Serum Levels of Interleukin 33 and Soluble ST2 Are Associated with the Extent of Disease Activity and Cutaneous Manifestations in Patients with Active Adult-onset Still's Disease

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ABSTRACT. ***Objective.*** Interleukin 33 (IL-33), a member of the IL-1 family and a ligand of the orphan receptor ST2, plays key roles in innate and adaptive immunity. We examined the associations between IL-33/ST2 levels and clinical manifestations of patients with active adult-onset Still's disease (AOSD).

Methods. Blood samples were collected from 40 patients with active AOSD, 28 patients with rheumatoid arthritis (RA), and 27 healthy controls (HC). The serum levels of IL-33 and soluble ST2 were determined using ELISA. Expression levels of IL-33 and ST2 in biopsy specimens obtained from 34 AOSD patients with rash were immunohistochemically investigated.

Results. IL-33 levels of patients with AOSD were higher than those of patients with RA and HC. Soluble ST2 levels of patients with AOSD were higher than those of HC, but not of patients with RA. Serum IL-33 levels correlated with systemic score, erythrocyte sedimentation rate, ferritin levels, and aspartate transaminase levels. However, serum soluble ST2 levels correlated only with ferritin levels. The numbers of inflammatory cells expressing IL-33 and ST2 were elevated in skin lesions of patients with AOSD compared to HC, but did not differ from those of the skin lesions of eczema or psoriasis.

Conclusion. We found significantly higher serum IL-33 and soluble ST2 levels in patients with active AOSD. Results indicate that the IL-33/ST2 signaling pathway may play a role in the pathogenesis of the acute inflammation and skin manifestations associated with AOSD. (First Release April 1 2017; J Rheumatol 2017;44:740–7; doi:10.3899/jrheum.170020)

Key Indexing Terms:
ADULT ONSET STILL'S DISEASE | INTERLEUKIN 33 | SOLUBLE ST2
BIOMARKER | DISEASE ACTIVITY

Adult-onset Still's disease (AOSD) is a rare systemic inflammatory disorder of unknown etiology. The major clinical features of AOSD are a high spiking fever, an evanescent rash, polyarthralgia, arthritis, and hepatosplenomegaly. Although laboratory data are nonspecific, also observed are leukocytosis with neutrophilia, thrombocytosis, elevated levels of acute-phase reactants such as the C-reactive protein (CRP) and ferritin, and elevated levels of liver enzymes. The pathogenesis of AOSD remains unclear, but the condition is considered an autoinflammatory syndrome[1]. Several factors, including infection, a genetic predisposition, and environmental variables have been suggested as triggers for the development of immune dysregulation. Tumor necrosis factor-α (TNF-α) and proinflammatory cytokines interleukin 1β (IL-1β), IL-6, IL-8, and IL-18 have been suspected of involvement in the acute inflammation associated with AOSD[2,3]. In particular, IL-1β is thought to play an important clinical role in AOSD, being involved in the development of fever, rash, and peripheral neutrophilia by activating the thermoregulatory center, the endothelium, and the bone marrow[4,5].

IL-33, a member of the IL-1 family and a ligand of the orphan receptor ST2, is important in innate and adaptive immunity[6]. IL-33 is constitutively expressed in the steady-state in the nuclei of several cell types, including epithelial, endothelial, and fibroblast cells[7,8]. IL-33 is

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released by damaged epithelial or endothelial cells, and might act as a dual-function alarmin with both intracellular and extracellular functions similar to those of the high-mobility group box 1 (HMGB1) and S100 proteins[9]. IL-33 binds the 2 different isoforms of ST2: ST2L, which is membrane-bound and activates intracellular signaling; and ST2, which is soluble, acting as a decoy to prevent ligand binding to the membrane-bound form[10]. Because IL-33 released upon cell death and during stress can induce excessive inflammation, IL-33/ST2 signaling has been evaluated in several infectious and inflammatory diseases[8]. The cytokine and its receptor appear to be involved in several chronic inflammatory conditions, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren syndrome, and inflammatory bowel disease[11,12,13,14]. A study showed that soluble serum ST2 levels correlated with the clinical extent of disease activity in patients with systemic juvenile idiopathic arthritis (JIA)[15].

Previously, we showed that levels of S100 and HMGB1 proteins were elevated in patients with AOSD, and that these levels correlated with the extent of AOSD clinical activity[16,17,18]. Such results suggested that alarmins may significantly affect acute inflammatory pathogenesis of AOSD. However, no study has yet addressed the roles played by IL-33 and soluble ST2 in patients with AOSD. Therefore, we measured the serum levels of IL-33 and soluble ST2 and explored their associations with clinical activity in patients with active AOSD. Also, we immunohistochemically analyzed skin biopsy material for IL-33 and ST2 expression in 34 patients with active untreated AOSD to explore the *in vivo* relevance of these markers to AOSD.

MATERIALS AND METHODS

Subjects. Forty patients with active AOSD, 28 patients with RA, and 27 healthy controls (HC) were enrolled. Informed consent was obtained from all subjects; blood samples were then collected. Patients with AOSD were diagnosed using Yamaguchi's criteria after exclusion of those with infectious, autoimmune, or neoplastic disorders[19]. Also, we excluded patients who had a history of allergic diseases. Patients with RA satisfied the American College of Rheumatology 1987 revised criteria for the classification of RA[20]. HC were recruited by public advertisements and screened (by questionnaire) for any history of autoimmune, rheumatic, or any other diseases. Followup samples were collected from 16 of the 40 patients with AOSD 8.6 ± 8.3 months later. Medical histories, clinical features, and the outcomes of physical examination were entered into a database at the time of blood sampling. Each sample underwent a complete blood count, measurement of erythrocyte sedimentation rate (ESR), and CRP assay, rheumatoid factor, antinuclear antibody, and ferritin levels (normal 13–150 ng/ml for women and 30-400 ng/ml for men). AOSD disease activity was evaluated as previously described. The scores ranged from 0 to 12 with 1 point being given for each of the following manifestations: fever, a typical rash, pleuritis, pneumonia, pericarditis, hepatomegaly or an abnormal liver function test, splenomegaly, lymphadenopathy, leukocytosis ≥ 15,000/mm^2, a sore throat, myalgia, or abdominal pain[21]. The study was approved by the Ajou University Hospital Institutional Review Board (IRB No: BMR-OBS-16-099).

IL-33 and soluble ST2 assays. All sera were prepared by centrifugation of fresh peripheral blood samples and stored at –70°C immediately after collection. IL-33 levels were measured using a commercial ELISA kit (Quantikine, R&D Systems) according to the manufacturer's instructions. Soluble ST2 levels were also determined using an ELISA kit (Quantikine, R&D Systems) according to the manufacturer's instructions.

Immunohistochemical evaluation of skin biopsy samples. Skin biopsies were obtained from 34 patients with AOSD who had skin lesions; H&E sections were examined. For comparison, skin biopsies were obtained from 5 HC, 5 patients with psoriasis, and 5 with eczema. All slides were independently examined by 3 pathologists (JHH, JEK, and HY) for several skin histological variables, including epidermal changes, the extent of inflammatory cell infiltration, and the presence of karyorrhexis, vasculitis, and interstitial mucin deposition.

Immunohistochemistry was performed on formalin-fixed paraffin-embedded sections prepared using a Benchmark XT automated staining system (Ventana Medical Systems Inc.). The primary antibodies used were anti-IL-33 (1:50) and anti-ST2 (1:200; R&D Systems). Antigens were detected using a Ventana Optiview DAB kit (Ventana Medical Systems). Scores were calculated by dividing the number of positive inflammatory cells by the number of all inflammatory cells, and were graded on a scale from 1 to 3: 1, 1%-33%; 2, 34%-66%; and 3, 67%-100%. The scores for endothelial cells and keratinocytes were calculated similarly. The pathologists were blinded and there were some differences among the pathologists. The mean values of the reading scores were determined to be final results.

Statistical analyses. The levels of serum IL-33 and soluble ST2 are shown as means ± SD. Differences between the groups were evaluated using the independent t test or the Mann-Whitney U test. Correlations between disease activity markers and cytokine levels were evaluated using Pearson's or Spearman's correlation test. The Wilcoxon signed-rank test was used to compare cytokine levels in patients who underwent followup serum sampling. All statistical analyses were performed using SPSS version 20.0. A p value < 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of the patients. Table 1 shows the clinical characteristics of the 40 patients with AOSD, those with RA, and HC. The mean age of patients with AOSD was 42.5 ± 17.2 years, and 85% of all patients were women. There was no significant difference in age or sex among the groups. The clinical symptoms of patients with AOSD included a high spiking fever (95%), rash (85%), sore throat (52.5%), arthritis (50%), and hepatomegaly (17.5%). All patients with AOSD were in the initial stage of active disease prior to treatment. The symptom duration of patients with AOSD was 1.0 ± 0.6 months. The disease duration in patients with RA was 41.4 ± 27.7 months. Among patients with RA, 18 (64.3%) were treated with methotrexate (MTX) at the time of sampling, 22 (78.6%) with hydroxychloroquine (HCQ), and 5 (17.8%) with leflunomide. Daily glucocorticosteroid dose of the patients with RA was 2.28 ± 1.87 mg prednisolone equivalent.

Serum IL-33 and ST2 levels in patients with AOSD. Figure 1 shows serum IL-33 and soluble ST2 levels of patients with AOSD, patients with RA, and HC. IL-33 levels of patients with AOSD (14.2 ± 6.9 ng/ml) were higher than those of patients with RA (7.9 ± 5.4 ng/ml, $p < 0.001$) and HC (7.3 ± 3.2 ng/ml, $p < 0.001$; Figure 1A). Soluble ST2 levels of patients with AOSD (1031.9 ± 431.2 pg/ml) were higher than those of HC (507.9 ± 221.5 pg/ml, $p < 0.001$) but not those of patients with RA (922.0 ± 206.7 pg/ml, $p = 0.167$; Figure 1B).

Table 1. Clinical characteristics of patients. All values are presented as n (percent) or mean ± SD.

Characteristics	Active AOSD, n = 40	Inactive AOSD, n = 16	RA, n = 28	HC, n = 27
Age, yrs	42.5 ± 17.2	38.9 ± 15.6	48 ± 9.5	42.8 ± 10.5
Sex, F/M	34/6	12/4	24/4	24/3
Symptom duration or disease duration, mos	1.0 ± 0.6	8.6 ± 8.3	41.4 ± 27.7	
Fever	38 (95)	0 (0)		
Sore throat	21 (52.5)	0 (0)		
Rash	34 (85)	1 (6.3)		
Lymphadenopathy	14 (35)	0 (0)		
Splenomegaly	8 (20)	0 (0)		
Hepatomegaly	7 (17.5)	0 (0)		
Pericarditis	6 (15)	0 (0)		
Pleuritis	5 (12.5)	0 (0)		
Arthralgia	36 (90)	2 (12.5)		
Arthritis	20 (50)	1 (6.3)		
Hemoglobin, g/dl	11.2 ± 1.7	12.7 ± 1.7	12.9 ± 1.4	
Leukocyte, /μl	13,689 ± 4920	8256 ± 1660	7692.9 ± 3437	
Platelet, $\times 10^3$/μl	307.3 ± 113.2	258.3 ± 57.2	253.8 ± 61.6	
Ferritin, ng/ml	6775.4 ± 10,307.6	159.8 ± 206.1		
ESR, mm/h	57.8 ± 21.5	10.0 ± 8.7	28.5 ± 17.0	
CRP, mg/dl	8.76 ± 6.34	0.17 ± 0.19	0.93 ± 2.26	
AST, mg/dl	77.8 ± 64.3	23.9 ± 7.8	24.8 ± 10.6	
ALT, mg/dl	79.3 ± 92.2	23.1 ± 12.2	23.5 ± 18.0	
Bilirubin, mg/dl	0.70 ± 0.77	0.76 ± 0.33	0.58 ± 0.21	
Albumin, g/dl	3.70 ± 0.65	4.46 ± 0.22	4.44 ± 0.29	
ANA positivity	4 (10)		7 (25)	
RF positivity	1 (2.5)		26 (75)	
Systemic score	5.37 ± 1.30	0.19 ± 0.40		
DAS28			3.87 ± 1.13	
Methotrexate		12 (75.0)	18 (64.3)	
Cyclosporine		2 (12.5)	0 (0)	
Leflunomide		0 (0)	5 (17.8)	
Hydroxychloroquine		5 (31.3)	22 (78.6)	
Daily corticosteroid (mg prednisolone equivalent)		5.86 ± 3.11	2.28 ± 1.87	

The systemic scoring system of Pouchot, *et al*[21] assigns a score from 0 to 12 with 1 point for each of the following manifestations: fever, typical rash, pleuritis, pneumonia, pericarditis, hepatomegaly or abnormal liver function test data, splenomegaly, lymphadenopathy, leukocytosis ≥ 15,000/mm^2, sore throat, myalgia, and abdominal pain. AOSD: adult-onset Still's disease; RA: rheumatoid arthritis; HC: healthy controls; LDH: lactate dehydrogenase; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AST: aspartate transaminase; ALT: alanine transaminase; ANA: antinuclear antibody; RF: rheumatoid factor; DAS28: 28-joint Disease Activity Score.

Followup samples were collected from 16 patients with AOSD 8.6 ± 8.3 months after the first samplings. The systemic scores were 0.19 ± 0.40 (Table 1). In followup patients with AOSD, 12 (75%) were treated with MTX at the time of the sampling, 5 (31.3%) with HCQ, and 2 (12.5%) with cyclosporine. Daily glucocorticosteroid dose of the patients was 5.86 ± 3.11 mg prednisolone equivalent. Mean IL-33 level was 8.2 ± 6.1 ng/ml, and that of soluble ST2 was 1045.2 ± 377.0 pg/ml. Systemic score had decreased to 0.19 ± 0.40. Serum IL-33 level fell after disease activity resolved (p = 0.008; Figure 2A). However, soluble ST2 level did not change (p = 0.679; Figure 2B).

Correlations between serum IL-33 and soluble ST2 levels and disease activity in patients with AOSD. Table 2 shows the correlations between levels of disease activity markers and those of serum IL-33 or soluble ST2 in patients with AOSD. Serum IL-33 level correlated with systemic disease scores (r = 0.476, p = 0.002), ESR (r = 0.530, p < 0.001), and with levels of ferritin (r = 0.333, p = 0.036) and aspartate transaminase (AST; r = 0.335, p = 0.035). However, serum soluble ST2 level correlated with only ferritin level (r = 0.379, p = 0.016). Also, serum IL-33 level did not correlate with that of soluble ST2.

We analyzed serum IL-33 and soluble ST2 levels regarding the manifestations of AOSD, especially arthritis. However, IL-33 levels were not different between patients with arthritis (15.4 ± 6.3 ng/ml) and those without (13.0 ± 7.4 ng/ml, p = 0.258). Also, soluble ST2 levels were not

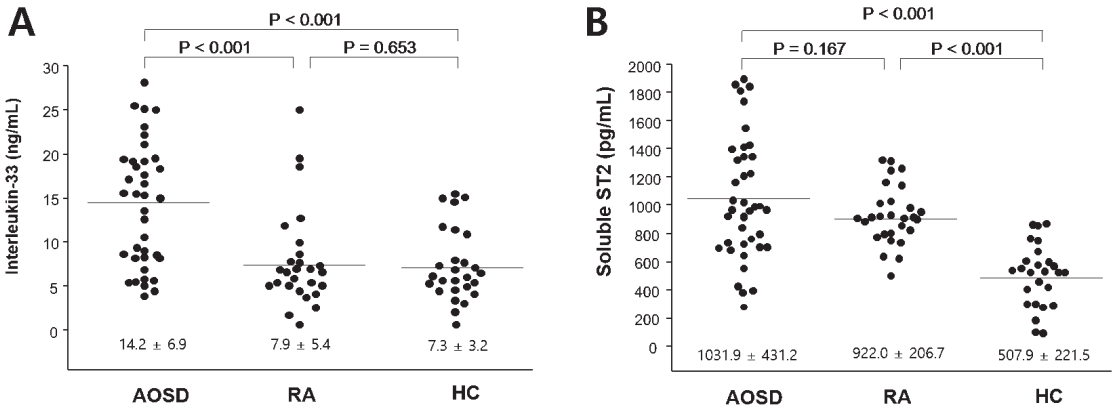

Figure 1. Levels of interleukin 33 (A) and soluble ST2 (B) in 40 patients with adult-onset Still's disease (AOSD), 28 patients with rheumatoid arthritis (RA), and 27 healthy controls (HC). Data are expressed as mean ± SD. The independent t test was used for statistical comparisons.

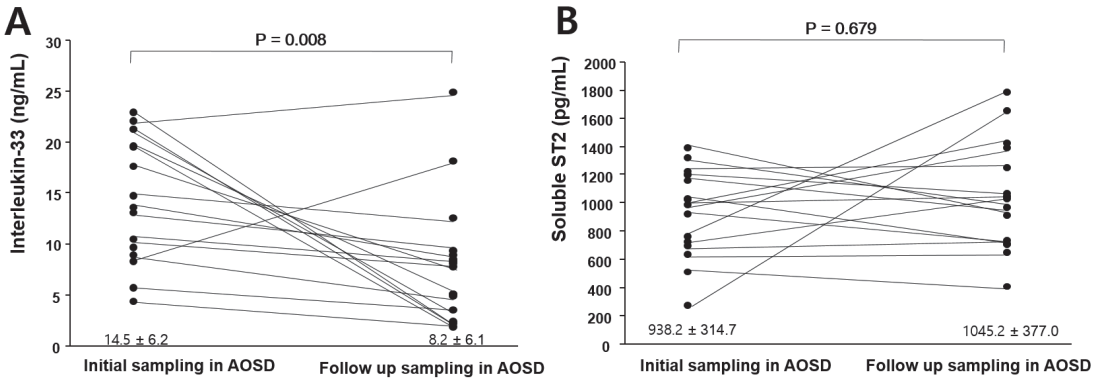

Figure 2. Levels of interleukin 33 (A) and soluble ST2 (B) in 16 patients with adult-onset Still's disease (AOSD), by the extent of disease activity. Data are expressed as mean ± SD. The Wilcoxon signed-rank test was used to perform statistical analysis.

different between patients with arthritis (1068.5 ± 384.9 pg/ml) and those without (995.2 ± 480.2 pg/ml; p = 0.597).

Histopathological characteristics of the skin, and immunohistochemical data. Almost all skin biopsies exhibited sparse lymphocytic or histiocytic infiltration in the upper dermis. More than half of all cases had nuclear debris in the dermis (20 cases; 58.8%). Neutrophil infiltrations were evident in some cases (10 cases; 29.4%). Mucin deposition was frequent in the dermis (18 cases; 52.9%). Immunohistochemical staining revealed that IL-33 and ST2 proteins were located in the nuclei of endothelial inflammatory cells and keratinocytes (Figure 3). Inflammatory cells also exhibited cytoplasmic staining patterns.

The results of immunohistochemical staining for IL-33 and ST2 are shown in Table 3. Most HC endothelial cells and keratinocytes were positive for IL-33 (Figure 3A). However, the numbers of such cells were reduced in skin biopsies from patients with AOSD (Figure 3B and 3C), eczema (Figure 3E), and psoriasis. Although only a few inflammatory cells were evident in most skin biopsy samples, the grade of IL-33–expressing inflammatory cells in patients with AOSD was higher than that of HC. Figure 3D shows a representative case in which IL-33–expressing inflammatory cells were frequent. Similarly, the numbers of ST2-positive endothelial cells and keratinocytes were somewhat reduced in the skin biopsies of patients with AOSD, although statistical significance was not attained (Figure 3F). However, ST2-positive inflammatory cells were more common than IL-33–positive cells (Figure 3F and 3G). Most inflammatory and endothelial cells in skin biopsies from patients with eczema and psoriasis were positive for ST2 (Figure 3H).

IL-33– or ST2-positive inflammatory cells did not differ in the extent of neutrophil infiltration, keratinocyte vacuolization, or karyorrhexis. However, the percentage of ST2-positive endothelial cells was higher in patients who exhibited necrosis than in those who did not (p = 0.014).

Table 2. Correlations between interleukin 33 (IL-33) and soluble ST2 levels and those of disease activity markers in 40 patients with adult-onset Still's disease.

Disease Activity Marker	Pearson's Correlation Coefficients, r (p)	
	IL-33	Soluble ST2
Systemic score	0.476 (0.002)	–0.014 (0.931)
Leukocyte	0.060 (0.713)	–0.067 (0.683)
Hemoglobin	–0.103 (0.528)	–0.210 (0.194)
Platelet	–0.009 (0.955)	–0.011 (0.946)
ESR	0.530 (< 0.001)	0.186 (0.251)
CRP	0.208 (0.197)	0.016 (0.924)
Ferritin	0.333 (0.036)	0.379 (0.016)
LDH	0.095 (0.560)	0.003 (0.986)
Albumin	–0.043 (0.791)	–0.246 (0.126)
Bilirubin	–0.237 (0.142)	–0.093 (0.570)
AST	0.335 (0.035)	0.11 (0.498)
ALT	0.231 (0.151)	–0.151 (0.354)
Soluble ST2	0.079 (0.626)	—

The systemic scoring system of Pouchot, *et al*[21] assigns a score from 0 to 12, with 1 point for each of the following manifestations: fever, typical rash, pleuritis, pneumonia, pericarditis, hepatomegaly or abnormal liver function test data, splenomegaly, lymphadenopathy, leukocytosis ≥ 15,000/mm^2, sore throat, myalgia, and abdominal pain. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; LDH: lactate dehydrogenase; AST: aspartate transaminase; ALT: alanine transaminase.

Similarly, the percentage of ST2-positive epithelial cells was also higher in patients who exhibited parakeratosis than in those who did not (p = 0.033).

DISCUSSION

In our current study, we found that the levels of serum IL-33 and soluble ST2 were significantly elevated in patients with active AOSD, and that the serum IL-33 level correlated with the levels of several markers of disease activity. Serum IL-33 levels fell when disease activity was reduced upon followup of patients with AOSD. We also confirmed that the expression of IL-33 and that of its receptor, ST2, were elevated in rash material from patients with active AOSD compared to the skin of HC. This is the first study, to our knowledge, to measure IL-33 and ST2 levels in serum and skin biopsy material from patients with active AOSD.

Several studies have found that levels of IL-33 and soluble ST2 are elevated in the serum and synovial fluid (SF) of patients with RA[22,23,24,25,26]. One study found that serum IL-33 levels were significantly higher in patients with RA, especially in those with high-level disease compared with low to moderate disease activity[25]. Another study measured SF and serum IL-33 levels in patients with RA, and found that the SF IL-33 level was elevated and correlated with increases in the 28-joint Disease Activity Score, ESR, and immunoglobulin level[22]. Also, serum and SF IL-33 levels were positively correlated. However, soluble serum ST2 was

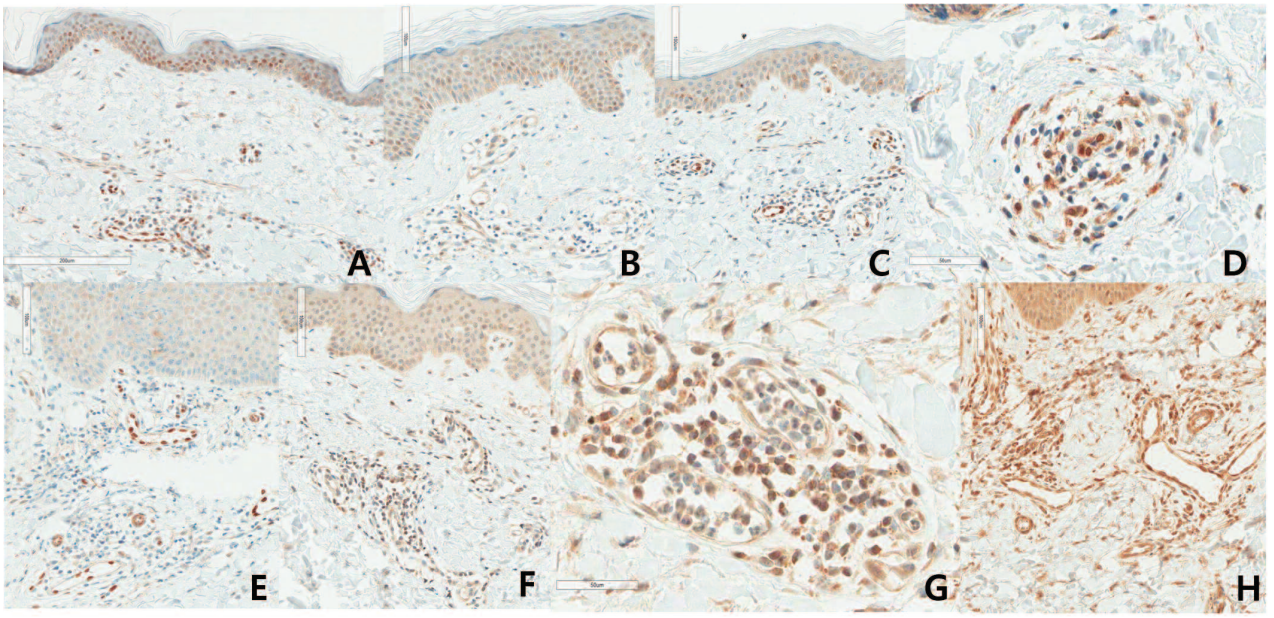

Figure 3. IL-33 (A, B, C, D, and E) and ST2 (F, G, and H) expression in skin biopsies of healthy controls (A), patients with adult-onset Still's disease (B, C, D, F, and G), and patients with eczema (E, H). Original magnifications ×200 (A, B, C, E, F, and H) and ×400 (D, G). A. IL-33 protein in the nuclei of keratinocytes and endothelial cells. B. IL-33–positive cells were rare among keratinocyte and endothelial and inflammatory cells. C, E. IL-33–positive cells were evident among endothelial cells. D. High magnification of IL-33–positive inflammatory cells. F. ST2-positive inflammatory cells were common. Keratinocytes and endothelial cells were weakly positive, or negative. G. High magnification of ST2-positive inflammatory cells. H. Most endothelial and inflammatory cells were positive. IL-33: interleukin 33.

Table 3. Interleukin 33 (IL-33) and ST2 immunohistochemical stain results in skin biopsies of 34 patients with adult-onset Still's disease (AOSD), 5 healthy controls (HC), 5 eczema samples, and 5 psoriasis samples.

	Grade	AOSD	HC	p (AOSD vs HC)	Eczema	p (AOSD vs eczema)	Psoriasis	p (AOSD vs psoriasis)
Positive inflammatory cells for IL-33	0	14 (41.2)	5 (100)	0.02	1 (20)	0.631	3 (60)	0.636
	1	20 (58.8)	0 (0)		4 (80)		2 (40)	
	2	0 (0)	0 (0)		0 (0)		0 (0)	
	3	0 (0)	0 (0)		0 (0)		0 (0)	
Positive endothelial cells for IL-33	0	0 (0)	0 (0)	0.006	0 (0)	0.056	0 (0)	0.239
	1	8 (23.5)	0 (0)		0 (0)		0 (0)	
	2	17 (50)	5 (100)		1 (20)		2 (40)	
	3	9 (26.5)	0 (0)		4 (80)		3 (60)	
Positive keratinocytes for IL-33	0	21 (61.8)	0 (0)	0.01	2 (40)	0.649	1 (20)	0.205
	1	9 (26.5)	1 (20)		2 (40)		3 (60)	
	2	4 (11.8)	3 (60)		1 (20)		1 (20)	
	3	0 (0)	1 (20)		0 (0)		0 (0)	
Positive inflammatory cells for ST2	0	1 (2.9)	5 (100)	< 0.001	0 (0)	0.017	0 (0)	0.118
	1	10 (29.4)	0 (0)		0 (0)		0 (0)	
	2	14 (41.2)	0 (0)		0 (0)		1 (20)	
	3	9 (26.5)	0 (0)		5 (100)		4 (80)	
Positive endothelial cells for ST2	0	2 (5.9)	0 (0)	0.265	0 (0)	0.009	0 (0)	0.009
	1	11 (32.4)	0 (0)		0 (0)		0 (0)	
	2	13 (38.2)	2 (40)		0 (0)		0 (0)	
	3	8 (23.5)	3 (60)		5 (100)		5 (100)	
Positive keratinocytes for ST2	0	14 (41.2)	2 (40)	0.235	0 (0)	0.133	0 (0)	0.36
	1	12 (35.3)	0 (0)		3 (60)		3 (60)	
	2	4 (11.8)	1 (20)		2 (40)		1 (20)	
	3	4 (11.8)	2 (40)		0 (0)		1 (20)	

These results used a Mann-Whitney U test.

detectable in only some patients with RA (24.1%), and the level did not correlate significantly with that of serum IL-33. Only 1 study, as far as we know, has measured IL-33 and soluble ST2 levels in patients with systemic JIA, the juvenile form of AOSD; in those patients, the soluble ST2 level was elevated, but not the IL-33 level[15]. Interestingly, the serum soluble ST2 level of patients with systemic JIA was elevated even in the inactive phase, during which other clinical variables were normal. In our current study, we found that the serum IL-33 levels of 40 patients with AOSD were significantly higher than those of patients with RA and HC. Serum IL-33 level correlated significantly with the levels of several markers of disease activity, including systemic score, ESR, and the ferritin and AST levels. Also, serum IL-33 levels fell significantly after symptoms improved in almost all followup patients with AOSD. However, the soluble ST2 levels of patients with AOSD were higher than those of HC but not of patients with RA, and correlated only with the ferritin level. Followup of patients with AOSD showed that soluble serum ST2 level did not decrease after symptoms improved. Interestingly, in this study, IL-33 levels of patients with RA were not higher than those of HC, whereas soluble ST2 levels were elevated. The reason our results differ from those of previous studies is not clear, but the disease activity of our patients with RA was relatively lower, and disease duration was different[22,27].

Although it is not clear why our results differ from those of a previous study on patients with systemic JIA, the discrepancy may be attributable to differences in study populations, such as levels of disease activity and treatments given[15]. Our patients had an average ferritin level of 6775.4 ± 10,307.6 ng/ml and an average AST level of 77.8 ± 64.3 u/l, but the previous patients with systemic JIA had a median ferritin level of 864 ng/ml and an AST level of 39 u/l. In addition, a few patients with systemic JIA were medicated; all of our patients were untreated prior to sampling. Also, in the earlier study, the inactive phase of systemic JIA in patients taking medication was defined as the phase in which no clinical symptoms recorded in the active phase were observed, and in which ESR and CRP levels were normal[28]. Remission was defined as 6 continuous months of inactive disease while taking medication. In our current study, the soluble serum ST2 levels in patients with AOSD were elevated not only during active disease but also during followup. This may be because some of our followup patients were in the inactive phase of disease, not in remission (as defined above). Previous studies showed that serum IL-33 levels are related to the severity of arthritis in patients with RA[22,26]. Also, studies have suggested that AOSD might be divided into 2 subsets: chronic arthritis type and systemic inflammatory type[29,30]. We compared serum IL-33 and soluble ST2 levels according to the presence or absence of

arthritis in AOSD and found that those levels were not different between patients with arthritis and those without. This result may be attributable to similar initial systemic manifestations in all patients with AOSD, although the clinical courses of patients are different in those prone to systemic inflammation and those prone to arthritis. When we compared the systemic scores for disease activity in patients with AOSD according to the presence of arthritis, mean systemic score was similar to 5.7 ± 1.3 (with arthritis) vs 5.1 ± 1.2 (without arthritis).

In tissue homeostasis and the immune responses associated with various inflammatory skin diseases, IL-33/ST2 signaling plays pivotal roles[31,32]. Several studies support the notion that human epidermal IL-33 is not a pre-stored alarmin, but rather a factor induced by inflammation[32,33,34]. In particular, IL-33/ST2 signaling is important in the immune responses to psoriasis and eczema[35,36,37]. Therefore, we measured the IL-33 and ST2 expression levels in skin biopsies from active untreated patients with AOSD, and compared the results to those of HC and the skin lesions of psoriasis and eczema. The numbers of inflammatory cells expressing IL-33 and ST2 were elevated in the skin lesions of patients with AOSD compared to HC, but did not differ from the levels in the lesions of patients with eczema or psoriasis. Interestingly, the IL-33 expression grade ranged from 0 to 3 among endothelial and epithelial cells of AOSD skin biopsies, but the cell numbers were lower than those of HC. Also, the ST2 expression grade ranged from 0 to 3 among the endothelial and epithelial cells of skin biopsies from patients with AOSD, but did not differ between patients with AOSD and HC. The numbers of endothelial cells expressing ST2 in patients with AOSD were somewhat lower than the numbers in patients with eczema or psoriasis. In our current study, we thus confirmed that IL-33 and ST2 were expressed by endothelial and epithelial cells of HC skin. Further, we suggest that IL-33 and ST2 expression of infiltrating inflammatory cells is involved in the dermal inflammation characteristic of AOSD. In addition, the high levels of serum IL-33 may be attributable to the loss or release of the materials from damaged endothelial cells. The cutaneous histological profile of AOSD is characterized by relatively milder infiltrations of lymphocytes and neutrophils and relatively fewer epidermal changes than those associated with psoriasis or eczema, although no prior study has compared AOSD skin lesions to those of psoriasis or eczema[38,39]. It has been suggested that in RA, IL-33/ST2 signaling is mediated through the induction of proinflammatory cytokines, including interferon-γ, TNF, and IL-17[8]. These cytokines are present in the serum, liver, and skin of patients with AOSD[2,3,40]. Such inflammatory conditions may lead to IL-33/ST2 signaling, sustaining the systemic and local inflammatory responses of AOSD through a vicious cycle. However, we did not compare initial IL-33/ST2 expression levels in patients with AOSD to those in followup skin biopsies. Also, we did not stain the AOSD skin biopsies for proinflammatory cytokines.

The levels of IL-33 and soluble ST2 were significantly higher in the sera of patients with active AOSD. Also, almost all followup patients with AOSD exhibited reductions in IL-33 levels after disease improved. We suggest that the high level of serum IL-33/ST2 is from recruitment of IL-33/ST2-positive inflammatory cells by proinflammatory cytokines and damage of endothelial cells and keratinocytes, resulting in release of IL-33/ST2. Thus, increased expression of IL-33 and ST2 may be a result of skin damage in AOSD. In addition, the increase of IL-33/ST2 may cause massive systemic inflammation with endothelial damage. These results indicate that the IL-33/ST2 signaling pathway may be involved in the pathogenesis of the acute inflammation and skin manifestations associated with AOSD.

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