Proinflammatory Cytokine Profiles in Sera and Pathological Tissues of Patients with Active Untreated Adult Onset Still's Disease

DER-YUAN CHEN, JOUNG-LIANG LAN, FANG-JU LIN, and TSU-YI HSIEH

ABSTRACT. ***Objective.*** To investigate concentrations of proinflammatory cytokines in the sera and their mRNA expression in biopsy specimens of evanescent rash and synovitis from patients with active untreated adult onset Still's disease (AOSD).

Methods. We measured serum levels of interleukin 6 (IL-6), IL-8, and tumor necrosis factor (TNF-α) by immunochemiluminescence method and serum IL-18 levels by ELISA in 50 patients with active untreated AOSD, 20 patients with active rheumatoid arthritis (RA), and 20 healthy controls. Multivariate analysis was used to evaluate the correlation between serum cytokine levels and disease activity and clinical features of AOSD. We also evaluated the expression of cytokine transcripts by real-time quantitative polymerase chain reaction in biopsy specimens of evanescent rash and synovitis from 12 patients with active untreated AOSD.

Results. Significantly higher levels of IL-6, IL-8, IL-18, and TNF-α in sera were found in patients with active untreated AOSD compared to healthy controls. Serum levels of IL-6 and IL-18 correlated well with clinical activity score of AOSD patients. Multiple logistic regression analysis showed that serum IL-6 level was a possible predictor for the occurrence of evanescent rash ($p = 0.0593$), serum IL-8 level was a significant predictor of persistent arthritis, and serum IL-18 level predicted occurrence of liver dysfunction. The levels of mRNA expression of IL-6, IL-18, and IL-8 were significantly higher in the biopsy tissue of Still's rash from AOSD patients compared with those in controls. Levels of mRNA expression of IL-18, IL-8, and TNF-α were significantly higher in the synovial membranes of AOSD patients compared with those in osteoarthritis controls. Significantly lower levels of TNF-α and IL-8 were found in the sera and in the synovial membranes of AOSD patients compared with those in RA patients. AOSD patients who had a chronic articular course had significantly higher levels of serum IL-8 compared with those who had a monocyclic systemic course.

Conclusion. Significantly higher levels of IL-6, IL-8, IL-18, and TNF-α were seen in both sera and pathological tissues of patients with active AOSD. The associations between levels of cytokine profile and distinct clinical manifestations and various patterns of disease course suggest the heterogeneity of pathogenesis in AOSD. (J Rheumatol 2004;31:2189–98)

Key Indexing Terms:
ADULT ONSET STILL'S DISEASE
CYTOKINE
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QUANTITATIVE POLYMERASE CHAIN REACTION

Adult onset Still's disease (AOSD) is an inflammatory disorder characterized by high spiking fever, evanescent rash, arthralgia or arthritis, sore throat, hepatosplenomegaly, variable multisystemic involvement, and laboratory abnormalities that include neutrophilic leukocytosis, abnormal liver function tests, increase in acute phase reactants, and elevated concentrations of serum ferritin[1,2]. Although studies have shown that serum ferritin level has a diagnostic value and can be used as an indicator of systemic disease activity in AOSD[3], little is known about the pathogenesis of AOSD and the factors responsible for its clinical features.

Several proinflammatory cytokines in AOSD have been investigated, but only to a limited extent[4-8]. Scheinberg, *et al* postulated interleukin 6 (IL-6) may be a possible marker of disease activity in AOSD[4]. Hoshino, *et al* also found that serum levels of IL-6, tumor necrosis factor (TNF-α), and interferon-γ (IFN-γ) were significantly increased in 12 patients with active AOSD compared with healthy controls,

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with IL-6 having the strongest associations with both articular and systemic manifestations[5]. A recent study showed that IL-18 elevation in active AOSD was much greater than that found in patients with rheumatoid arthritis (RA), and serum IL-18 levels were correlated with serum ferritin values and disease severity in AOSD, suggesting that IL-18 has a central role in the pathogenesis of this disease[6]. Fujii, *et al* reported serum levels of IL-18 were correlated with disease activity and C-reactive protein (CRP) values in chronic articular AOSD[7]. However, most of the cytokines are produced near their sites of action: quantification of cytokine gene expression at inflammatory sites would give a better reflection of *in vivo* cytokine profile.

Our aim was to determine and compare the serum concentrations of IL-6, IL-8, IL-18, and TNF-α in 50 patients with active untreated AOSD, in 20 patients with active RA, and in 20 healthy controls. We chose the RA patients as disease controls because several studies have documented the proinflammatory cytokine profile in this disease[9-11] and the similarity between articular manifestations in RA and chronic articular AOSD. Logistic regression was used to evaluate the simultaneous effects of serum cytokines on the occurrence of clinical features in patients with AOSD. To determine the *in vivo* involvement of cytokines in AOSD, we also investigated the expression of cytokine mRNA in biopsy specimens of Still's rash and synovitis from 12 patients with active untreated AOSD using a real-time quantitative polymerase chain reaction (RQ-PCR) technique. The changes in serum levels of cytokines of patients with treated AOSD were also studied during longitudinal followup.

MATERIALS AND METHODS

Patients and healthy controls. Fifty patients (33 women and 17 men, mean age 35.2 ± 14.7 yrs) fulfilling the Yamaguchi criteria for AOSD[12] were enrolled. Patients with infection, malignancy, or other rheumatic diseases were excluded. According to the criteria described by Pouchot, *et al* in 1991[13], the clinical activity score (range 0–12) for each patient with AOSD was assessed by assigning 1 point for each of the following manifestations: fever, evanescent rash, sore throat, arthritis, myalgias, pleuritis, pericarditis, pneumonitis, lymphadenopathy, hepatomegaly or abnormal liver function tests, abdominal pain, and leukocytosis ≥ 15,000/mm^3. After diagnosis of AOSD and initial investigation of cytokine profiles, all patients were treated with nonsteroidal antiinflammatory drugs (NSAID), corticosteroids, and disease modifying antirheumatic drugs (DMARD) including hydroxychloroquine, sulfasalazine and/or methotrexate (MTX). The mean duration of followup was 65.4 ± 39.1 months (range 24–178). Three patterns of disease course were determined and defined as follows: monocyclic systemic — only one episode of systemic manifestations, followed by complete remission within one year after disease onset; polycyclic systemic — more than one episode of systemic manifestations, followed by partial or complete remission after onset of the initial or the subsequent attack; and chronic articular — persistent arthritis involving at least one joint area, lasting > 6 months. Twenty age matched healthy volunteers were used as controls (18 women, 2 men, mean age 33.2 ± 7.5 yrs). Twenty patients with active RA (19 women, one man, mean age 47.5 ± 9.7 yrs) were included as disease controls. Inclusion criteria were fulfillment of the 1987 revised criteria of the American College of Rheumatology[14]; disease duration over one year; and active arthritis, defined as > 6 swollen and 6 tender joints for at least 3 months. All RA patients received low doses of oral prednisolone (5–10 mg daily) and NSAID but no DMARD except for oral MTX 12.5–17.5 mg weekly, at least one month prior to enrollment in the study. Sera were separated from freshly drawn blood taken at the same time of day to avoid circadian effects, and stored at –70°C for determination of cytokine profiles and other laboratory measures. This study protocol was approved by the Ethics Committee of Clinical Research, Taichung Veterans General Hospital, and informed consent was obtained from each participant.

Determination of serum levels of IL-6, IL-8, TNF-α, IL-18, and soluble IL-2 receptor (sIL-2R). Serum levels of IL-6, IL-8, and TNF-α were analyzed with a solid-phase, 2-site chemiluminescent immunometric assay according to the manufacturer's instructions (Immunite; Euro/DPC Ltd., Los Angeles, CA, USA). Serum levels of IL-18 were determined in 46 patients with active AOSD using ELISA according to the manufacturer's instructions (Bender MedSystems, Vienna, Austria). Serum levels of sIL-2R were determined by commercial ELISA kits (Cellfree; Endogen Inc., Woburn, MA, USA).

RNA extraction and real-time quantitative PCR for cytokine transcripts in biopsy specimens of evanescent rash and synovitis from patients with active untreated AOSD. Skin specimens were taken from 9 active AOSD patients with Still's rash, from 3 patients with quiescent AOSD, and from 4 healthy individuals undergoing plastic surgery. Synovial membranes were taken from 6 AOSD patients with active synovitis, 6 patients with RA, and 4 patients with osteoarthritis (OA). Total cellular RNA was isolated from biopsy tissues by the guanidinium isothiocyanate method[15]. RNA was quantitated by spectrophotometry at 260 nm. A 2.5 µg aliquot was reverse transcribed with 200 U of Moloney murine leukemia virus reverse transcriptase according to standard procedures (Boehringer Ingelheim).

The levels of IL-6, IL-8, IL-18, and TNF-α mRNA expression were quantified by real-time TaqMan PCR[16] according to the manufacturer's instructions (Corbette Research, Mortlake, NSW, Australia). The following oligonucleotide primers and TaqMan probes for IL-6, IL-8, IL-18, and TNF-α were designed and synthesized. For IL-6, sense primer 5'-CTC CTT CTC CAC AAG CGC CTTC-3' and antisense primer 5'-CGA CGA AAG TGT GTACAA TGAG-3', TaqMan probe 5'-CCT GCC CCA GTA CCC CCA GG-3'; for IL-8, sense primer 5'-GCT CTC TTG GCA GCC TTC CTGA-3' and antisense primer 5'-TGT TAT TAA AGA CAC AAC CGCG-3', TaqMan probe 5'-AGA ACT TAG ATG TCA GTG CA-3'; for IL-18, sense primer 5'-TTG ACC AAG GAA ATC GGC CTCT-3' and antisense primer 5'-GAT CCG ACC GAT AGA AAT ATG-3, TaqMan probe 5'-GAT TCT GAC TGT AGA GAT AA-3'; and for TNF-α, sense primer 5'-GAG CAC TGA AAG CAT GAT CC-3' and antisense primer 5'-CCG ACT CCT TGT TCG TGG CGGA-3', TaqMan probe 5'-GGA GCT GGC CGA GGA GGC GC-3'. The TaqMan probe consists of an oligonucleotide with a 5' reporter dye and a downstream 3' quencher dye. PCR was performed in a total volume of 50.0 µl containing 100 ng of cDNA, 0.5 µl Taq DNA polymerase (5 U/µl), 5.0 µl TaqMan probe, 5.0 µl each oligonucleotide primer, 25.0 µl PCR buffer, and 9.5 µl RNase-free water. PCR conditions were incubation for 2 min at 50°C, activation of Taq DNA polymerase for 10 min at 95°C, and then 40 cycles of 95°C for 15 s, followed by 58°C for 1.5 min. To standardize cytokine mRNA concentrations, transcript levels of the housekeeping gene ß-actin were determined in parallel for each sample. Final results were expressed as the copy ratio of specific cytokine/ß-actin transcripts.

Statistical methods. Results are presented as the mean ± SD unless otherwise specified. Differences among groups were determined by Kruskal-Wallis test for nonparametric analysis of variance or one-way analysis of variance with post-hoc multiple comparison using Scheffe's method. Spearman's correlations of serum cytokine levels with laboratory parameters were determined. Moreover, we constructed a multiple logistic regression model to evaluate the simultaneous effects of serum cytokines on the occurrence of clinical features in AOSD patients. For comparison of serum

levels of cytokines during followup for AOSD patients, the Wilcoxon signed-rank test was employed. A probability of less than 0.05 was considered to be significant.

RESULTS

Clinical features and laboratory findings of patients with active untreated AOSD. All patients with AOSD had high fever (> 39°C) and articular symptoms. Forty-six patients (92%) had generalized myalgia during the febrile episode. Typical evanescent rash was present in 44 patients (88%), and sore throat was seen in 41 patients (82%). Lymphadenopathy, hepatomegaly, splenomegaly, and serositis were noted in 11 patients (22%), 6 patients (12%), 6 patients (12%), and 5 patients (10%), respectively. Two AOSD patients presented with aseptic meningitis.

Forty-eight patients (96%) had elevated CRP values (> 1.0 mg/dl) and 47 patients (94%) had hyperferritinemia (> 300 µg/l). Increased erythrocyte sedimentation rates (ESR; > 40 mm/h) and elevated levels of serum sIL-2R (≥ 521 U/ml) were seen in 45 patients (90%). About two-thirds of patients had neutrophilic leukocytosis. Elevated hepatic enzyme levels (alanine aminotransferase, ALT, ≥ 40 IU/l) were present in 18 patients (36%). Disseminated intravascular coagulation (DIC) was noted in 5 patients (10%) with AOSD. All patients with DIC also had elevated hepatic enzyme levels.

Serum levels of cytokines in patients with active untreated AOSD and patients with active RA. As shown in Figure 1, the serum levels of IL-6, IL-18, IL-8, and TNF-α were significantly increased in patients with active untreated AOSD, compared with the healthy controls (IL-6 30.36 ± 37.09 vs 5.27 ± 0.42 pg/ml, $p < 0.001$; IL-18 506.18 ± 491.15 vs 60.23 ± 11.51 pg/ml, $p < 0.001$; IL-8 64.67 ± 109.10 vs 8.26 ± 5.10 pg/ml, $p < 0.05$; TNF-α 12.14 ± 7.45 vs 5.59 ± 0.86 pg/ml, $p < 0.001$) and were increased in patients with active

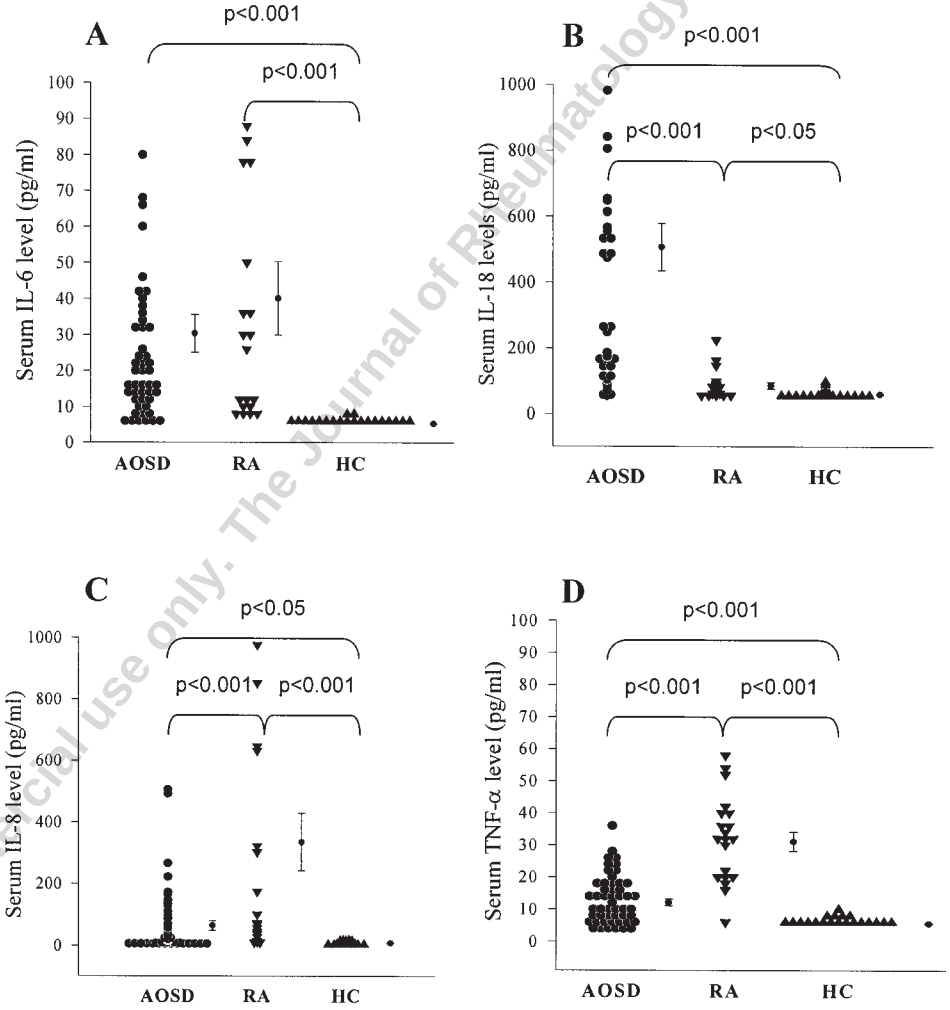

Figure 1. Serum levels of (A) IL-6, (B) IL-18, (C) IL-8, and (D) TNF-α in 50 patients with active untreated AOSD, 20 patients with active RA, and 20 healthy controls (HC). IL-18 levels were evaluated in 46 patients with AOSD. Individual values are plotted, with mean ± SD shown for each group. Values of p are indicated for comparisons of these 3 groups.

RA compared with healthy controls (IL-6 40.11 ± 45.60 vs 5.27 ± 0.42 pg/ml, $p < 0.001$; IL-18 86.22 ± 43.09 vs 60.23 ± 11.51 pg/ml, $p < 0.05$; IL-8 335.32 ± 416.79 vs 8.26 ± 5.10 pg/ml, $p < 0.001$; TNF-α 31.11 ± 13.38 vs 5.59 ± 0.86 pg/ml, $p < 0.001$). Significantly higher levels of serum IL-18 and lower levels of serum IL-8 and TNF-α were found in active untreated AOSD patients than in active RA patients (Figures 1B, 1C, 1D).

Cytokine mRNA expression in biopsy specimens of evanescent rash and synovitis from patients with active untreated AOSD. Significantly higher levels of IL-6 mRNA expression were found in biopsy specimens of Still's rash from active AOSD patients compared with those in skin specimens from quiescent AOSD patients (copy ratio to ß-actin: 2.36 ± 0.34 vs 0.68 ± 0.15, $p < 0.01$) and in healthy controls (copy ratio to ß-actin: 2.36 ± 0.34 vs 0.30 ± 0.06, $p < 0.01$; Figure 2A). The levels of IL-18 and IL-8 mRNA expression were significantly higher in biopsy specimens of Still's rash from active AOSD patients compared with skin specimens from healthy controls (copy ratio to ß-actin: 3.72 ± 1.08 vs 1.10 ± 0.25, $p < 0.05$; 1.46 ± 0.33 vs 0.34 ± 0.11, $p < 0.01$, respectively; Figures 2B, 2C). In contrast, there were no significant differences between AOSD patients and healthy controls in the levels of TNF-α mRNA expression in biopsy specimens of skin (Figure 2D). Regarding the expression of cytokine transcripts on the synovial membrane, levels of IL-18, IL-8, and TNF-α mRNA expression were significantly higher in patients with active untreated AOSD compared with OA controls (copy ratio to ß-actin: 1.52 ± 0.26 vs 0.52 ± 0.06, $p < 0.01$; 2.89 ± 0.31 vs 0.69 ± 0.07, $p < 0.01$; 5.06 ± 0.91 vs 1.51 ± 0.35, $p < 0.01$, respectively) and also higher in patients with active RA compared with OA controls (copy ratio to ß-actin: 1.07 ± 0.16 vs 0.52 ± 0.06, $p < 0.05$; 8.96 ± 2.59 vs 0.69 ± 0.07, $p < 0.01$; 16.65 ± 4.55 vs 1.51 ± 0.35, $p < 0.01$, respectively; Figures 3B, 3C, 3D). The levels of IL-8 and TNF-α mRNA expression on synovial membranes were significantly increased in active RA patients compared with those in patients with active untreat-

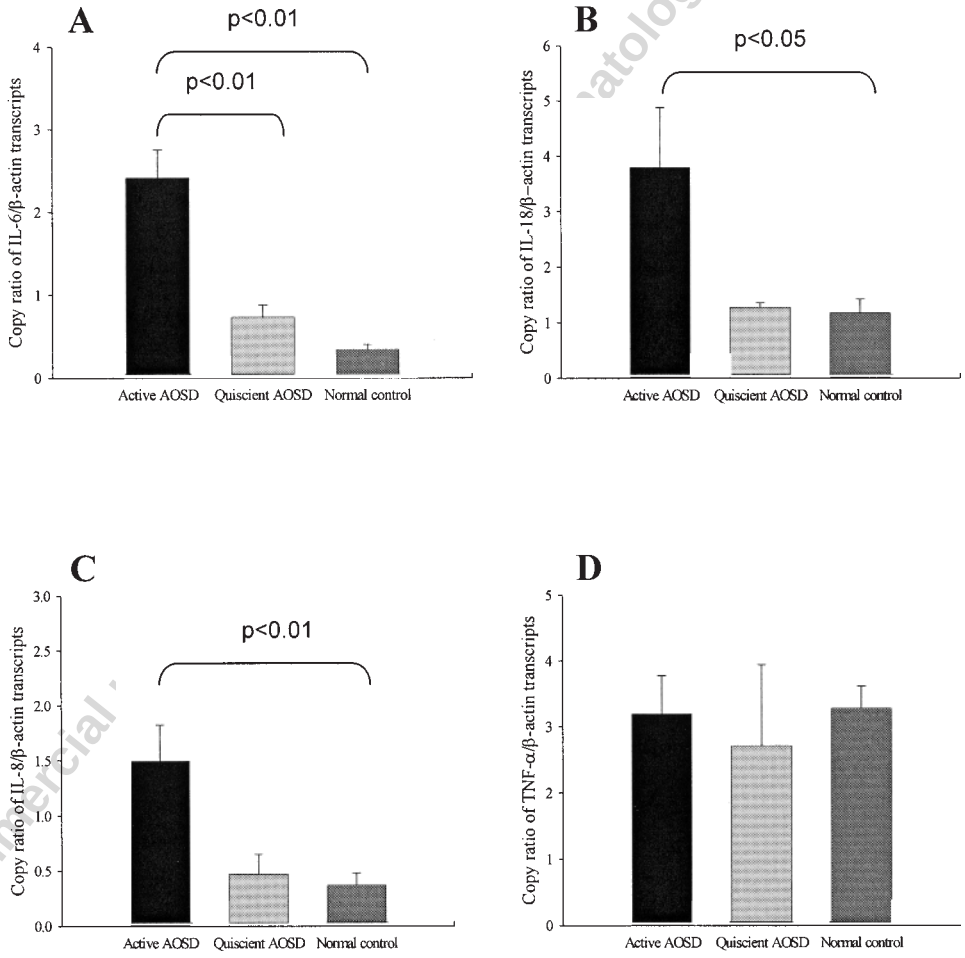

Figure 2. Levels of (A) IL-6, (B) IL-18, (C) IL-8, and (D) TNF-α mRNA expression in the biopsy skin specimens of Still's rash from patients with active AOSD (n = 9), from patients with quiescent AOSD (n = 3), and normal skin controls (n = 4) were analyzed by real-time TaqMan PCR method. Data are presented as mean ± SEM. The p values are provided for comparison of different groups.

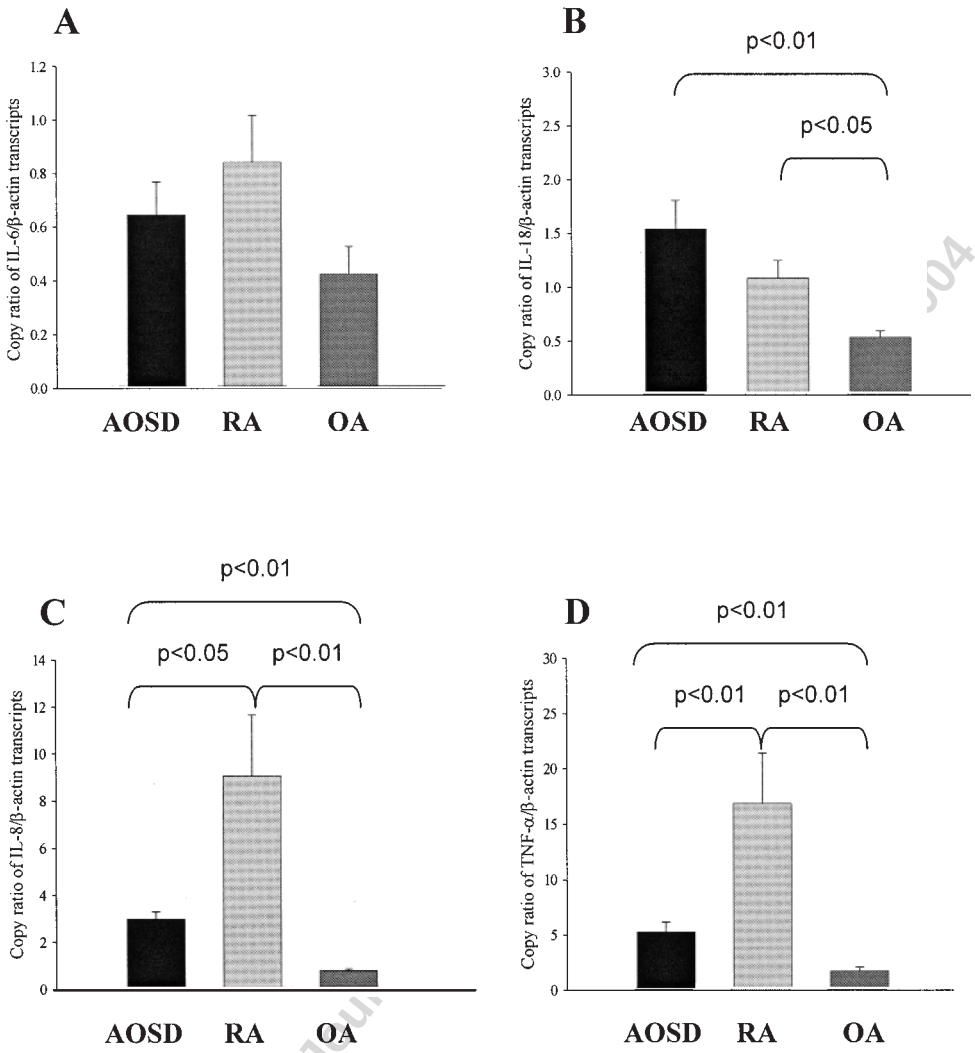

Figure 3. Levels of (A) IL-6, (B) IL-18, (C) IL-8, and (D) TNF-α mRNA expression in the synovial tissues during active arthritis from AOSD patients (n = 6), of active arthritis from RA patients (n = 6), and controls with OA (n = 4) were analyzed by real-time TaqMan PCR method. Data are presented as mean ± SEM. The p values are provided for comparison of different groups.

ed AOSD (copy ratio to ß-actin: 8.96 ± 2.59 vs 2.89 ± 0.31, $p < 0.05$; 16.65 ± 4.55 vs 5.06 ± 0.91, $p < 0.01$, respectively; Figures 3C, 3D). No significant differences were seen between AOSD patients and OA controls with regard to levels of IL-6 mRNA expression on synovial membranes.

Multiple logistic regression analysis of the effects of serum cytokines on occurrence of clinical features in AOSD patients. In AOSD patients, multiple logistic regression analysis was performed to evaluate the simultaneous effects of proinflammatory cytokine profiles on the occurrence of clinical features. In a model with serum cytokines (IL-6, TNF-α, IL-8, and IL-18), serum IL-6 level was identified as a possible predictor of evanescent rash ($p = 0.0593$). Serum IL-8 value was found to be a significant predictor of persistent arthritis ($p = 0.0202$), and serum IL-18 value predicted occurrence of liver dysfunction ($p = 0.0027$) (Table 1).

Correlations of serum cytokine levels with clinical activity score and biological measures of inflammation in patients with AOSD. As illustrated in Table 2, clinical activity scores significantly correlated with serum levels of IL-6 and IL-18. The serum levels of IL-6 correlated well with white blood cell (WBC) count, platelet count, ESR, serum CRP value, and serum sIL-2R level. Serum levels of IL-18 were well correlated with serum ferritin values and serum sIL-2R levels. Statistical significance was not confirmed, however, for correlations between serum levels of IL-8 or TNF-α and clinical activity scores, ESR, serum CRP level, serum ferritin values, or sIL-2R levels in patients with AOSD.

Changes in serum levels of cytokine profiles in patients with AOSD after therapy. To investigate the serial changes of serum levels of IL-6, IL-18, IL-8, and TNF-α, 8 AOSD patients with polycyclic systemic pattern of clinical course

Table 1. Multiple logistic regression analysis of the effects of serum cytokines on the occurrence of clinical features in patients with AOSD (n = 46).

	Evanescent Rash			Persistent Arthritis			Liver Dysfunction		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
IL-6	1.264	0.9908–1.6135	0.0593	0.997	0.9761–1.0187	0.7955	1.009	0.9921–1.0271	0.2867
IL-18	1.001	0.9984–1.0037	0.4223	0.999	0.9967–1.0010	0.2789	1.003	1.0009–1.0043	0.0027**
IL-8	1.014	0.9843–1.0454	0.3537	1.012	1.0019–1.0226	0.0202*	0.998	0.9908–1.0054	0.6076
TNF-α	0.944	0.8163–1.0916	0.4370	0.986	0.8880–1.0959	0.7999	1.093	0.9884–1.2094	0.0831

Persistent arthritis: arthritis involving at least one joint area and lasting longer than 6 months; liver dysfunction: elevated hepatic enzyme-alanine aminotransferase ≥ 40 IU/l; IL-6. * $p < 0.05$, ** $p < 0.005$ (Wald's correlation).

Table 2. Correlation coefficients (r) and levels of significance (p) of the correlation of serum cytokine levels with clinical activity score and laboratory measures in patients with AOSD (n = 50).

	IL-6		IL-18†		TNF-α		IL-8	
	r	p	r	p	r	p	r	p
Systemic activity score	0.715	0.000	0.450	0.002	–0.129	NS	0.132	NS
WBC count	0.453	0.001	0.165	NS	–0.036	NS	–0.187	NS
Platelet count	0.369	0.008	–0.109	NS	–0.188	NS	0.078	NS
ESR	0.360	0.010	0.034	NS	–0.118	NS	–0.007	NS
Serum CRP level	0.440	0.001	0.215	NS	–0.096	NS	–0.142	NS
Serum ferritin level	0.226	NS	0.373	0.011	0.161	NS	0.215	NS
Serum sIL-2R level	0.342	0.015	0.474	0.001	0.041	NS	0.279	NS

WBC count: white cell count in peripheral blood; sIL-2R: soluble interleukin 2 receptor; NS: not significant.
† IL-18 levels were evaluated in 46 patients with AOSD.

were available for examination at the active phase and the remission phase. The serum levels of IL-6 and IL-18 declined significantly, paralleling the clinical remission ($p < 0.05$ for both; Figure 4A). But this association with clinical activity was not observed in serum levels of IL-8 or TNF-α. We also examined the kinetics of cytokine profiles in one AOSD patient and observed that serum levels of IL-6 and IL-18 paralleled the change of body temperature and the severity of Still's rash (Figure 4B).

Serum cytokine profiles in AOSD patients grouped according to 3 patterns of disease course. A followup analysis of 50 patients with AOSD was performed over a mean period of 65.4 ± 39.1 months (range 24–178 mo). As shown in Table 3, 24 patients (48%) had the polycyclic systemic pattern, 14 patients (28%) had monocyclic systemic pattern, and the remaining 12 patients (24%) had a chronic articular pattern. Significantly higher levels of serum IL-8 in the active stage were seen for AOSD patients who had a chronic articular pattern compared with those who had a monocyclic systemic pattern. Articular manifestations were significantly different among the 3 groups, but age at onset, followup duration, and the main clinical features were nonsignificant (data not shown).

DISCUSSION

This study is the first to investigate proinflammatory cytokine profiles in both the sera and pathological tissues of patients with AOSD and to evaluate associations of the levels of cytokine profiles with distinct clinical manifestations and the various patterns of disease course in this disease. The results demonstrate that serum concentrations of IL-6, IL-8, IL-18, and TNF-α were significantly higher in patients with active untreated AOSD compared with those in the healthy controls. The levels of IL-6, IL-8, and IL-18 mRNA expression in biopsy tissues of Still's rash from AOSD patients were significantly higher compared with skin of healthy controls. Similarly, the levels of mRNA expression of IL-18, IL-8, and TNF-α were also higher in the synovial membranes of AOSD patients compared with those of osteoarthritis controls. Among the cytokine profiles examined, serum levels of IL-6 and IL-18 correlated well with clinical activity scores and biological indicators of inflammation in patients with AOSD.

IL-6 is a multifunctional cytokine that participates in the regulation of immune and inflammatory responses[17-19]. Among its proinflammatory activities are the induction of fever, synthesis of acute phase protein, leukocytosis, and

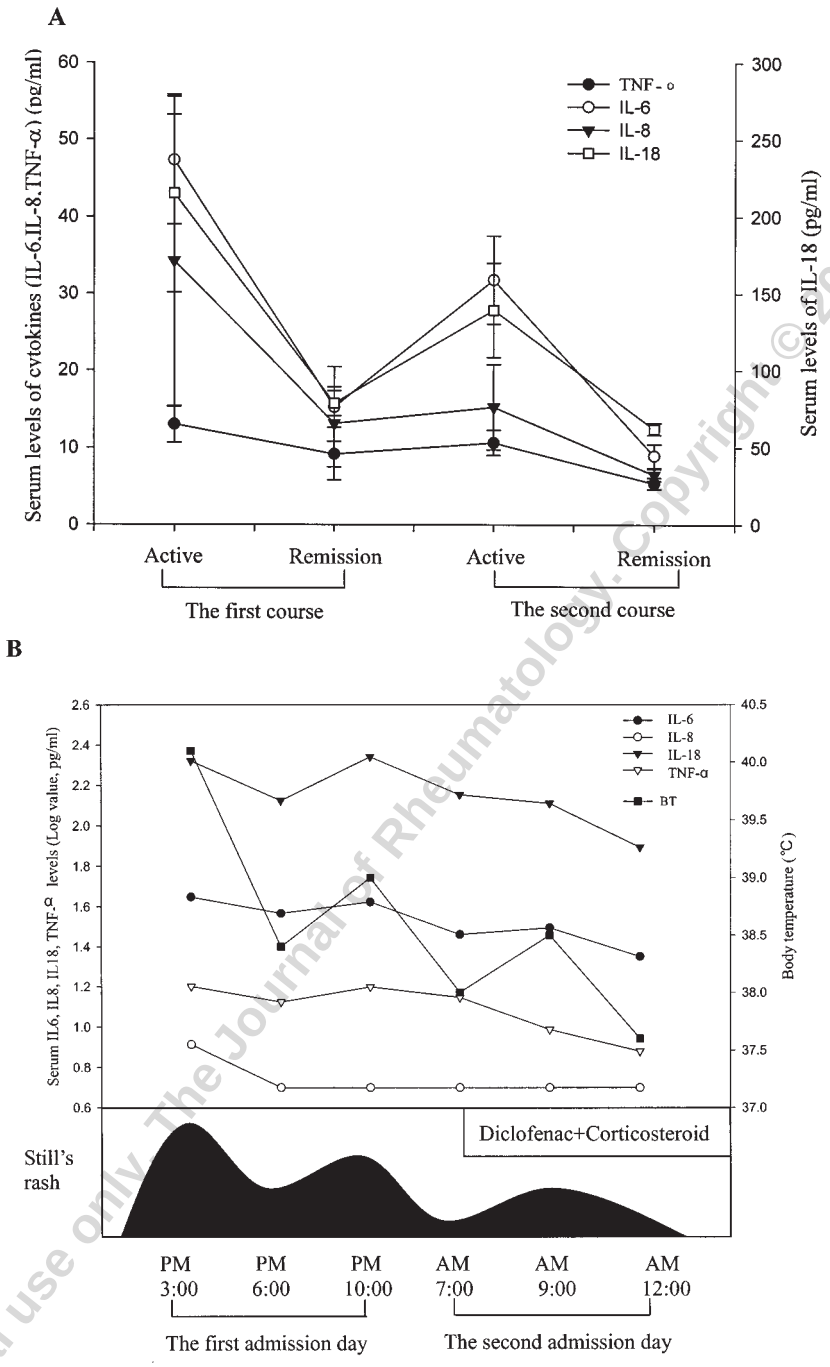

Figure 4. A. Serial changes in the serum levels of IL-6, IL-8, IL-18, and TNF-α during active and remission phase in 8 AOSD patients with polycyclic systemic pattern of clinical course. Data are presented as mean ± SEM. Serum levels of IL-6 and IL-18 declined significantly, paralleling the clinical remission ($p < 0.05$ for both). B. The daily kinetics of serum levels of IL-6, IL-8, IL-18, and TNF-α, body temperature, and skin expression in one patient with AOSD.

thrombocytosis[17-19]. We found that serum levels of IL-6 were significantly higher in patients with active AOSD than in healthy controls (Figure 1A). Serum levels of IL-6 correlated well with clinical activity score, WBC count, platelet count, ESR, and serum CRP levels (Table 2). The strong correlation of serum IL-6 levels with ESR and CRP may be explained by the major role of IL-6 in the induction of acute-phase proteins[17]. During a longitudinal followup, we

Table 3. Demographic data and serum levels of cytokine in patients with AOSD during active stage, grouped according to disease course.

	Monocyclic Systemic, n = 14	Polycyclic Systemic, n = 24	Chronic Articular, n = 12
Age at onset, yrs	28.4 ± 7.1	39.1 ± 16.7	35.4 ± 15.1
Male: female	4 : 10	7 : 17	6 : 6
Serum IL-6 levels, pg/ml	21.09 ± 21.17	35.33 ± 45.51	31.27 ± 33.20
Serum IL-8 levels, pg/ml	10.75 ± 7.67	59.25 ± 69.09	138.43 ± 182.41*
Serum TNF-α levels, pg/ml	11.46 ± 6.25	12.88 ± 8.90	11.44 ± 5.75
Serum IL-18 levels, pg/ml†	606.54 ± 653.66	561.40 ± 439.88	273.05 ± 250.62

† Values are shown as means ± standard deviation. IL-18 levels were evaluated in 46 patients with AOSD. * $p < 0.05$ versus monocyclic systemic.

found that serum IL-6 levels declined markedly, paralleling the clinical remission (Figure 4A). These findings are consistent with those of a previous study, which indicate that serum IL-6 is a sensitive marker of disease activity in AOSD[5]. In addition, serum IL-6 level was a possible predictor of evanescent rash (odds ratio 1.264, $p = 0.0593$). The pathogenic role of IL-6 in the cutaneous manifestation is further supported by our results, which showed that levels of IL-6 mRNA expression were significantly higher in biopsy specimens of Still's rash from active AOSD patients than in skin specimens from patients with quiescent AOSD and healthy controls (Figure 2A).

IL-18, a recently described member of the IL-1 cytokine family, promotes Th1 immune response and stimulates the production of IFN-γ in T cells and natural killer cells in synergy with IL-12[20,21]. Serum IL-18 levels are increased in several inflammatory diseases, including AOSD and RA, during the active stage[6-8,11]. We observed significantly higher levels of serum IL-18 in patients with AOSD and RA compared with healthy controls (Figure 1B), consistent with the findings of previous studies[6-8]. Serum levels of IL-18 decreased significantly with antiinflammatory therapy in our AOSD patients ($p < 0.05$; Figure 4A). In addition, the serum IL-18 levels in AOSD patients were significantly correlated with clinical activity scores ($r = 0.450$, $p < 0.005$); serum sIL-2R levels ($r = 0.474$, $p < 0.005$), which reflected T cell activation[22] and might be used to monitor disease activity of AOSD[8]; and serum ferritin values ($r = 0.373$, $p < 0.05$), which were considered an activity marker for AOSD[3]. The pathogenic role of IL-18 in this disease is further supported by our results showing elevated levels of IL-18 transcripts in the biopsy specimens of Still's rash and synovitis from patients with AOSD (Figures 2B, 3B).

Compared with active RA patients, serum levels of IL-18 were markedly higher in patients with active untreated AOSD (Figure 1B). IL-18 is produced mainly by activated macrophage-lineage cells, which included Kupffer cells in liver[20]. A previous report suggested that liver dysfunction in AOSD reflects the underlying disease[23]. Liver dysfunction was found in 71.6% of AOSD patients in one review[2] and in 36% of our patients. Further, we found that IL-18 level in AOSD patients was significantly correlated with hepatic enzyme value ($r = 0.390$, $p < 0.01$) and was a predictor of liver dysfunction (OR 1.003, 95% CI 1.0009–1.0043, $p < 0.005$). It is possible that IL-18 might be released into circulation because of liver injury, which is commonly observed in AOSD patients, but is unusual in patients with RA. However, a bias may have been introduced as a result of MTX therapy in RA patients prior to this investigation.

We found that serum levels of TNF-α were higher in AOSD patients compared with healthy controls (Figure 1D), consistent with findings of other reports[5], and this suggests that TNF-α is a potent inducer of inflammatory response in this disease. Recent findings that TNF-α antagonists (infliximab and etanercept) were effective for treatment of patients with AOSD refractory to conventional therapy[24,25] support this hypothesis. In contrast to IL-6 and IL-18, the serum levels of TNF-α were significantly higher in RA patients compared with those in AOSD patients (Figure 1D), and the levels of TNF-α mRNA expression in synovial membranes were significantly higher in the RA patients than in AOSD patients (Figure 3D). TNF-α is known to play a pivotal role in the pathogenesis of RA[26,27] and anti-TNF therapy is effective in this disease[28,29]. The *in vitro* and *in vivo* studies showed that TNF-α was an osteoclast-activating factor that could promote cartilage and bone resorption[30,31]. Clinically, TNF-α antagonist arrests radiographic progression of bone erosions and joint damage in a large number of RA patients[29]. The lower levels of TNF-α in both the serum and the pathological tissue of patients with active untreated AOSD confer some protection against the detrimental articular effects in this disease. A large prospective cohort study should be conducted to confirm these findings.

IL-8 is a potent angiogenic factor and acts as a proinflammatory chemokine for leukocytes, and it is an important contributor to the angiogenic activity found in inflamed joints in RA[32,33]. We found that serum levels of IL-8 were significantly higher in patients with active AOSD than in healthy controls (Figure 1C). Serum IL-8 value was a significant predictor of persistent arthritis (OR 1.012, 95% CI

1.0019–1.0226, $p < 0.05$). Further, we found levels of IL-8 transcripts were significantly higher in synovial membranes from AOSD patients compared with those in the OA controls (Figure 3C). These results suggest that focal expression of IL-8 in synovial membranes attracts different inflammatory cells that contribute to the development of synovitis in patients with AOSD. Compared with RA patients, significantly lower levels of IL-8 in the sera and its transcripts in the synovial membranes were found in patients with AOSD (Figures 1C, 3C). Previous histopathological studies have described a high expression of IL-8 in RA synovial membranes[10,34]. The differences in the levels of IL-8 in both the sera and the synovial membranes between patients with AOSD and RA may account for the differences in the perpetuation of the immune response and the frequency of joint destruction in both diseases[35,36].

The disease course and prognosis for patients with AOSD may vary considerably. We investigated whether the serum cytokine profile differs between patients with distinctly different courses of this disease. We found that serum levels of IL-8 in active disease stage were higher in AOSD patients who had the chronic articular pattern compared with those who had the monocyclic systemic pattern (Table 3). This suggests that IL-8 may play an important role in the perpetuation of the chronic arthritis in AOSD.

Our results showed significantly higher levels of IL-6, IL-8, IL-18, and TNF-α in both sera and pathological tissues of patients with active AOSD. Increased IL-6 production may play a role in systemic inflammation and evanescent rash, and may represent a reliable marker of disease activity in AOSD. Elevated IL-8 production may result in articular damage and may predict chronic arthritis in patients with AOSD. Serum IL-18 levels correlated significantly with clinical activity scores and hepatic enzyme levels, and may contribute to hyperferritinemia. However, every proinflammatory cytokine is counteracted by either antiinflammatory cytokines or cytokine inhibitors, and thus it is the relative level of a proinflammatory cytokine to its antagonist that is crucial in determining its final effect.

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