Natural Killer Cell Cytolytic Function in Korean Patients with Adult-onset Still's Disease

JEONG HA PARK, HEE-SUN KIM, JIN SOOK LEE, JIN JU KIM, KYONG-HEE JUNG, YONG-WOOK PARK, and DAE-HYUN YOO

ABSTRACT. ***Objective.*** To investigate natural killer (NK) cell proportions, NK cell cytotoxicity, and interleukin 18 (IL-18) expression, in patients with adult-onset Still's disease (AOSD).

Methods. Forty-five patients with AOSD (active = 22, inactive = 23) and 32 healthy controls were included. The proportions of NK cells among peripheral blood mononuclear cells were assessed by flow cytometry. IL-18 and IL-18-binding protein (IL-18BP) concentrations were measured by ELISA. Twenty-four patients with AOSD and 18 controls were examined for cytotoxic activity of NK cells by co-incubating NK cells with NK-sensitive K562 cells. The association of NK cell function with clinical and laboratory measures was investigated.

Results. The proportions of NK cells were significantly lower in patients with active AOSD than in patients with inactive disease and controls. NK cell cytotoxic function was significantly lower in patients with AOSD than in controls. NK cell proportions and cytotoxic functions were reexamined in 11 and 6 patients, respectively, after treatment. Low NK cell proportion and cytotoxic dysfunction were improved with clinical improvements of the patients. IL-18 and IL-18BP levels were much higher in patients with active AOSD than in controls. NK cell cytotoxic functions were consistently low and IL-18 and IL-18BP levels were constantly high in patients with AOSD, regardless of disease activity.

Conclusion. Low NK cell proportion, defective cytotoxic function, and elevated IL-18 levels may be significant features of AOSD. After resolution of the acute phase, low NK cell proportion was recovered and NK cell cytolytic function was restored along with clinical improvement. These findings possibly contribute to immunologic abnormalities in AOSD. (First Release Aug 1 2012; J Rheumatol 2012;39:2000–7; doi:10.3899/jrheum.111500)

Key Indexing Terms:
ADULT-ONSET STILL'S DISEASE MACROPHAGE ACTIVATION SYNDROME
NATURAL KILLER CELL INTERLEUKIN 18 INTERLEUKIN 18 BINDING PROTEIN

From the Department of Rheumatology, Wonju Christian Hospital, Wonju College of Medicine, Yonsei University, Wonju City, Kangwon-do; Department of Rheumatology, Guro Hospital, Korea University, Seoul; The Institute of Rheumatology, and the Division of Rheumatology, Department of Internal Medicine, Hanyang University College of Medicine and the Hospital for Rheumatic Diseases, Hanyang University, Seoul; and Division of Rheumatology, Department of Internal Medicine, Inha University Hospital, Incheon, South Korea.

J.H. Park, MD, Clinical Assistant Professor, Department of Rheumatology, Wonju Christian Hospital, Wonju College of Medicine, Yonsei University; H-S. Kim, MD, Department of Rheumatology, Guro Hospital, Korea University; J.S. Lee, BS, The Institute of Rheumatology, Hanyang University; J.J. Kim, MD, Department of Internal Medicine, Hanyang University College of Medicine and the Hospital for Rheumatic Diseases, Hanyang University; K-H. Jung, MD, PhD, Division of Rheumatology, Department of Internal Medicine, Inha University Hospital; Y-W. Park, MD, PhD, Department of Rheumatology, Chonnam National University Medical School; D-H. Yoo, MD, PhD, Professor, Chairman, Department of Medicine, Hanyang University College of Medicine, The Institute of Rheumatology, Hanyang University, Division of Rheumatology, Department of Internal Medicine, the Hospital for Rheumatic Diseases.

Address correspondence to Dr. D-H. Yoo, The Hospital for Rheumatic Diseases, Hanyang University Medical Center, 17 Haengdang-Dong, Seongdong-Gu, Seoul 133-792, South Korea.
E-mail: dhyoo@hanyang.ac.kr

Accepted for publication June 18, 2012.

Macrophage activation syndrome (MAS) is a potentially fatal condition characterized by fever, cytopenia, hepatosplenomegaly, and lymphadenopathy accompanying excessive activation and proliferation of macrophages. MAS is quite similar to hemophagocytic lymphohistiocytosis (HLH), a not uncommon complication in adult-onset Still's disease (AOSD)[1,2,3]. Decreased natural killer (NK) cell proportion and defective NK cell cytotoxicity have been reported in children with HLH[4,5]. Despite the similarity with MAS and HLH, the exact relationship between phenotype and NK cell function in AOSD is not clear. It has been reported that interleukin 18 (IL-18) levels are much higher in patients with AOSD than in patients with other autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)[6,7,8]. High levels of IL-18 and macrophage activation are observed often in AOSD, and MAS has been reported with defective NK cell cytotoxic function in systemic onset juvenile idiopathic arthritis (soJIA)[9,10]. Hence NK cell function and serum IL-18 profiles seem to be dysregulated in AOSD. We investigated NK cell proportion

and NK cell cytotoxicity, as well as IL-18 expression, in patients with AOSD.

MATERIALS AND METHODS

Patients and controls. Forty-five patients meeting Yamaguchi's criteria for AOSD[11] and 32 age-matched and sex-matched healthy control subjects who had no history of autoimmune disease, infectious disease, or malignancy were included in our study in the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea. The study was approved by the institutional review board of Hanyang University Medical Center. Clinical and laboratory data were collected when patients visited the clinic. We recorded sex, age, medications, duration of disease, and the clinical features of AOSD: typical rash, arthralgia/arthritis, myalgia, sore throat, serositis, and lymphadenopathy. As laboratory measures, we determined white blood cell counts, platelet counts and hemoglobin levels in peripheral blood, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum level of ferritin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, NK cell proportions among peripheral blood mononuclear cells (PBMC), perforin, granzyme B expression, and cytokines IL-18 and IL-18-binding protein (IL-18BP). Active disease was defined as 2 or more of the following major diagnostic criteria of AOSD: fever ≥ 39°C, arthralgia/arthritis, typical rash, and leukocytosis including 80% or more of granulocytes[12].

Twenty-four patients with AOSD, including 16 with active disease and 8 inactive type, and 18 healthy controls were analyzed for NK cell cytotoxicities, perforin, and granzyme B expression.

Flow cytometric analysis. Proportions of NK cells as well as perforin and granzyme B expression were examined as described[13,14]. In brief, isolated PBMC using Ficoll-Paque (Amersham Bioscience, Buckinghamshire, UK) were stained with the following antibodies: FITC-labeled CD3 and phycoerythrin-labeled CD56 (Pharmingen, San Diego, CA, USA) for 20 min at 4°C. Cells were stained with antibodies against cell-surface molecules, and fixed and permeabilized with Cytofix/Cytoperm (Pharmingen).

After permeabilization, cells were stained with FITC-conjugated anti-perforin and FITC-conjugated granzyme B antibodies (Pharmingen) for 30 min at 4°C. Then the cells were washed and analyzed with a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA). The flow cytometry samples were gated on NK cells (defined as CD3−CD56+). Acquired flow cytometric data were analyzed using Cell Quest software (Becton Dickinson). The perforin and granzyme B-positive regions were compared to matching isotype controls.

NK cell cytotoxicity. NK cells were isolated from PBMC by negative selection using a MACS NK Cell isolation kit (Miltenyi Biotec Inc., Auburn, CA, USA) according to the manufacturer's instructions. NK cell cytotoxic activity was assessed after co-incubation of effector cells with target cells at different effector-to-target (E:T) ratios (2:1−16:1). As effector cells, we used NK cells isolated from PBMC in patients with AOSD and controls. Cells of the NK-sensitive K562 cell line were used as target cells. After incubation, 15 µl of CD45-FITC (Becton Dickinson) antibodies were added to each tube and 20 µl of propidium iodide (PI; Invitrogen) at 1 µl/ml was added to each tube at 10−15 min before acquisition. Flow cytometry was performed with a FACS Calibur flow cytometer. Percentages of dead cells were calculated from the PI-positive cells in the samples. Percentage-specific cytotoxicity was obtained by subtracting background cell death (total cell death minus spontaneous cell death).

IL-18, free IL-18, and IL-18BP measurements. IL-18 (Medical and Biological Laboratories, Nagoya, Japan) was measured using commercial ELISA kits. The minimum detectable concentration of the IL-18 was 12.5 pg/ml. If the absorbance of a sample exceeded 1000 pg/ml, the sample was diluted 5~20-fold. For serum levels of IL-18BP, ELISA plates were coated with anti IL-18BPa monoclonal antibodies [2 µg/ml in phosphate buffered saline (PBS), R&D Systems, Minneapolis, MN, USA] and incubated overnight at room temperature. The plates were washed with wash buffer followed by blocking with reagent diluent added to each well and incubated at room temperature for a minimum of 1 h. After further washing, samples and standards were added and the plates were incubated for 2 h at room temperature. After more washing, anti-IL-18BPa monoclonal antibody (200 ng/ml in PBS) was added and the plates were incubated for 2 h at room temperature. The plates were again washed and streptavidin conjugated to horseradish peroxidase was added and the plates were incubated for 20 min at room temperature. The reactions were stopped with 2 N H_2SO_4 and the optical density of each well was determined with a VersaMax microplate reader set to 450 nm (Molecular Devices, Sunnyvale, CA, USA). Free IL-18 was calculated by applying the law of mass action, using 1:1 stoichiometry for the complex of IL-18 and IL-18BPa and a dissociation constant of 0.4 nm[15,16,17].

Statistical analysis. Nonparametric analysis was used to compare NK cell proportion, cytotoxic activity, perforin, granzyme B expression, and serum IL-18 and IL-18BP expression in healthy controls and patients with active and those with inactive AOSD. Statistical difference in the marker levels between controls and patients with active and inactive AOSD were analyzed by Mann-Whitney U test. Results are represented as the median [interquartile range (IQR)], unless otherwise specified. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical presentation. Forty-five patients with AOSD (89% women; mean age 36 ± 10 yrs) and 32 healthy controls (84% women; mean age 34 ± 9 yrs) were studied. Forty-five patients with AOSD were assigned to the active (n = 22) or inactive (n = 23) disease group. Baseline characteristics and biologic findings of the 2 groups were summarized in Table 1. Median levels of biologic markers that represent disease activity of AOSD such as serum ferritin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were higher in the active disease group than in the patients with inactive disease. There were 11 (50%) steroid-naive untreated patients with active disease. Methotrexate (MTX) was used to treat 10 patients (45.5%) with active AOSD and 14 (60.9%) with inactive AOSD. Bone marrow aspiration was performed in 18 patients, but only 1 displayed hemophagocytic features. That patient also had the typical clinical features of hemophagocytosis, namely high fever, cytopenia, high ferritin level, and hepatosplenomegaly.

NK cell proportions and NK cell cytotoxicity. NK cell percentages were significantly lower in patients with active AOSD than in controls (median 5.5%, IQR 4.1−11.5, vs 11.1%, IQR 7.9−16.6; $p < 0.05$) and in patients with inactive AOSD as well (median 5.5%, IQR 4.1−11.5, vs 11.8%, IQR 7.7−15.5; $p < 0.05$; Figure 1A). Thirteen (28.9%, 13/45) of the patients with AOSD had NK cell proportions below the normal range (< 5%), compared with only 1 (3.1%, 1/32) among the controls. There were 11 patients with active AOSD (50%, 11/22) and 2 patients with inactive disease (8.7%, 2/23) who showed NK proportion below the normal range. The cytotoxicity of NK cells was positively dependent on the E:T ratio in both AOSD and controls, but the NK cell cytotoxicity was significantly lower in the patients with active AOSD and the patients with inactive disease than in

Table 1. Clinical characteristics and laboratory findings of patients with active and patients with inactive adult-onset Still's disease.

Measure	Active, n = 22	Inactive, n = 23	p
Female/male, n	18/4	22/1	NS
Age, yrs (range)	33.5 (18–62)	35.0 (26–56)	NS
Disease duration, mo (range)	12.0 (0–65)	31.5 (5–179)	< 0.001
Biochemistry, median (IQR)			
Hemoglobin, g/dl	11.1 (10.4–11.9)	11.5 (10.3–13.3)	NS
Leukocyte, 10^9/l	11.2 (8.0–15.2)	7.0 (6.1–8.8)	< 0.01
PMN, 10^9/l	0.8 (0.7–0.9)	0.7 (0.6–0.8)	< 0.01
Platelet, 10^9/l	256.5 (170.0–381.2)	250.0 (193.0–276.5)	NS
AST/ALT, U/l	32/22.5 (16/15–49/40)	19/15 (15/11–22/22)	< 0.05
LDH, U/l	277.5 (160.5–423.8)	166.0 (127.3–267.0)	NS
ESR, mm/h	79.0 (26.0–99.7)	20.5 (7.0–46.3)	< 0.001
CRP, mg/dl	4.5 (1.7–11.4)	0.2 (0.2–2.5)	< 0.001
Ferritin, ng/ml	1046.6 (148.5–3467.8)	24.5 (18.3–70.0)	< 0.001
Prednisolone dose, mg (range)	15.0 (0–50)	5.0 (0–15)	< 0.001
Receiving medication, n (%)			
Methotrexate	10 (45.5)	14 (60.9)	
Leflunomide	5 (22.7)	8 (37.8)	
Cyclosporine	4 (18.2)	2 (8.7)	

IQR: interquartile range; PMN: polymorphonuclear neutrophil; AST: aspartate transaminase; ALT: alanine transaminase; LDH: lactate dehydrogenase; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; NS: not significant.

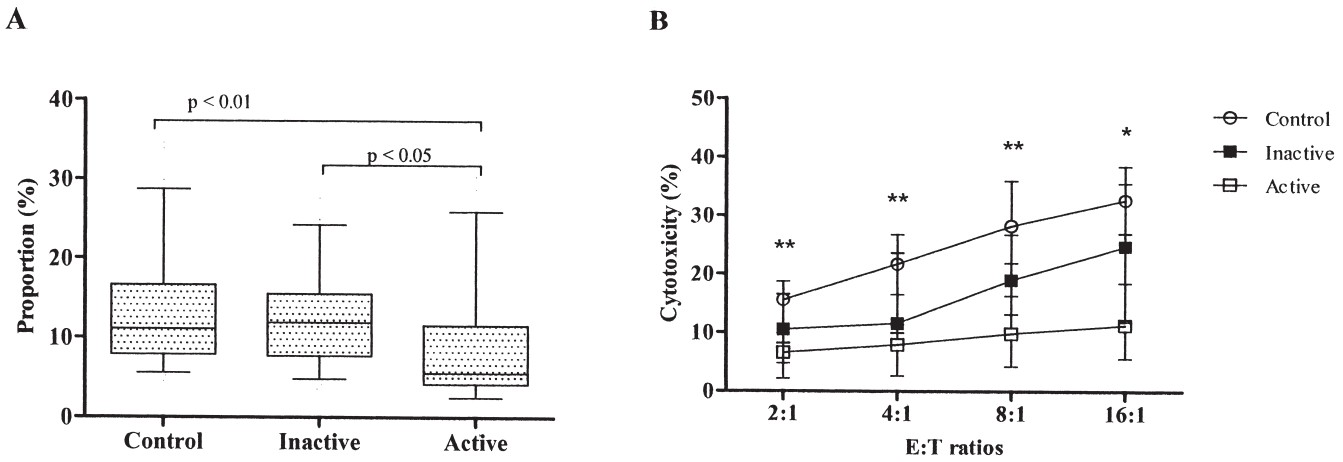

Figure 1. Proportion and cytotoxicity of natural killer (NK) cells in patients with adult-onset Still's disease (AOSD). A. Proportions of circulating NK cells in the 22 patients with active AOSD were reduced more than in the 23 patients with inactive AOSD and 32 healthy controls. Each box represents the 25th to 75th percentiles. Lines inside boxes represent the median. Whiskers represent 10th and 90th percentiles. B. Cytotoxic activity of NK cells against K562 cells. The cytotoxic activities of NK cells isolated from the peripheral blood mononuclear cells of 16 patients with active AOSD, 8 patients with inactive disease, and 18 healthy controls by negative selection were examined after co-incubation with NK-sensitive K562 cells at different effector-to-target (E:T) ratios. NK cell cytotoxic activity was significantly lower in patients with active AOSD than in patients with inactive AOSD and controls. The cytotoxicity of NK cells was positively dependent on the E:T ratio in both patients and controls. When E:T ratio was 16:1, NK cell cytotoxic activity was lower in patients with active AOSD than in the inactive AOSD, but the difference was not statistically significant (p = 0.08). All values in the figure are the medians. **p < 0.05, control vs active AOSD and inactive AOSD vs active AOSD, respectively. *p < 0.05, control vs active AOSD; p = 0.08, inactive AOSD vs active AOSD.

controls. When the E:T ratio was 16:1, NK cell cytotoxic activity was lower in the patients with active AOSD than in those with inactive AOSD, but the difference was not statistically significant (p = 0.08; Figure 1B). In the patients with AOSD, NK cell cytotoxicity and NK cell proportion were positively correlated (data not shown). Of 22 patients with active AOSD, 11 (50%) were steroid-naive untreated patients. NK proportion was not affected by use of systemic steroid (median 4.7%, IQR 3.3–11.8, vs 9.6%, IQR 4.3–14.9; p = 0.36) in patients with active AOSD. NK cell

cytotoxic function was similar in treated and steroid-naive untreated patients with active AOSD.

NK cell proportions were examined twice in 11 patients (at the onset and after treatment with systemic glucocorticoid and immunosuppressive agents) and in 6 of these, cytotoxic activity was also reexamined after 3 months of treatment. NK cell proportion increased in 9 (81.8%, 9/11) of the 11 patients, and their clinical features also improved. In 5 (83.3%, 5/6) of the 6 patients, NK cell function also improved (Figure 2). NK cell cytotoxic activity actually declined in 1 patient, and her clinical features also worsened.

Expression of perforin and granzyme B. Perforin expression was highly variable in our study. Although the proportions of perforin-positive cells appeared to be slightly reduced in the patients with AOSD, the effect was not statistically significant between 24 patients with AOSD (16 with active disease and 8 with inactive disease) and 18 controls (median 20.8% vs 29.8%, respectively; p = 0.09). Figure 3 shows perforin and granzyme B expression of a healthy control, a patient with inactive AOSD, and a patient with active AOSD. The proportions of granzyme B-positive cells were slightly decreased in patients with AOSD compared with controls (median 22.1% vs 27.4%; p = 0.09). However, there was no significant difference in perforin and granzyme B expression levels between 16 patients with active AOSD and 8 with inactive AOSD (median 20.2% vs 25.1%, p = 0.83; 20.2% vs 24.6%, p = 0.79, respectively), or between patients with active disease and healthy controls (median 20.2% vs 29.8%, p = 0.07; 20.2% vs 27.4%, p = 0.07, respectively).

IL-18 and IL-18BP levels. Serum IL-18, free IL-18, and IL-18BP levels were significantly higher in the patients with AOSD than in healthy controls (median 2463.2 pg/ml vs 170.3 pg/ml, $p < 0.001$; and 8289.0 pg/ml vs 1269.0 pg/ml, $p < 0.001$; 8289.0 pg/ml vs 1269.0 pg/ml, $p < 0.001$, respectively). Figures 4A and 4B show that serum IL-18 and free IL-18 levels were higher in patients with active AOSD than in patients with inactive AOSD (median 19,352.0 pg/ml vs 1648.7 pg/ml, $p < 0.005$; 15,384.8 pg/ml vs 1100.5 pg/ml, $p < 0.001$, respectively). Serum IL-18BP levels were lower in patients with active AOSD than in patients with inactive

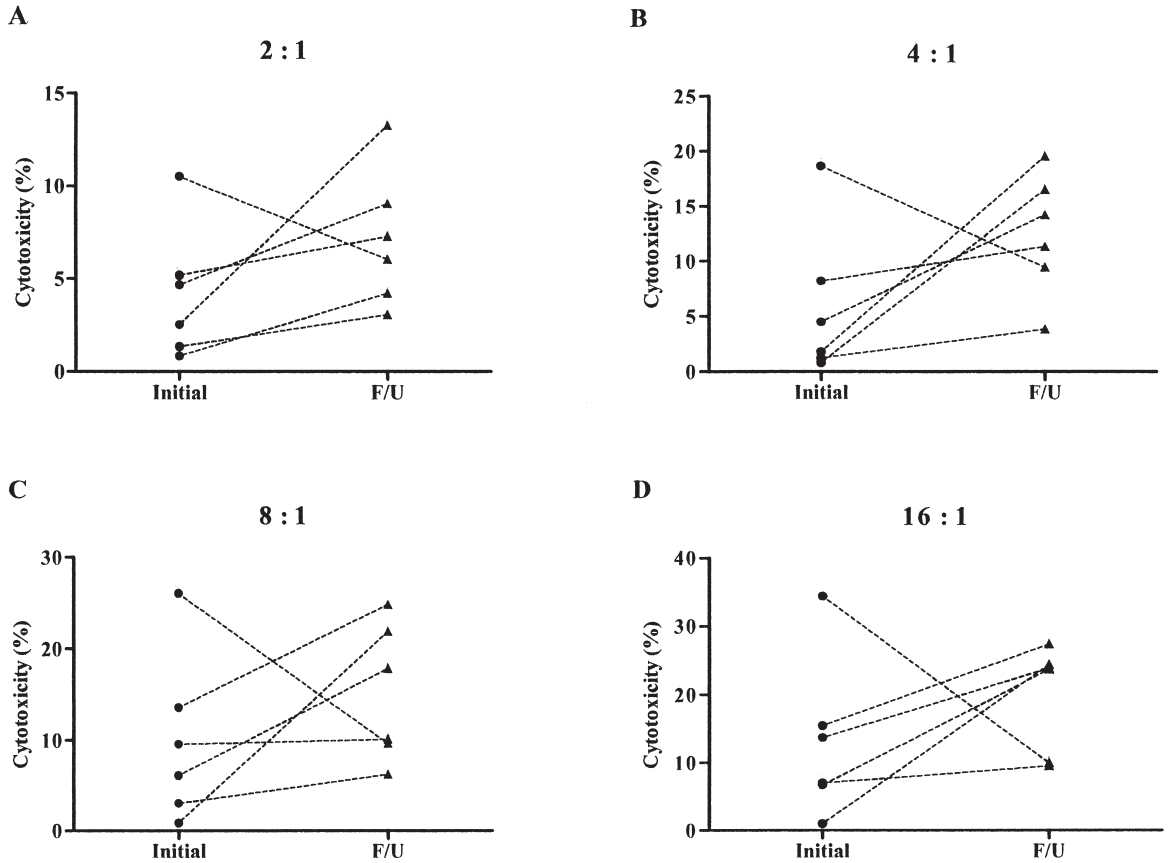

Figure 2. Natural killer (NK) cell cytotoxicity as a function after treatment of adult-onset Still's disease (AOSD). NK cell-mediated cytotoxicity against K562 cell line was evaluated at the indicated effector-to-target (E:T) ratios in 6 patients with AOSD in whom cytotoxic function was reexamined 3 months after proper management. NK cell cytotoxic activity increased in 5 of the patients (83.3%, 5/6) along with clinical improvement. NK cell cytotoxic activity declined in 1 patient, and her clinical features also worsened.

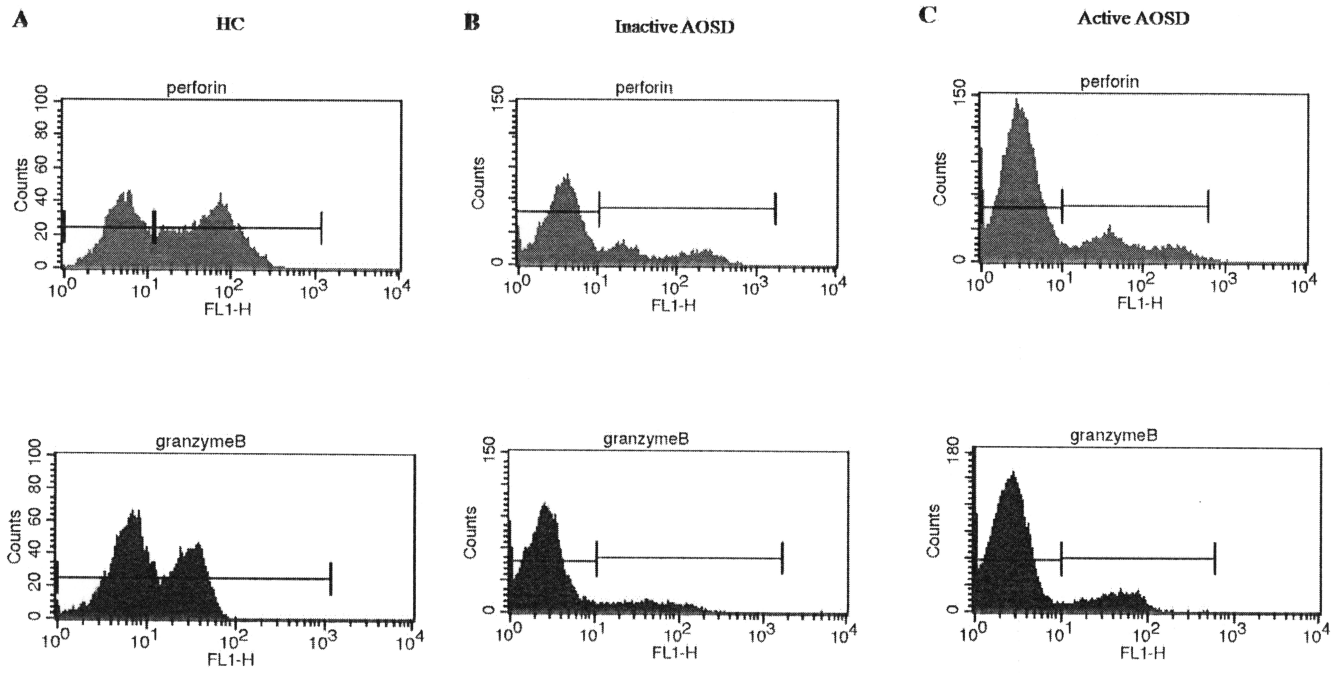

Figure 3. Flow cytometric analysis of perforin and granzyme B expression in natural killer (NK) cells. Expression of perforin and granzyme B in NK cells from a healthy control, a patient with inactive, and a patient with active adult-onset Still's disease (AOSD), as determined by intracellular flow cytometry. A. In a healthy control (HC) subject, NK cells express high levels of granzyme B and perforin. B. In a patient with inactive AOSD, a slightly decreased proportion of perforin and granzyme B positive NK cells. C. In a patient with active AOSD, perforin and granzyme B expression were mildly decreased in NK cells.

AOSD (median 7941.2 pg/m vs 8894.8 pg/m, $p = 0.36$), but these values were not statistically significant (Figure 4C). Clinical improvement was accompanied by decreased IL-18 and free IL-18 levels, when those cytokine levels were compared with patients with active AOSD and patients with inactive AOSD. However, serum IL-18, free IL-18, and IL-18BP levels were constantly higher in patients with AOSD than in controls, regardless of disease activity. In the 7 patients whose cytokine profiles were followed up, median IL-18 levels decreased and IL-18BP levels were a little decreased; the differences were small (median 6824.6 pg/ml to 413.6 pg/ml, 6240.1 pg/ml to 6448.3 pg/ml, respectively) and there was no statistical significance (Figure 4D). IL-18 levels were proportional to the levels of several conventional biomarkers, such as ferritin, ESR, and CRP ($r = 0.34$, 0.29, and 0.29, respectively, $p < 0.05$). Similar proportional results were noted for free IL-18 levels, but not for IL-18BP levels, compared to the levels of conventional biomarkers (data not shown).

DISCUSSION

Several reports have suggested that depressed NK cell function may be a feature of soJIA[9,18] as well as several other rheumatic diseases[19,20,21]. We found that NK cell proportions and cytotoxic activities were decreased in patients with active AOSD. The reason NK cells behave abnormally in patients with AOSD is not well understood. AOSD and soJIA share several clinical and laboratory characteristics and are often seen as related to MAS[1]. Hence, the immunologic abnormalities seen in MAS may be relevant to the pathogenesis of AOSD. Arlet and colleagues demonstrated that macrophages were highly activated and IL-18 levels were increased in AOSD and that IL-18 seemed to be a key cytokine in pathogenesis of AOSD[22]. Several studies have shown that defective functioning of cytotoxic T lymphocytes and NK cells is associated with reduced expression of perforin and granzyme B, which may be responsible for the cytotoxic activity of these cells. Defective cytolytic function may be responsible for the inability to destroy pathogens and persistent lymphocyte and macrophage activation[9,18,23,24]. There is a report that corticosteroid treatment suppresses NK cell function[13]. Nevertheless, the depressed NK cell cytotoxicity seems to be a feature of AOSD. In our study of 22 patients with active AOSD, 11 (50%) were steroid-naive untreated patients. Active cases, including untreated patients, showed more prominent low proportions of NK cells and poor cytotoxic function as well. Patients with active AOSD had shorter disease duration, so they might be less exposed to immunosuppressive agents. Although active patients received high-dose systemic steroids, half the patients were steroid-naive. NK cell dysfunction and low NK cell proportions were not affected by systemic steroid. Impaired NK function might occur regardless of steroid administration. In addition, several patients

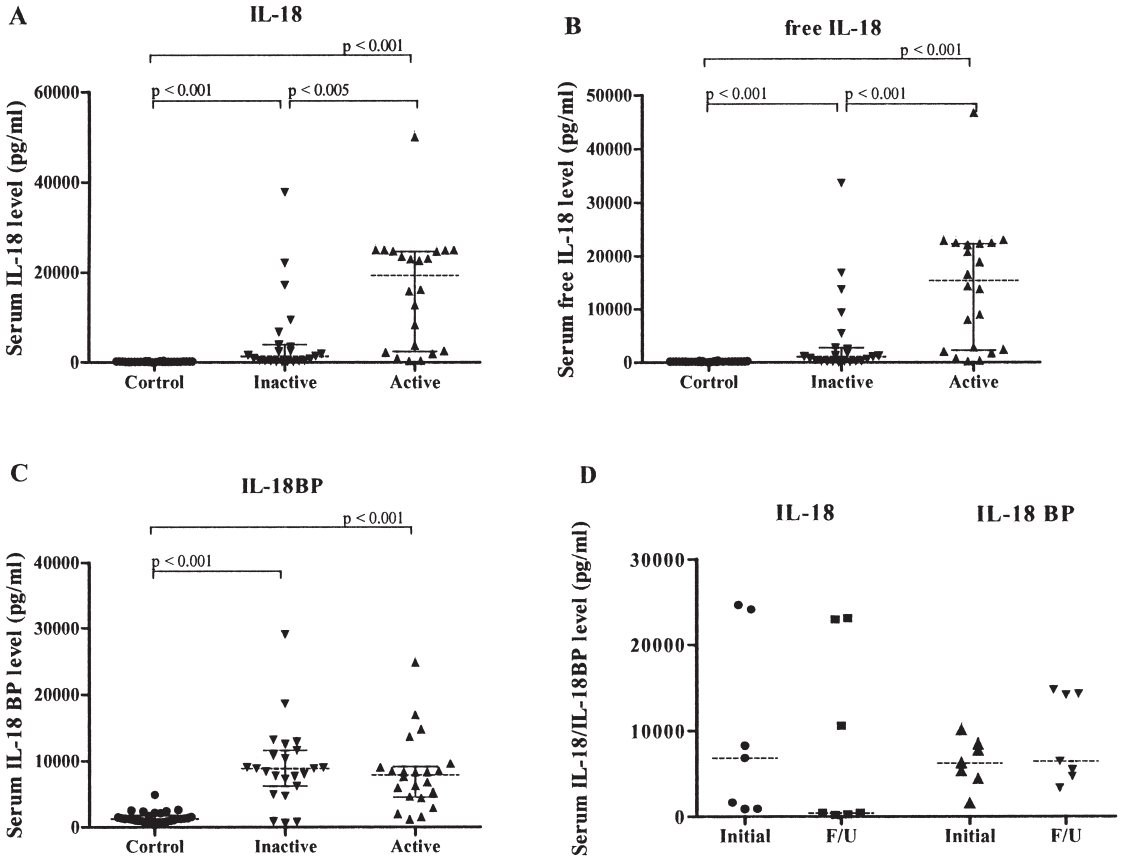

Figure 4. Serum interleukin 18 (IL-18), free IL-18, and IL-18-binding protein (IL-18BP) levels in the 45 patients with adult-onset Still's disease (AOSD; active = 22, inactive = 23) and 32 healthy controls. A, B, and C: Serum IL-18, free IL-18, and IL-18BP levels, respectively; levels were much higher in patients with AOSD than in controls, regardless of disease activity. Serum IL-18BP levels were lower in patients with active than in patients with inactive AOSD, but these values were not statistically significant. D. IL-18 levels and IL-18BP levels in 7 patients with AOSD whose cytokine profiles were followed after 3 months of treatment. There were no statistically significant differences. All measures are median. FU: followup.

with soJIA who received only nonsteroidal antiinflammatory drugs had markedly depressed NK cell activity[9], and NK cell function returned to normal levels after autologous stem cell transplantation[23]. Although no other control disease groups were included in our study, low NK cell proportion was more prominent in patients with active AOSD and correlated significantly with NK cell cytotoxicities, suggesting that defective NK cell functions are likely to be associated with NK deficiency.

We found that there were no significantly different perforin and granzyme B expressions in patients with AOSD and in controls, even between patients with active AOSD and controls. Our data suggest that expression of perforin and granzyme B is not related to the NK cell dysfunction in patients with AOSD, as it is thought to be in patients with soJIA[9]. The defective NK cell cytotoxic function could be associated with the other possible cytotoxic enzymes rather than perforin and granzyme B.

In our study, NK cell proportions and cytotoxic functions were reexamined in 11 and in 6 patients, respectively. In the second test, NK cell proportions increased slightly in 9 of 11 patients. Interestingly, NK cell cytotoxic function was lower in patients with AOSD than in healthy controls, even in the patients who displayed no clinical disease activity. NK cell proportions were significantly lower in patients with active AOSD than in controls. Low NK cell numbers have been reported in 1 study[25], and another study noted a moderate correlation between NK cell function and NK cell number in soJIA[9]. Our data demonstrate that low NK cell proportion and impaired cytotoxic function were attenuated in patients with inactive AOSD compared with patients with active disease. These findings suggest that low NK cell proportions could be recovered after treatment and NK cell cytolytic function could be restored along with clinical improvement; hence our results show that NK cell dysfunction and low NK cell proportions could be relevant to the development of AOSD. However, our data showed that low NK cell percentage was improved with treatment, but that function was not fully recovered and was lower than in healthy controls until the second examination. Because we did not perform

complete blood counts in the controls, we do not know the exact numbers of NK cells in them, and that is one limitation of our study.

It has been reported that several proinflammatory cytokines such as IL-6, IL-8, tumor necrosis factor, and IL-18 are increased in AOSD[7,12,26]. We did not evaluate other proinflammatory cytokines; however, IL-18, in particular, seems to play a crucial role in AOSD[6,22,27,28]. We found that serum IL-18 and IL-18BP levels were profoundly higher in patients with AOSD than in healthy controls and that serum IL-18 levels were higher in patients with active AOSD than in those with inactive disease. IL-18 is considered a marker of active disease and IL-18BP is considered a natural IL-18 inhibitor, with very high affinity (400 pmol/l) for IL-18[17,28,29]. Clinical improvement was accompanied by decreased IL-18 and free IL-18 levels, and IL-18BP levels showed a slight tendency to increase in the patients with no clinical activity, compared with patients with active AOSD and those with inactive disease. IL-18 profiles were reexamined for only 7 patients, hence further evaluations were needed to determine the correlation between clinical disease activity and IL-18 profiles. However, IL-18, free IL-18, and IL-18BP were constantly high in patients with AOSD, regardless of disease activity. Decreased proportion and dysfunction of NK cells in AOSD flares could be considered a consequence of excess cytokines. In our study, NK cell cytotoxic function was consistently lower and IL-18 profiles were higher in patients with AOSD than in healthy controls, regardless of clinical disease activity. The functional derangement of the IL-18-NK cell axis needs to be further investigated.

Decreased NK cell proportions and defective NK cell function may be relevant features of active AOSD. NK cell proportion was elevated after treatment and NK cell cytolytic function was restored along with clinical improvement. However, NK function was not fully recovered and was lower than in healthy controls, and IL-18 and IL-18BP were constantly high in patients with AOSD, regardless of disease activity. These findings possibly contribute to immunologic abnormalities in AOSD. Further studies are needed to clarify the role of the IL-18-NK cell axis in AOSD.

REFERENCES

1. Grom AA. Macrophage activation syndrome and reactive hemophagocytic lymphohistiocytosis: The same entities? Curr Opin Rheumatol 2003;15:587-90.
2. Arlet JB, Le TH, Marinho A, Amoura Z, Wechsler B, Papo T, et al. Reactive haemophagocytic syndrome in adult-onset Still's disease: A report of six patients and a review of the literature. Ann Rheum Dis 2006;65:1596-601.
3. Dhote R, Simon J, Papo T, Detournay B, Sailler L, Andre MH, et al. Reactive hemophagocytic syndrome in adult systemic disease: Report of twenty-six cases and literature review. Arthritis Rheum 2003;49:633-9.
4. Sullivan KE, Delaat CA, Douglas SD, Filipovich AH. Defective natural killer cell function in patients with hemophagocytic lymphohistiocytosis and in first degree relatives. Pediatr Res 1998;44:465-8.
5. Egeler RM, Shapiro R, Loechelt B, Filipovich A. Characteristic immune abnormalities in hemophagocytic lymphohistiocytosis. J Pediatr Hematol Oncol 1996;18:340-5.
6. Kawashima M, Yamamura M, Taniai M, Yamauchi H, Tanimoto T, Kurimoto M, et al. Levels of interleukin-18 and its binding inhibitors in the blood circulation of patients with adult-onset Still's disease. Arthritis Rheum 2001;44:550-60.
7. Chen DY, Lan JL, Lin FJ, Hsieh TY. Proinflammatory cytokine profiles in sera and pathological tissues of patients with active untreated adult onset Still's disease. J Rheumatol 2004;31:2189-98.
8. Maruyama J, Inokuma S. Cytokine profiles of macrophage activation syndrome associated with rheumatic diseases. J Rheumatol 2010;37:967-73.
9. Villanueva J, Lee S, Giannini EH, Graham TB, Passo MH, Filipovich A, et al. Natural killer cell dysfunction is a distinguishing feature of systemic onset juvenile rheumatoid arthritis and macrophage activation syndrome. Arthritis Res Ther 2005;7:R30-7.
10. Grom AA. Natural killer cell dysfunction: A common pathway in systemic-onset juvenile rheumatoid arthritis, macrophage activation syndrome, and hemophagocytic lymphohistiocytosis? Arthritis Rheum 2004;50:689-98.
11. Yamaguchi M, Ohta A, Tsunematsu T, Kasukawa R, Mizushima Y, Kashiwagi H, et al. Preliminary criteria for classification of adult Still's disease. J Rheumatol 1992;19:424-30.
12. Fujii T, Nojima T, Yasuoka H, Satoh S, Nakamura K, Kuwana M, et al. Cytokine and immunogenetic profiles in Japanese patients with adult Still's disease. Association with chronic articular disease. Rheumatology 2001;40:1398-404.
13. Nair MP, Schwartz SA. Immunomodulatory effects of corticosteroids on natural killer and antibody-dependent cellular cytotoxic activities of human lymphocytes. J Immunol 1984;132:2876-82.
14. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood 2002;99:61-6.
15. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: A novel modulator of the Th1 cytokine response. Immunity 1999;10:127-36.
16. Novick D, Schwartsburd B, Pinkus R, Suissa D, Belzer I, Sthoeger Z, et al. A novel IL-18BP ELISA shows elevated serum IL-18BP in sepsis and extensive decrease of free IL-18. Cytokine 2001; 14:334-42.
17. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci USA 2000;97:1190-5.
18. Grom AA, Villanueva J, Lee S, Goldmuntz EA, Passo MH, Filipovich A. Natural killer cell dysfunction in patients with systemic-onset juvenile rheumatoid arthritis and macrophage activation syndrome. J Pediatr 2003;142:292-6.
19. Papo T, Andre MH, Amoura Z, Lortholary O, Tribout B, Guillevin L, et al. The spectrum of reactive hemophagocytic syndrome in systemic lupus erythematosus. J Rheumatol 1999;26:927-30.
20. Baxter AG, Smyth MJ. The role of NK cells in autoimmune disease. Autoimmunity 2002;35:1-14.
21. Deane S, Selmi C, Teuber SS, Gershwin ME. Macrophage activation syndrome in autoimmune disease. Int Arch Allergy Immunol 2010;153:109-20.
22. Arlet JB, Le Thi Huong DB, Pouchot J, Piette JC. [Current concepts on the physiopathology of adult-onset Still's disease]. Rev Med Interne 2005;26:549-56.

23. Wulffraat NM, Rijkers GT, Elst E, Brooimans R, Kuis W. Reduced perforin expression in systemic juvenile idiopathic arthritis is restored by autologous stem-cell transplantation. Rheumatology 2003;42:375-9.
24. Takada H, Nomura A, Ohga S, Hara T. Interleukin-18 in hemophagocytic lymphohistiocytosis. Leuk Lymphoma 2001;42:21-8.
25. Wouters CH, Ceuppens JL, Stevens EA. Different circulating lymphocyte profiles in patients with different subtypes of juvenile idiopathic arthritis. Clin Exp Rheumatol 2002;20:239-48.
26. Scheinberg MA, Chapira E, Fernandes ML, Hubscher O. Interleukin 6: A possible marker of disease activity in adult onset Still's disease. Clin Exp Rheumatol 1996;14:653-5.
27. Sugiura T, Kawaguchi Y, Harigai M, Terajima-Ichida H, Kitamura Y, Furuya T, et al. Association between adult-onset Still's disease and interleukin-18 gene polymorphisms. Genes Immun 2002; 3:394-9.
28. Choi JH, Suh CH, Lee YM, Suh YJ, Lee SK, Kim SS, et al. Serum cytokine profiles in patients with adult onset Still's disease. J Rheumatol 2003;30:2422-7.
29. Banda NK, Vondracek A, Kraus D, Dinarello CA, Kim SH, Bendele A, et al. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. J Immunol 2003;170:2100-5.