24 and every 12 weeks after week 24. The following JIA core criteria were used to calculate ACR Pedi scores: Physician's Global Assessment of disease activity (PhGA), Patient's Global Assessment of well-being (PaGA), active joint count in 73 joints (AJC73), limitation of motion in 69 joints (LOM69), Childhood Health Assessment Questionnaire (CHAQ) to assess physical function, and C-reactive protein (CRP) concentration. ACR Pediatric 30 (ACR Pedi 30) response was defined as ≥30 % improvement in at least three of six JIA core criteria and ≥30 % worsening in not more than one of six JIA core criteria. ACR Pedi 50/70/90 responses were defined similarly according to the respective

level of response for at least three of the six JIA core criteria and ≥30 % worsening in not more than one of six JIA core criteria.

Pharmacokinetics and immunogenicity

Blood samples for analysis of serum adalimumab concentrations and anti-adalimumab antibodies (AAAs) were collected at baseline and weeks 2, 4, 8, 16, 24, 36, 48, and 60. A validated enzyme-linked immunoadsorbent assay method based on a double-antigen technique was used to measure adalimumab and AAA concentrations. The lower limit of quantitation (LLOQ) for adalimumab was 3.13 ng/mL in diluted (1:10) serum or 31.3 ng/mL in undiluted serum. The LLOQ for AAAs was 1.0 ng/mL in diluted serum or 10 ng/mL in undiluted serum. Samples were considered AAA-positive if the measured AAA concentration was >20 ng/mL in undiluted serum, and the sample was collected within 30 days after an adalimumab dose. Serum samples with adalimumab concentrations >2 μg/mL were not analysed for AAAs.

Safety

The investigator monitored each patient for clinical and laboratory evidence of adverse events (AEs) on a routine basis throughout the study. AEs were also assessed at a follow-up visit 28 days after study completion and 70 days after the last dose of study drug (by site visit or telephone). Duration of exposure was calculated as the number of days between the first dose of study drug and the last dose plus 14 days.

Statistical analysis

As per the prespecified analysis plan, efficacy analyses were conducted on the full analysis set, which was defined as all patients who received at least one dose of study drug. The primary efficacy endpoint, ACR Pedi 30 response rate at week 16, was analysed with non-responder imputation (NRI), whereby patients with missing data at week 16 or who failed to meet ACR Pedi 30 response at week 16 were considered non-responders. Secondary endpoints, including longitudinal data for ACR Pedi 30/50/70/90 response rates and the JIA core variables from week 16 through week 60, were analysed using observed cases (i.e., only patients who were in the study at the time point being evaluated were included). Serum adalimumab and AAA concentrations were summarised descriptively. The numbers and percentages of patients who experienced AEs with an onset on or after the first adalimumab injection up to 70 days (five half-lives) after the last injection were summarised for the safety analysis set, which was identical to the full analysis set.

Table 1 Demographics and clinical characteristics at baseline

	With MTX (*N*=20)	Without MTX (*N*=5)	All adalimumab (*N*=25)
Age			
Mean (SD), years	13.2 (3.22)	12.6 (4.39)	13.0 (3.38)
4–12 years, *n* (%)	9 (45)	3 (60)	12 (48)
13–17 years, *n* (%)	11 (55)	2 (40)	13 (52)
Female, *n* (%)	15 (75)	5 (100)	20 (80)
Body weight			
Mean (SD), kg	40.5 (11.28)	35.3 (16.64)	39.5 (12.31)
≥30 kg, *n* (%)	14 (70)	3 (60)	17 (68)
Duration of JIA, mean (SD), years	4.8 (3.97)	4.2 (2.75)	4.7 (3.72)
RF positive, *n* (%)	14 (70)	3 (60)	17 (68)
Anti-CCP antibody, mean (SD), U/mL	105.5 (135.67)	8.5 (15.50)	86.1 (127.19)
LOM69, mean (SD)	8.6 (5.65)	5.8 (2.05)	8.0 (5.22)
AJC73, mean (SD)	12.0 (6.10)	13.6 (9.32)	12.3 (6.66)
CRP concentration			
Abnormal (>0.3 mg/dL), *n* (%)	11 (55)	3 (60)	14 (56)
Mean (SD), mg/dL	1.0 (1.32)	3.6 (3.86)	1.5 (2.22)
CHAQ (0–3), mean (SD)	0.8 (0.79)	0.7 (1.11)	0.8 (0.84)
PhGA (0–100 mm), mean (SD)	56.5 (18.49)	58.6 (25.83)	56.9 (19.56)
PaGA (0–100 mm), mean (SD)	44.6 (24.84)	48.6 (34.20)	45.4 (26.19)

AJC active joint count, *CCP* cyclic citrullinated protein, *CHAQ* Childhood Health Assessment Questionnaire, *CRP* C-reactive protein, *JIA* juvenile idiopathic arthritis, *LOM* limitation of motion, *MTX* methotrexate, *PaGA* Patient's Global Assessment, *PhGA* Physician's Global Assessment, *RF* rheumatoid factor, *SD* standard deviation

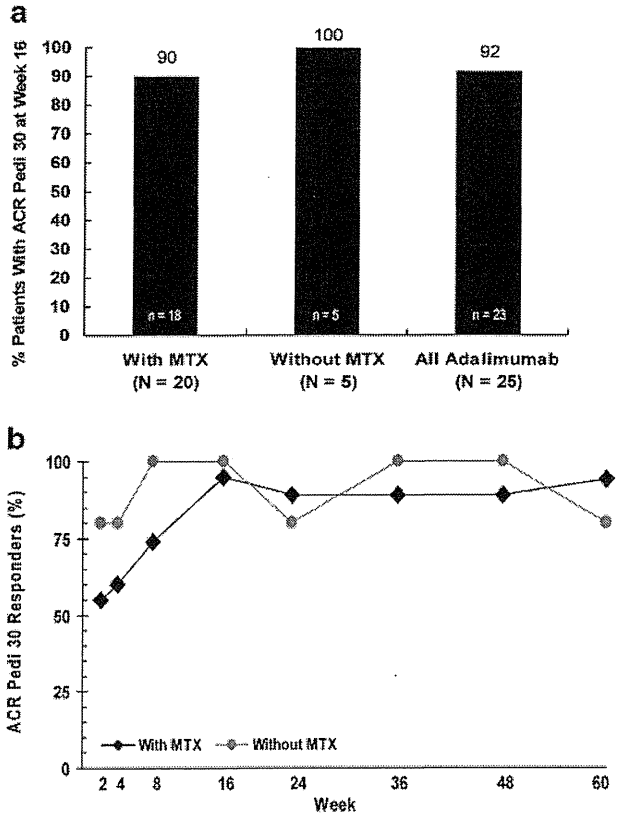


Fig. 1 ACR Pedi 30 response rates. **a** Primary efficacy outcome: ACR Pedi 30 response rates at week 16 of adalimumab therapy (NRI). **b** ACR Pedi 30 response rates over time with adalimumab therapy (as observed) (*black diamond* with MTX, *grey circle* without MTX)

Results

Patient disposition and duration of treatment

A total of 25 children with JIA were treated with adalimumab, of which 20 were receiving concomitant MTX at baseline. Twenty-four patients completed 24 weeks of therapy, including the primary endpoint assessment at week 16, and 22 patients completed up to 60 weeks. The three patients who discontinued the study, all due to lack of efficacy, were in the concomitant MTX group. The average duration of adalimumab treatment through week 60 was 396 days (390 days for patients receiving concomitant MTX at baseline and 421 days for patients without MTX).

Baseline demographics and clinical characteristics

At baseline, the mean age was approximately 13 years, 80 % of the patients were female, and 32 % weighed <30 kg (Table 1). For patients who received MTX (N=20) and those who did not (N=5), respectively, the duration of JIA was 4.8 and 4.2 years, 70 and 60 % were rheumatoid factor positive (RF positive), and 55 and 60 % had abnormal (>3 mg/dL) CRP concentrations. All patients had polyarticular JIA at disease onset, except for one patient (not receiving MTX) who had pauciarticular JIA at disease onset.

Baseline PhGA, PaGA, and CHAQ scores were similar between groups. Baseline anti-cyclic citrullinated protein (anti-CCP) antibody concentrations were considerably greater in patients who were receiving MTX compared with

Table 2 JIA core variables at weeks 16 and 60 of adalimumab therapy (as observed)

	Adalimumab with MTX			Adalimumab without MTX		
	Mean baseline value	Mean visit value	Mean % change	Mean baseline value	Mean visit value	Mean % change
Week 16						
PhGA (mm)	55.8	20.6	−64.8	58.6	10.6	−83.8
PaGA (mm)	44.7	22.1	−50.5	48.6	15.4	−74.5
AJC73	11.0	3.8	−59.9	13.6	3.2	−80.3
LOM69	7.5	3.7	−38.3	5.8	1.6	−76.7
CHAQ	0.7	0.4	−32.8	0.7	0.4	−35.7
CRP (mg/dL)	0.9	0.3	−23.8	3.6	1.7	−65.1
Week 60						
PhGA (mm)	55.0	16.7	−74.0	58.6	12.8	−81.5
PaGA (mm)	42.9	16.6	−51.7	48.6	27.0	−48.6
AJC73	11.1	1.9	−81.5	13.6	3.0	−79.8
LOM69	7.6	1.5	−78.7	5.8	2.4	−72.2
CHAQ	0.7	0.2	−46.9	0.7	0.5	−41.0
CRP (mg/dL)	1.0	0.4	−46.6	3.6	1.4	−4.0

AJC active joint count, *CHAQ* Childhood Health Assessment Questionnaire, *CRP* C-reactive protein, *LOM* limitation of motion, *MTX* methotrexate, *PaGA* Patient's Global Assessment, *PhGA*, Physician's Global Assessment

those not receiving MTX. All patients had received DMARDs previously. At baseline, all patients were receiving concomitant NSAIDs, and most patients were also receiving concomitant systemic corticosteroids (70 % in the MTX group and 80 % in the non-MTX group).

Efficacy

Primary endpoint: ACR Pedi 30 response

Overall, 92 % of patients achieved ACR Pedi 30 at week16 of adalimumab therapy (90 % in the MTX group and 100 % in the non-MTX group) (Fig. 1a). ACR Pedi 30 response at week 60 was observed for 94 % of patients with concomitant MTX and 80 % of patients without concomitant MTX (Fig. 1b).

ACR Pedi 50/70/90 responses

ACR Pedi 50/70 response rates at week 16 of adalimumab therapy were generally consistent with those at week 60, with approximately 90 and 75 % of patients achieving these levels of response. Overall, ACR Pedi 90 response rates increased from ≤20 % at week 16 to 50 % at week 60.

JIA core variables over time

Adalimumab therapy was associated with improvements in each of the six JIA core variables over time (Table 2). Mean decreases in disease activity generally started as early as week 2 (data not shown) and remained consistent with improvements observed through week 60.

Pharmacokinetics and immunogenicity

Mean adalimumab concentrations for patients who received adalimumab 40 mg eow plus MTX were 10.4 μg/mL (range, 0–19.4 μg/mL; *N*=14) at week 16 and 14.4 μg/mL (range, 0–21.6 μg/mL; *N*=14) at week 60 (Fig. 2a). Mean adalimumab concentrations for patients who received adalimumab 20 mg eow plus MTX were 6.73 μg/mL (range, 0–13.2 μg/mL; *N*=6) at week 16 and 14.3 μg/mL (range, 0–24.6 μg/mL; *N*=6) at week 60; two of the six patients initially receiving the 20-mg dosage increased to 40 mg by week 60 due to changes in body weight. For patients receiving adalimumab without MTX, mean adalimumab concentrations were consistent with mean concentrations in patients receiving adalimumab plus MTX (Fig. 2b).

At week 24 of adalimumab therapy, 16 % (4 of 25) patients had at least one AAA-positive serum sample (3 of 20 with MTX [15 %] and 1 of 5 without MTX [20 %]). At week 60, 15 % (3 of 20) of patients receiving adalimumab plus MTX and 60 % (3 of 5) of patients receiving adalimumab without

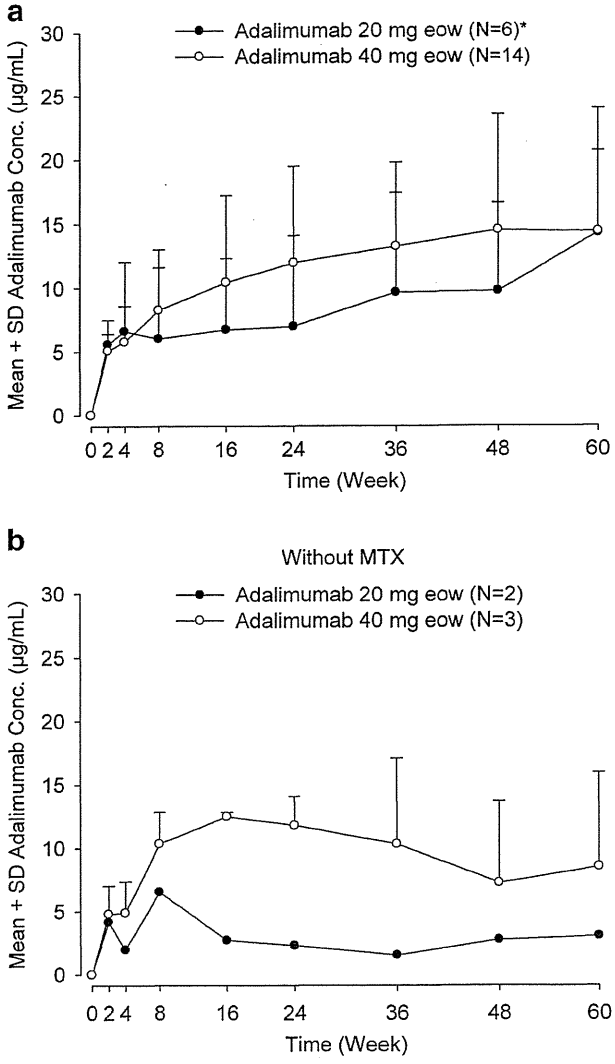


Fig. 2 Serum adalimumab concentrations. **a** Mean concentration for all patients (*N*=20) with concomitant MTX by dosage of adalimumab (*black circle* 20 mg eow, *open circle* 40 mg eow). **b** Mean concentration in patients without concomitant MTX (*N*=5) by dosage of adalimumab (*black circle* 20 mg eow, *open circle* 40 mg eow). *Asterisk* Adalimumab dosages were increased from 20 to 40 mg eow at week 16 for two patients, at week 36 for one patient, and at week 48 for one patient owing to body weight increases (<30 kg at baseline to ≥30 kg at week 16), as per protocol

MTX had detectable AAAs (6 of 25 patients [24 %] overall). Two patients first had detectable AAAs at week 8, two at week 16, and two at week 60. Of these six patients, three patients were receiving the 20-mg dosage (one with MTX and two without MTX) and three were receiving the 40 mg dosage (two with MTX and one without MTX).

Trough concentrations of adalimumab <2 g/mL were transient for three of the four patients in whom AAAs were detected prior to week 60. For two of these four patients (one with MTX and one without MTX), the lack of

measurable serum adalimumab at a given study visit was followed by detectable adalimumab concentrations that increased at later time points through week 60. Another patient (with MTX) had AAAs detected at week 8 but had measurable adalimumab concentrations at weeks 36 and 48 following dosage increase to 40 mg eow at week 16; the patient's adalimumab concentration was again undetectable at week 60. The remaining patient (with MTX) had undetectable adalimumab concentrations from week 8 to week 60. All six patients with AAA at any time point achieved ACR Pedi 30 response at week 16 and completed the study; four of the six patients exhibited ACR Pedi 30 response at week 60.

Safety

Through week 60, all patients had experienced at least one AE (Table 3). Six patients (all with concurrent MTX) experienced nine serious AEs as follows: JIA, pyrexia, and arthralgia; pneumonia; hepatitis B infection; pharyngitis; dehydration, pharyngeal pain, and pyrexia. Consistent with study exclusion criteria, the patient with hepatitis B infection had been negative for hepatitis Bs antigen and antibodies against HBs and HBc at the start of the trial; no risk factors for transmission (i.e., travel to endemic areas, injection drug use, or other exposure) were reported, and the patient discontinued the study because of this event. Of the six patients with serious AEs, four occurred in AAA-negative patients and two occurred in AAA-positive patients (one with serious AE of JIA; one with serious AEs of dehydration, pharyngeal pain, and pneumonia).

AEs of special interest

Infections occurred in 80 % of patients receiving MTX and 100 % of patients not receiving MTX, of which three in the MTX group were serious (acute pharyngitis, pneumonia, and hepatitis B infection). Six patients had an injection-site reaction (erythema, swelling, warmth, all in the MTX group) and three had a hepatic event (two in the MTX group, hepatic function abnormal and blood alkaline phosphatase increased; one in the non-MTX group, hepatic function abnormal). No deaths, malignancies, opportunistic infections, congestive heart failure, demyelinating disease, allergic reactions, lupus-like syndrome, or blood dyscrasias were reported in any patient through week 60 of the study.

Discussion

Polyarticular JIA with RF positivity, which represents approximately 5 % of patients with JIA, tends to be more aggressive than RF-negative polyarticular JIA and is associated with early joint destruction and disability [18, 19]. In addition, RF-positive JIA patients have been found to be less likely to achieve a state of disease inactivity and spend more time in an active disease state compared with RF-negative patients [20]. In the present study, 68 % of patients were RF positive, thus representing a population of patients with potentially severe and difficult-to-treat JIA who are candidates for intensive therapy. Results of this study demonstrate the efficacy of adalimumab in reducing disease activity and maintaining response through 60 weeks of therapy in pediatric patients with polyarticular JIA in Japan. Because the majority of patients in the study were receiving concomitant MTX, firm conclusions regarding the efficacy of adalimumab monotherapy vs. combination therapy are limited; however, adalimumab-treated patients receiving combination therapy with MTX in the Western JIA study had numerically higher ACR Pedi 30, 50, 70, and 90 responses compared with those not receiving MTX [16].

AAAs may develop in some patients during adalimumab therapy. In pediatric patients with JIA in Japan, an AAA-positive result was not associated with study discontinuation or lack of efficacy; all six patients with AAAs completed the study, and four achieved at least ACR Pedi 30 at week 60. AAA positive rates in pediatric patients on concomitant MTX were numerically different, with a higher rate in

Table 3 Patients with treatment-emergent adverse events

Adverse Event	Adalimumab with MTX (*N*=20) *n* (%)	Adalimumab without MTX (*N*=5) *n* (%)	All adalimumab (*N*=25) *n* (%)
Any AE	20 (100)	5 (100)	25 (100)
Any severe AE	0	0	0
Any AE leading to discontinuation of study drug	1 (5)	0	1 (4)
Any serious AE	6 (30)	0	6 (24)
Any infectious AE	16 (80)	5 (100)	21 (84)
Any serious infectious AE	3 (15)	0	3 (12)
Injection-site reaction	6 (30)	0	6 (24)
Hepatic-related AE	2 (10)	1 (20)	3 (12)

AE adverse event, *MTX* methotrexate

Japanese vs. Western patients, 15 % (3/20) vs. 5.9 % (5/85), respectively [16]. JIA rates appear consistent with rates observed in Japanese and Western adult RA populations; however, limited pediatric data preclude any correlation with adult RA. In this study, the overall sample size, particularly of the non-MTX group, was too small to make any meaningful comparison of AAA rates to the Western JIA study population.

The similarity of the inclusion criteria between the present study conducted in Japan and the Western JIA study [16] allows for comparison of the results. Disease duration (approximately 4 years) and baseline JIA core criteria generally were similar between patients in both studies; however, a greater percentage of patients in Japan were RF positive at baseline and had received prior DMARD therapy. Notably, ACR Pedi 30 rates at week 16 for patients receiving adalimumab plus MTX were comparable between both studies, with >90 % of patients in each study achieving this efficacy endpoint.

In adult RA, the overall safety profile of adalimumab in Japanese studies is consistent with that observed in Western studies [21–23]. Similarly, the safety profile of adalimumab in patients with polyarticular JIA in Japan was as expected, based on the safety profile observed in the Western JIA study [16], and no new AEs of interest were observed. Although infections were the most common AE of interest in the present study, no opportunistic infections or tuberculosis were reported through 60 weeks of adalimumab therapy. One event of hepatitis B infection was reported in the present study, and hepatitis B evaluation is recommended for patients receiving biologic therapy.

In addition to adalimumab, two other biologic agents (etanercept and tocilizumab) are approved for the treatment of JIA in Japan. Although head-to-head trials have not been conducted, clinical efficacy as measured by ACR Pedi 30 response rates appears to be relatively similar among these drugs. In a study of DMARD-refractory polyarticular JIA in Japan, etanercept treatment resulted in an ACR Pedi 30 response rate of 91 % at week 12 of the primary study and 94 % at week 96 of the open-label extension [12]. For the anti-interleukin-6 receptor antibody tocilizumab, approved for DMARD-refractory polyarticular JIA and systemic JIA [11], the ACR Pedi 30 response rate was 95 % at week 12 and 100 % at week 48 [24].

Limitations of this study include the open-label study design and small sample size, especially for patients not receiving MTX. In addition, the effect of adalimumab on radiographic progression could not be assessed because X-ray data were not collected. Although the absence of valid and reliable radiographic scoring systems in children with JIA is the primary reason radiographic outcomes have not been included in pivotal JIA trials, measures such as the adapted Sharp-van der Heijde score have been validated and are recommended for use in future pediatric JIA studies [25, 26].

The efficacy, safety, and tolerability of adalimumab were demonstrated and maintained through 60 weeks of therapy in patients with JIA in Japan. The results of the present study were similar to those from a JIA study conducted in the USA and Europe [16]. AAA-positive rates were numerically greater in JIA patients in Japan; however, the development of these antibodies was not associated with study discontinuation or with lack of efficacy. In conclusion, fixed-dose adalimumab (20 or 40 mg eow) was effective in reducing disease activity in pediatric patients with polyarticular JIA in Japan.

Acknowledgments Cathryn M. Carter, MS, of Arbor Communications, Inc., funded by Abbott, and Kathleen V. Kastenholz, PharmD, MS, of Abbott, drafted and revised the manuscript. Abbott Laboratories and Eisai Co., Ltd. funded this study (NCT00690573).

Conflict of interest TI has received research grants from Abbott/Eisai and Novartis, consulting fees from Abbott/Eisai, and speaker's fees from Abbott/Eisai, Chugai, and Mitsubishi Pharma; ST has received research grants, consulting fees, and/or speaker's fees from Eisai, Chugai, Takeda, Bristol-Myers KK, Teijin, Pfizer, Mylan, Mitsubishi Tanebe, Asahi Kasei, and Astellas; HU has received indirect research grants from Abbott and Eisai and speaker's fees from Pfizer and Chugai; KY has received research grants from Eisai/Abbott; YI has received research grants, consulting fees, and/or speaker's fees from Abbott, Eisai, and Chugai; TK has received research grants from Abbott, Eisai, and Chugai; NI has received research grants and speaker's fees from Eisai/Abbott; TM has received research grants from Eisai/Abbott; IO has received research grants from Abbott and Eisai; MMiyoshi has received research grants from Abbott and Eisai; YO has received research grants from Abbott; YK has received indirect research grants from Eisai and Chugai; NK has received research grants from Abbott, Eisai, and Chugai; MMori has received research grants from Abbott, Eisai, Pfizer, and MSD, consulting fees from Pfizer and MSD, and speaker's fees from Abbott, Pfizer, Chugai, Dainippon Sumitomo, and Astellas; SS was an employee of Abbott at the time of manuscript preparation and submission and may hold Abbott stock; he is currently an employee of GlaxoSmithKline and may hold GSK stock or options; SK has received research grants, consulting fees, and/or speaker's fees from Abbott, Astellas, Bristol-Myers Squibb, Chugai, Daiichi-Sankyo, Eisai, Hisamitsu, Janssen, Mitsubishi-Tanabe, Pfizer, Santen, and Takeda; SY has received research grants from Abbott/Eisai and Novartis, consulting fees from Abbott/Eisai and Chugai, and speaker's fees from Chugai, Novartis, and GSK; NM, HK, and GP are employees of Abbott and may hold Abbott stock or options.

References

1. Ravelli A, Martini A (2007) Juvenile idiopathic arthritis. Lancet 369:767–778
2. Shaw KL, Southwood TR, Duffy CM, McDonagh JE (2006) Health-related quality of life in adolescents with juvenile idiopathic arthritis. Arthritis Rheum 55:199–207

3. Oliveira S, Ravelli A, Pistorio A, Castell E, Malattia C, Prieur AM, Pediatric Rheumatology International Trials Organization (PRINTO) et al (2007) Proxy-reported health-related quality of life of patients with juvenile idiopathic arthritis: the Pediatric Rheumatology International Trials Organization multinational quality of life cohort study. Arthritis Rheum 57:35–43
4. Amine B, Rostom S, Benbouazza K, Abouqal R, Hajjaj-Hassouni N (2009) Health related quality of life survey about children and adolescents with juvenile idiopathic arthritis. Rheumatol Int 29:275–279
5. Feinstein AB, Forman EM, Masuda A, Cohen LL, Herbert JD, Moorthy LN, Goldsmith DP (2011) Pain intensity, psychological inflexibility, and acceptance of pain as predictors of functioning in adolescents with juvenile idiopathic arthritis: a preliminary investigation. J Clin Psychol Med Settings 18:291–298
6. Yokota S, Mori M, Imagawa T, Takei S, Murata T, Tomiita M, Ito Y, Fujikawa S (2007) Proposal for juvenile idiopathic arthritis guidance on diagnosis and treatment for primary care pediatricians and nonpediatric rheumatologists. Mod Rheumatol 17:353–363
7. Hashkes PJ, Laxer RM (2005) Medical treatment of juvenile idiopathic arthritis. JAMA 294:1671–1684
8. Flatø B, Vinje O, Forre O (1998) Toxicity of antirheumatic and antiinflammatory drugs in children. Clin Rheumatol 17:505–510
9. Wallace CA (1998) The use of methotrexate in childhood rheumatic diseases. Arthritis Rheum 41:381–391
10. Beukelman T, Patkar NM, Saag KG, Tolleson-Rinehart S, Cron RQ, DeWitt EM et al (2011) 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. Arthritis Care Res (Hoboken) 63:465–482
11. Yokota S, Imagawa T, Takei S, Murata T, Tomiita M, Itoh Y, Fujikawa S, Mori M (2011) Guidance on using tocilizumab for juvenile idiopathic arthritis. Mod Rheumatol 21:563–571
12. Mori M, Takei S, Imagawa T, Imanaka H, Nerome Y, Kurosawa R et al (2011) Etanercept in the treatment of disease-modifying antirheumatic drug (DMARD)-refractory polyarticular course juvenile idiopathic arthritis: experience from Japanese clinical trials. Mod Rheumatol 21:572–578
13. Humira [Japanese Prescribing Information] (2011) Abbott Japan Co., Ltd. Mita, Minato-Ku, Tokyo. http://www.info.pmda.go.jp/go/pack/3999426G1024_1_13/. Accessed 01 Aug 2012
14. Humira [Prescribing Information] (2012) Abbott Laboratories. http://www.rxabbott.com/pdf/humira.pdf. Accessed 01 Aug 2012
15. Humira [Summary of Product Characteristics] (2012) Abbott Biotechnology Deutschland GmbH. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000481/WC500050870.pdf. Accessed 01 Aug 2012
16. Lovell D, Ruperto N, Goodman S, Reiff A, Jung L, Jarosova K, Pediatric Rheumatology Collaborative Study Group, Pediatric Rheumatology International Trials Organization et al (2008) Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. N Engl J Med 359:810–820
17. Lovell DJ, Ruperto N, Reiff A, Jung LK, Higgins G, Kone-Paut I et al (2011) Long-term efficacy and safety of adalimumab for up to 6 years in patients with juvenile idiopathic arthritis. Arthritis Rheum 63(10 Suppl):S99
18. Goldzweig O, Hashkes PJ (2011) Abatacept in the treatment of polyarticular JIA: development, clinical utility, and place in therapy. Drug Des Devel Ther 5:61–70
19. Wallace CA, Levinson JE (1991) Juvenile rheumatoid arthritis: outcome and treatment for the 1990s. Rheum Dis Clin North Am 17:891–905
20. Ringold S, Seidel KD, Koepsell TD, Wallace CA (2009) Inactive disease in polyarticular juvenile idiopathic arthritis: current patterns and associations. Rheumatology (Oxford) 48:972–977
21. Miyasaka N, CHANGE Study Investigators (2008) Clinical investigation in highly disease-affected rheumatoid arthritis patients in Japan with adalimumab applying standard and general evaluation: the CHANGE study. Mod Rheumatol 18:252–262
22. Burmester GR, Mease P, Dijkmans BA, Gordon K, Lovell D, Panaccione R, Perez J, Pangan AL (2009) Adalimumab safety and mortality rates from global clinical trials of six immune-mediated inflammatory diseases. Ann Rheum Dis 68:1863–1869
23. Schiff MH, Burmester GR, Kent JD, Pangan AL, Kupper H, Fitzpatrick SB, Donovan C (2006) Safety analyses of adalimumab (HUMIRA) in global clinical trials and US postmarketing surveillance of patients with rheumatoid arthritis. Ann Rheum Dis 65:889–894
24. Imagawa T, Yokota S, Mori M, Miyamae T, Takei S, Imanaka H et al (2012) Safety and efficacy of tocilizumab, an anti-IL-6-receptor monoclonal antibody, in patients with polyarticular-course juvenile idiopathic arthritis. Mod Rheumatol 22:109–115
25. Ravelli A (2008) The time has come to include assessment of radiographic progression in juvenile idiopathic arthritis clinical trials. J Rheumatol 35:553–557
26. Ravelli A, Ioseliani M, Norambuena X, Sato J, Pistorio A, Rossi F et al (2007) Adapted versions of the Sharp-van der Heijde score are reliable and valid for the assessment of radiographic progression in juvenile idiopathic arthritis. Arthritis Rheum 56: 3087–3095

Mod Rheumatol (2013) 23:8–18
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

Nahoko Tanaka · Natsuko Kusunoki · Yoshie Kusunoki · Tomoko Hasunuma · Shinichi Kawai

Received: 9 November 2011 / Accepted: 14 February 2012 / Published online: 21 March 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

N. Tanaka · N. Kusunoki · Y. Kusunoki · T. Hasunuma · S. Kawai (✉)
Division of Rheumatology, Department of Internal Medicine, School of Medicine, Faculty of Medicine, Toho University, 6-11-1 Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan
e-mail: skawai@med.toho-u.ac.jp

T. Hasunuma
Research Center for Clinical Pharmacology, Kitasato University, Tokyo, Japan

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.

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×*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)]

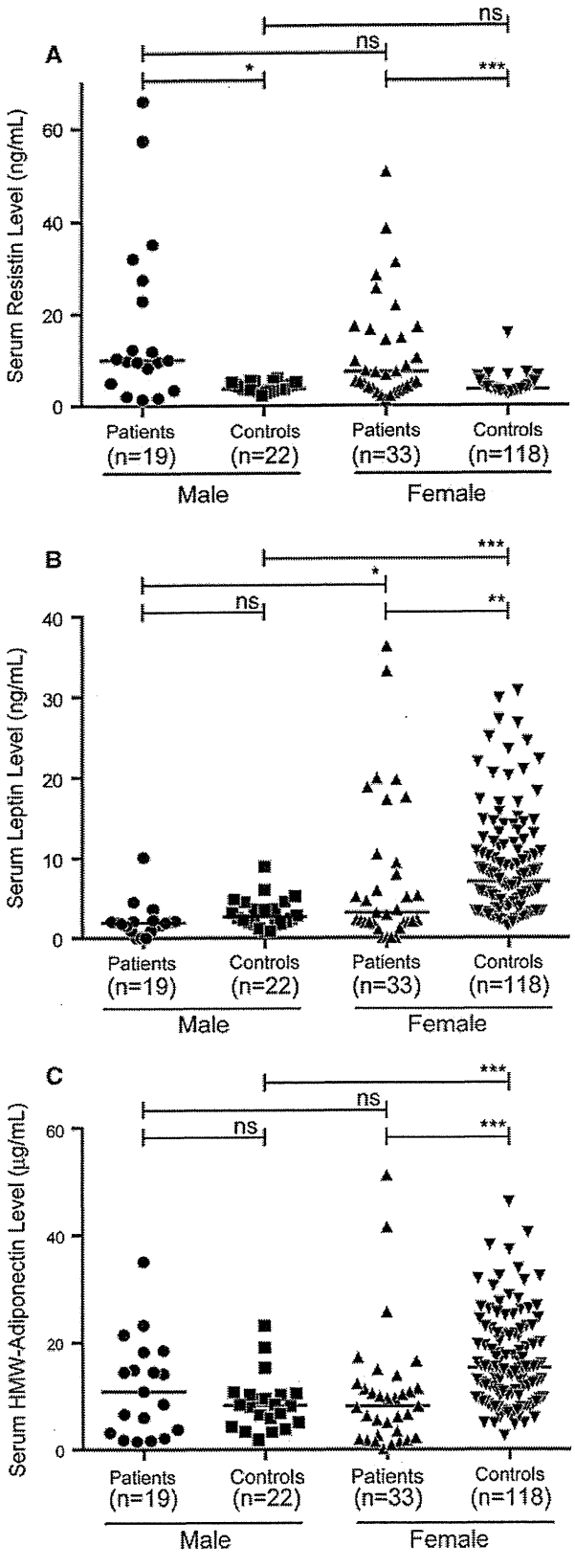
[c] Azathioprine (n = 1), cyclophosphamide (n = 14), cyclosporin A (n = 3), methotrexate (n = 4), or tacrolimus (n = 2) was administered 2–4 weeks after glucocorticoid therapy

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