Serum Cytokine Profiles in Patients with Adult Onset Still's Disease

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ABSTRACT. ***Objective.*** Adult onset Still's disease (AOSD) is a systemic inflammatory disorder characterized by fever, arthritis, and rash. Although the pathogenesis is not known, immunologically mediated inflammation occurs in active AOSD. To evaluate the pathogenesis and disease activity of AOSD, we measured serial serum concentrations of several cytokines in patients with active and inactive disease.

Methods. Seventeen patients diagnosed as having AOSD were enrolled. We analyzed clinical and laboratory findings retrospectively. Serial serum samples were obtained from 14 patients with active and inactive AOSD. Interleukin 18 (IL-18), soluble IL-2 receptor (sIL-2R), IL-6, interferon-γ (IFN-γ), and IL-8 were determined by ELISA.

Results. Serum levels of IL-18, IFN-γ, and IL-8 were significantly higher in patients with AOSD than in healthy controls ($p < 0.01$), but there were no significant differences between patients with active and inactive AOSD. Serum sIL-2R levels tended to be higher in the active state than in healthy controls, but there was no statistically significant difference between the 2 groups. Serum sIL-2R levels decreased significantly with antiinflammatory therapy ($p < 0.05$). Serum IL-18 and sIL-2R levels correlated significantly with serum ferritin levels in the active AOSD group ($p < 0.05$).

Conclusion. Overproduction of IL-18 may contribute to the pathogenic mechanism of AOSD, and serum sIL-2R levels may be used as a marker for monitoring disease activity in AOSD. (J Rheumatol 2003;30:2422–7)

Key Indexing Terms:
ADULT ONSET STILL'S DISEASE
CYTOKINE
INTERLEUKIN 18
SOLUBLE IL-2 RECEPTOR

Adult onset Still's disease (AOSD) is a systemic inflammatory disorder of unknown origin characterized by polyarthritis, high spiking fever, evanescent salmon-colored rash, lymphadenopathy, and hepatosplenomegaly. Laboratory findings reveal evidence of nonspecific inflammation such as leukocytosis, anemia, increased levels of serum acute phase reactants, and elevation of hepatic enzymes[1-5]. Hyperferritinemia has been considered to be correlated with disease activity[6,7]. Although the pathogenesis is not yet clear, immunologically mediated inflammation occurs in active AOSD. Recently, several studies described increased interleukin 6 (IL-6) or γδ T cell activation in the acute phase of AOSD[8-10]. Kawashima, *et al* suggested that IL-18 overproduction may be closely related to the pathogenesis of AOSD and that it was correlated with disease activity[11]. Fujii, *et al* also reported that soluble IL-2 receptor (sIL-2R) and IL-18 concentrations were correlated with disease activity in chronic articular AOSD, suggesting that AOSD with chronic articular disease has a distinct clinical and immunogenetic profile[12]. But there have been only a few studies on serial changes of serum cytokine concentrations involving an adequate number of patients with AOSD.

To evaluate the role of cytokines in the pathogenesis and disease activity of AOSD, we compared serum IL-18, sIL-2R, IL-6, interferon-γ (IFN-γ), and IL-8 concentrations between patients with active and inactive disease states and monitored their changes with antiinflammatory therapy.

MATERIALS AND METHODS

Patients. Seventeen patients diagnosed with AOSD in the Allergy and Rheumatology Clinic, Ajou University Hospital, from February 1998 through August 2001 were enrolled. There were 13 women and 4 men with a mean (SD) age of 33.9 (8.5) years. All patients satisfied the criteria of Yamaguchi, *et al*[4]. We analyzed clinical and laboratory findings of the patients retrospectively. Three of the major criteria, such as fever, rash, and arthralgia, were observed in more than 80% of the patients (Table 1). Oligoarticular involvement (≤ 4 joints) was observed in 9 patients (52.9%), and the arthritis was chronic in 4 patients (23.5%). Large joints (especially the shoulder, knee, and elbow) were more commonly affected than small joints. Patients were treated with nonsteroidal antiinflammatory drugs (NSAID) in 10 cases (58.8%) and systemic steroids in 16 (94.1%). All patients received intravenous immunoglobulin (IVIG) for disease control and steroid-sparing effects. After the systemic manifestations disappeared,

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Table 1. Clinical manifestations of the study subjects (n = 17).

Variable	n	%
Fever	16	94.1
Rash/urticaria	15	88.2
Arthritis	14	82.4
Myalgia	10	58.8
Sore throat	9	52.9
Hepatosplenomegaly	7	41.2
Lymphadenopathy	6	35.3
Morning stiffness	4	23.5
Pleuritis	3	17.6
Pneumonitis	1	5.9

weekly low dose methotrexate (7.5–12.5 mg) was added for steroid-sparing effect and for controlling arthritis in 12 patients (70.6%).

Serial serum samples were collected twice from 14 patients in both active and inactive states. The initial samples were taken in the active state before treatment with antiinflammatory agents, and followup samples were taken when the antiinflammatory therapy was finished and systemic symptoms improved one to 4 weeks after the initial samples were taken. The third samples were taken during completely inactive state in 7 of the 14 patients, usually 3 to 12 weeks after the initial samples were taken, when the laboratory findings of inflammation were normalized. We were also able to obtain serum samples from 2 patients who had recurrences. Sera from 15 healthy controls (7 men, 8 women) were also collected; the mean (SD) age of controls was 31.8 (10.7) years.

ELISA for IL-18, sIL-2R, IL-6, IFN-γ. Serum concentrations of sIL-2R, IL-6, and IFN-γ were measured in duplicate using ELISA kits (Pierce-Endogen, Rockford, IL, USA). In the case of IL-18, the ELISA kit produced by MBL (Nagoya, Japan) was used.

ELISA for IL-8. In 13 of the 14 patients, serum IL-8 concentrations were determined. Microplates (Costar, Cambridge, MA, USA) were coated with anti-human IL-8 monoclonal antibody (Endogen, Woburn, MA, USA). After serum loading, biotin-labeled anti-human IL-8 Mab (Endogen) was added. The wells were incubated with streptavidin-peroxidase (Sigma Chemical Co., St. Louis, MO, USA). Then colorimetric reaction was developed with TMB (3,3',5,5'–tetramethylbenzidine) substrate solution. The reaction was stopped by the addition of 2 N sulfuric acid and the absorbance was read at 450 nm (reference 570 nm) by an automated microplate reader (Benchmark, Bio-Rad, Hercules, CA, USA). Amounts of IL-8 in the samples were calculated from standard curves made by optical densities against the concentration of recombinant human IL-8 (Endogen) in the standard wells.

Statistical analysis. SPSS software (Chicago, IL, USA) version 10.0 was used to analyze the data. All results were expressed as mean ± SEM. Mann-Whitney U tests were applied to evaluate the statistical differences of serum cytokine concentrations between patients and controls. Wilcoxon signed-rank tests were applied to evaluate statistical differences between each of 2 matched values. The correlation coefficient was obtained by Spearman's correlation tests. A p value ≤ 0.05 was regarded as significant.

RESULTS

Laboratory findings. Elevated levels of serum erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and ferritin were noted in more than 80% of the patients (Table 2). White blood cell counts were normal during active illness in 4 patients, and leukopenia (minimum 3300/mm^3) was noted in 3 patients with no predisposing causes. Two patients showed positive antinuclear antibodies (ANA)

Table 2. Laboratory findings in the study subjects (n = 17).

Variable	n	%
Leukocytosis (> 10,000)	7	41.2
Neutrophilia (> 80%)	11	64.7
Anemia (< 10 g/dl)	3	17.6
Thrombocytosis (> 400,000/mm^3)	3	17.6
ESR (> 20 mm/h)	14	82.4
CRP (> 0.8 mg/dl)	15	88.2
LDH (> 220 U/l)	11	78.6
Hyperferritinemia (> 284 ng/ml)	14	82.4
Hypoalbuminemia (< 3.5 g/dl)	11	64.7
Elevated AST/ALT	11	64.7
Negative ANA	15	88.2
Negative RF	16	94.1
IgG (> 1796 mg/dl)	4	23.5
IgA (> 365 mg/dl)	2	11.8
IgM (> 200 mg/dl)	3	17.6
C3 (> 125 mg/dl)	9	56.3
C4 (> 37 mg/dl)	1	6.3

LDH: lactate dehydrogenase, RF: rheumatoid factor.

(one had a titer of 1:40, the other 1:320). However, anti-dsDNA antibody and serum complement (C3/C4) levels were in normal ranges and there were no clinical manifestations suggesting systemic lupus erythematosus (SLE). Rheumatoid factor was positive with low titer in one patient.

Changes of serum cytokines in patients with AOSD. Serum levels of IL-18, IFN-γ, and IL-8 in both active and inactive AOSD were significantly higher than those of controls ($p < 0.01$), while no significant difference was noted between the active and inactive states (Table 3, Figure 1). Serum concentrations of sIL-2R in the active state tended to be higher than in controls, although statistical significance was not noted. However, serum sIL-2R levels in the patients with active AOSD were significantly higher than those of the inactive AOSD ($p < 0.01$). Two patients with recurrence showed no change of IL-18 level, but there was an elevation of sIL-2R level in one patient. There was no significant difference in IL-6 between patients and controls.

Changes of acute phase reactants in patients with AOSD. Serum ferritin levels (2467.8 ± 3417.9 ng/ml) in the active AOSD were significantly higher than those of the inactive AOSD (190.1 ± 227.1 ng/ml; $p < 0.05$). ESR and CRP levels in the active state were also significantly higher than in the inactive state ($p < 0.05$). Ferritin, ESR, and CRP levels were normalized when disease activity was controlled in all patients (Figure 2).

Correlation between acute phase reactants and serum cytokine levels in active AOSD. Serum ferritin levels showed a positive correlation with IL-18 ($r = 0.54$, $p < 0.05$) and sIL-2R levels in the active AOSD patients ($r = 0.64$, $p < 0.05$; Figure 3). There was no significant correlation between ferritin and IL-6, IL-8, and IFN-γ levels. ESR and

Table 3. Comparison of serum cytokines between patients with active and inactive AOSD (15 healthy controls were enrolled).

	IL-18, pg/ml	sIL-2R, U/ml	IFN-γ, pg/ml	IL-8, pg/ml	IL-6, pg/ml
Patients					
Active	772.8 ± 105.1	1152.1 ± 341.2†	142.6 ± 84.5	65.9 ± 27.3	11.6 ± 3.3
Inactive	788.2 ± 112.7	247.0 ± 51.0	11.8 ± 9.0	60.7 ± 22.0	9.7 ± 2.8
n	14	14	14	13	14
Controls	196.4 ± 16.8	341.5 ± 25.0	0	10.0 ± 0.5	6.3 ± 1.0
p*	< 0.01	> 0.05	< 0.01	< 0.05	> 0.05

All values presented as mean ± SEM. * Between active AOSD and controls, and between inactive AOSD and controls, respectively. † $p < 0.01$, active versus inactive AOSD.

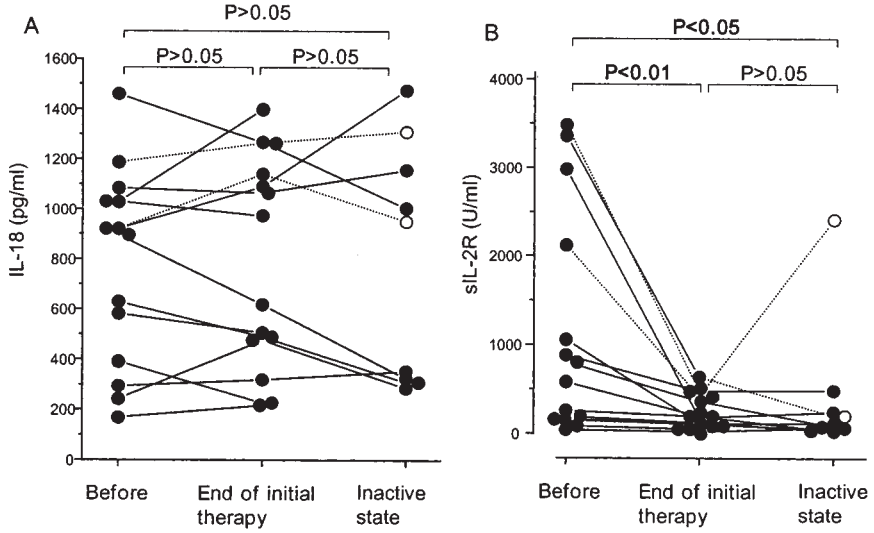

Figure 1. Changes of serum cytokines in active and inactive AOSD. A. Serum IL-18 levels were higher than those of controls, even in remission. B. Serum sIL-2R levels in active AOSD were higher than those of inactive AOSD. ○: patients with disease recurrence.

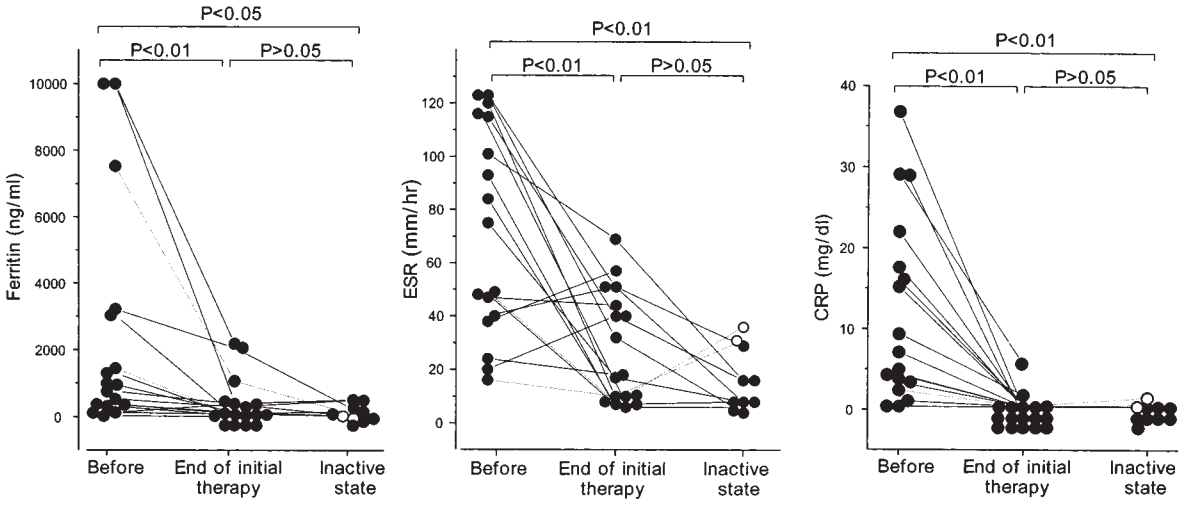

Figure 2. Changes of acute phase reactants in active and inactive AOSD. Elevated serum ferritin, ESR, and CRP levels in active AOSD were normalized with antiinflammatory therapy. ○: patients with disease recurrence.

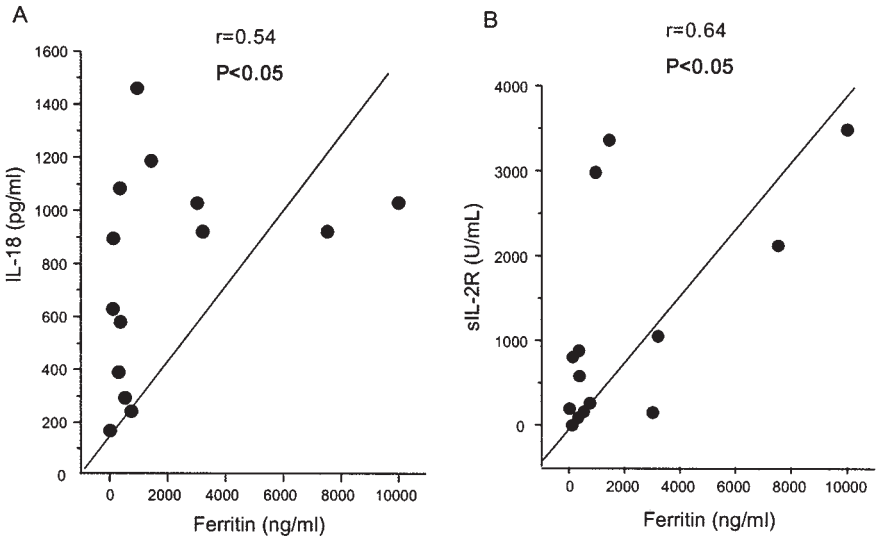

Figure 3. Correlations between serum ferritin levels and cytokines in active AOSD. Serum ferritin levels showed a positive correlation with sIL-2R (A) and IL-18 levels (B).

CRP were not significantly correlated with any cytokine measured in this study (data not shown).

DISCUSSION

We determined serum IL-18, sIL-2R, IL-6, IFN-γ, and IL-8 concentrations serially according to disease activity in Korean patients with AOSD. Our data suggest that overproduction of serum IL-18 and sIL-2R may contribute to the pathogenic mechanism of AOSD, and serum sIL-2R concentrations may be used as a marker to monitor disease activity in AOSD.

IL-18 is an 18 kDa protein and a novel cytokine with pleiotropic activities, and was originally described as an IFN-γ inducing factor[13-16]. IL-18 is produced mainly by activated macrophage and Kupffer cells[17]. IL-18 promotes the proliferation of activated T lymphocytes and production of IL-2, tumor necrosis factor-α, nitric oxide (NO), and granulocyte-macrophage colony-stimulating factor, and augments the cytotoxic activity of natural killer cells through upregulation of Fas ligand[15,16]. Several studies report that serum and synovial fluid IL-18 concentrations in patients with rheumatoid arthritis (RA) were higher than those of osteoarthritis (OA) and healthy controls[14,18], and that it also facilitates the development of erosive, inflammatory arthritis[14,15]. Recently, serum levels of IL-18 were reported to be elevated in patients with active AOSD compared to those with SLE, RA, juvenile RA, systemic sclerosis (SSc), polymyositis/dermatomyositis, Sjögren's syndrome, and infection. As well, the authors observed increased IL-18 mRNA expression in blood monocytes and T cells and increased IL-18 bioactivity in sera of patients with active AOSD, and serum IL-18 concentrations diminished and then gradually normalized after the disease was successfully controlled[11]. Also, serum IL-18 levels in the steroid nonresponders were significantly higher than in the steroid responders, suggesting that IL-18 levels were related with disease severity[19]. Fujii, *et al* reported increased levels of serum IL-18 in systemic and chronic articular AOSD, even in remission, but correlation with disease activity was noted only in chronic articular AOSD[12]. We found elevated concentrations of IL-18 in patients with active and inactive AOSD, but there was no significant difference between the active and the inactive states. Also, in patients with active disease, serum IL-18 levels showed significant correlation with ferritin levels, which represented the disease activity. Although we observed no changes of IL-18 levels with therapeutic response, these results suggest that elevated serum IL-18 levels from activated macrophages and Kupffer cells may contribute to the pathogenesis of AOSD.

IFN-γ and IL-8 have been detected at high levels in blood in patients with AOSD[8,9]. IFN-γ and IL-8 are induced by IL-18, which indicates the participation of Th1-related pathways in AOSD[15]. We also found that serum IFN-γ and IL-8 levels in active and inactive AOSD were significantly increased compared to those in controls. However, serum IFN-γ and IL-8 levels were not significantly different between the active and the inactive disease states.

Soluble IL-2 receptor is a 45 kDa molecule released by activated lymphocytes[20,21]. Significantly elevated levels of sIL-2R have been reported in patients with RA[22,23], juvenile RA[24-27], SLE[28,29], polymyositis[21], and SSc[30]. However, serum sIL-2R levels in patients with OA or crystal–induced inflammatory disease were comparable with levels in healthy controls. In patients with RA, sIL-2R levels in synovial fluid were significantly elevated not only in comparison with those in OA, but also in comparison with concurrent serum sIL-2R in the same RA patients. These findings provide further evidence that implicates local

(synovial) immune activation in this disease[22]. Results from serial measurements of serum sIL-2R in juvenile RA suggest a positive correlation between serum sIL-2R levels and changes in disease activity[24-27]. Increased serum sIL-2R levels were noted in patients with active AOSD and were significantly correlated with disease activity only in those with chronic articular patterns of AOSD[12]. In addition, increased γδ T cells were found in peripheral blood lymphocytes in the active state in AOSD[10]. Our results show that serum sIL-2R levels tended to be higher in patients with active AOSD, although levels were not significantly different from those of controls. However, serum sIL-2R levels were significantly higher in active AOSD than in inactive AOSD. They were correlated with ferritin levels in active AOSD. These results suggest that sIL-2R levels produced by activated T lymphocytes may contribute to the pathogenesis of AOSD, and serum sIL-2R level may be used as a marker to monitor disease activity.

Rheumatic diseases are associated with elevated concentrations of the acute phase proteins, particularly CRP[31]. IL-6 is a multifunctional 26 kDa cytokine that is produced by a range of cells[32], and it is widely accepted that IL-6 is one of the major contributors to the acute phase protein response[31-33]. In inflammatory arthritis, IL-6 concentrations in serum and synovial fluid were increased in conjunction with ESR and CRP[33]. In addition, elevated serum IL-6 levels were noted in patients with active AOSD[8,9]. But it is surprising that there was no significant elevation of IL-6 level in our patients in spite of increased levels of the acute phase reactants and other cytokines. Further studies will be needed to confirm these conflicting results.

Overproduction of IL-18 from active inflammatory cells may contribute to systemic inflammation in patients with AOSD by activating T lymphocytes that produce sIL-2R, IFN-γ, and IL-8, and serum sIL-2R concentration may be used as a marker monitoring therapeutic response in AOSD.

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