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Characteristic patterns of HLA presentation and T cell differentiation in adult-onset Still's disease

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Abstract

We examined the expression of human leukocyte antigen (HLA) and composition of differentiated T cells in the peripheral blood to understand the characteristics of the immune changes in patients with adult-onset Still's disease (AOSD). This study enrolled patients with AOSD (n = 14), patients with rheumatoid arthritis (RA, n = 20), and healthy controls (HC, n = 20). The percentage of surface-stained cells with HLA-DP, DQ, and DR alleles and the composition of differentiated T cells in peripheral blood leukocytes (PBLs) were evaluated by flow cytometry. AOSD patients exhibited significantly higher percentages of lymphocytes presenting HLA-DP and HLA-DR, and lower percentages of cells presenting HLA-DQ, than RA patients or HC. The proportions of CD4+, CD4+CCR7+, CD4+CD62L–, and CD8+CD62L– cells from PBLs were decreased in AOSD patients relative to RA patients or HCs. By contrast, AOSD patients had higher proportions of CD8+naïve T cells in whole blood relative to RA patients or HC. The proportions of CD4+ effector memory T cells, CD8+ naïve T cells, and CD8+ effector memory T cells in whole blood cells and CD4+ effector memory T cell in lymphocytes were significantly associated with the systemic score. While the proportions of CD4+, CD8+, CCR7+, CD4+CCR7+, CD4+CD62L–, and CD8+CD62L– cells were significantly decreased in AOSD patients, and the proportion of CD8+naïve T cells was elevated in AOSD and correlated with the systemic score. Further studies of a large cohort of AOSD patients will be necessary to evaluate these markers in the pathogenesis of AOSD.

Keywords

adult-onset still's disease, biomarker, disease activity, human leukocyte antigen, T cell

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Background

Adult-onset Still's disease (AOSD) is a systemic inflammatory disease characterized with various systemic symptoms, including a spiking fever, sore throat, maculopapular rash, and arthritis.[1] This disease is typically described as the adult type of systemic juvenile idiopathic arthritis (JIA), presenting as a combination of auto-inflammatory and autoimmune conditions. The pathogenesis of AOSD is thought to involve an environmental trigger in the presence of a permissive genetic background, leading to excessive cytokine production and the

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activation of innate immunity. Evidence for such activation can be seen in the form of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-17, and IL-18, which occur in higher concentrations in the serum of patients with AOSD. Further activation of innate immune cells is triggered by the interaction of specific damage-associated molecular patterns (DAMPs) and pattern recognition receptors (PRRs), and several PRRs are responsible for the pathological pathways of JIA and AOSD.[2]

The role of T cells in AOSD pathogenesis remains controversial. In rheumatic diseases, a human leukocyte antigen (HLA)-restricted T cell response to antigen has been shown to affect disease progression, with several HLA alleles associated with disease severity.[3] It was revealed that HLA-DRB1*11 and variant major histocompatibility complex (MHC) class II contributed to the development of JIA, while a "shared epitope" encoded by HLA-DRB1 alleles was correlated with the typical characteristics of both JIA and rheumatoid arthritis (RA). An expressions of HLA-DR4 and HLA-DRw6+ were correlated with articular symptoms in patients with AOSD.[4] After receiving signals, naive CD4+ and CD8+ T cells differentiate into effector or memory T cells.[5] T cells expressing L-selectin (CD62L) are regarded as central memory T cells, with CCR7+T cells recruited to the T cell zone of the lymph nodes via direct binding to CCL19 and CCL21.[6] Central memory cells exist in secondary lymphoid organs, where they proliferate or expand in response to re-stimulation by antigen. Loss of CCR7 expression enables transition into effector memory T cells that have the ability to react with the inflammatory cytokines in peripheral tissues. However, no alterations of such differentiated or expanded T cell populations or their roles have been identified in JIA and AOSD.

Here, we determined the frequencies of cells presenting HLA-DP, DQ, and DR and the composition of differentiated T cells in peripheral blood leukocytes (PBLs) of AOSD patients. The frequencies of these markers were then compared based on clinical activity.

Materials and methods

We enrolled 14 untreated active AOSD and 20 RA patients and 20 healthy controls (HCs). Among patients with typical clinical features of AOSD including prolonged fever, salmon-colored rash, arthralgia, and myalgia, the workup had been conducted. The patients were enrolled when the findings and symptoms met Yamaguchi et al.'s[7] criteria after infectious, malignant, and other inflammatory diseases could be ruled out.

PBLs and serum were isolated from blood of the AOSD patients, RA patients, and HCs. Follow-up PBLs and serum samples were obtained in six AOSD patients after their symptoms resolved. Clinical information and laboratory findings were obtained from a review of the patients' medical records. Disease activity of AOSD was estimated using the systemic score from 0 to 12.[8] Inactive status was defined as the sign of active disease was absent with normalized inflammatory markers. The Institutional Review Board of Ajou University Hospital approved this study (AJIRB-BMR-SMP-13-380) and the informed consent was signed from all patients and HCs.

After red blood cells' (RBCs) lysis with ammonium chloride potassium solution, the PBLs were washed in phosphate-buffered saline (PBS) and applied to staining with each tube containing 1×10^6 cells. Then, the samples were incubated with phycoerythrin (PE)-labeled anti-human HLA-DR (catalog #12-9952; eBioscience, San Diego, CA, USA), fluorescein isothiocyanate (FITC)-labeled anti-human HLA-DQ (catalog #11-9881; eBioscience), FITC-labeled anti-human HLA-DP (catalog 251042; Abbiotec, San Diego, CA, USA), allophycocyanin (APC-eFluor 780)-labeled anti-human CD4 (catalog 47-0047; eBioscience), FITC-labeled anti-human CD8 (catalog #551347; BD PharMingen, San Jose, CA, USA), PE-labeled anti-human CCR7 (catalog #12-1979; eBioscience), and peridinin chlorophyll protein (PerCP-eFluor 710)-labeled anti-human CD62L (L-selectin, catalog #46-0629; eBioscience). After the stained cells were washed with PBS, they were analyzed with a FACSAria III flow cytometer (Becton Dickinson, San Jose, CA, USA). The cells were gated according to forward and size scatter and 10,000 cells were analyzed. The fluorescence-activated cell sorter (FACS) data were done according to the gating of whole blood, granulocytes, lymphocytes, and monocytes, after which specific surface markers were overlaid to identify differences in marker expression relative to gated populations.

The results are presented as means ± SD or frequencies with the percentage. Differences of the evaluated cell surface markers were analyzed using the Mann–Whitney U test. Correlations between the frequencies of cells and disease activity markers were analyzed with Spearman's correlation

test. The statistical analyses were performed with SPSS for Windows (ver. 23.0; IBM, Armonk, NY, USA), and P values <0.05 were considered statistically significant.

Results

Clinical characteristics of subjects

A total of 14 AOSD and 20 RA patients were included, along with 20 HC (Supplement Table 1). Of our 14 AOSD patients, 11 were females (78.6%), and their mean age was 52.5 ± 20.4 years. The mean age was not different significantly among the patients with AOSD and RA and HC. Of the 14 patients with AOSD, 8 were newly diagnosed, and the remaining 6 patients were experiencing disease flare-ups with having stopped their medication prior to disease flare-up. Follow-up samples of 6 patients were collected after resolution of their disease activity.

Surface expression of HLA-DP, DR, and DQ in AOSD, RA, and HC

Representative histograms for patients with AOSD and RA and HC are shown in Supplement Figure 1. Mean frequencies of surface-stained cells presenting HLA-DQ were significantly decreased in patients with AOSD compared to HCs ($P=0.012$), with similar expression to that seen in RA (Figure 1). No differences were evident in the expression of HLA-DP or DR among the groups. Among monocytes, HLA-DP and DR expression was significantly decreased in the RA patients relative in those with AOSD and HC ($P=0.012$ and $P=0.017$, respectively). No differences in HLA expression were evident between the AOSD patients and HC. The frequencies of lymphocytes presenting HLA-DP and DR were elevated in AOSD patients relative to HC ($P=0.002$ and $P=0.001$, respectively) and RA patients ($P=0.003$ for both). The frequencies of granulocytes presenting HLA-DR in AOSD patients were elevated in comparison with those of RA patients ($P=0.033$).

Percentage of differentiated T cells of the subjects

The representative flow cytometry dot plots of surface-stained cells presenting CD4+CCR7+, CD8+CCR7+, CD4+CD62L– and CD8+CD62L– cells from PBLs were shown in Supplement Figure 2. The mean frequencies of CD4+ and CD4+CCR7+ cells from whole blood were significantly lower in AOSD patients compared with RA patients ($P<0.001$ and $P=0.023$) and HCs ($P<0.001$ and $P=0.004$; Figure 2). Significantly lower mean frequencies of CD8+, CCR7+, and CD8+CCR7+ cells in whole blood were observed in AOSD patients relative to HCs ($P<0.001$ and $P=0.006$ and 0.005, respectively). Significantly lower mean frequencies of CD4+CD62L– and CD8+CD62L– cells in whole blood were detected in AOSD patients compared to RA patients ($P=0.005$ and $P=0.03$, respectively) and HCs ($P=0.001$ and $P<0.001$, respectively). The mean frequencies of CD8+ naïve T cells were significantly higher in patients with AOSD compared to the patients with RA and HCs ($P=0.002$ and $P=0.004$, respectively).

Correlation between cell expression of HLA-DP, DQ, and DR and several markers for disease activity in patients with AOSD

The frequencies of cells presenting HLA-DP in whole blood (r=0.56, $P=0.037$), monocytes (r=0.695, $P=0.006$), lymphocytes (r=0.6, $P=0.023$), and granulocytes (r=0.554, $P=0.04$) were significantly associated with lactate dehydrogenase (LDH) expression, while HLA-DP was correlated with aspartate transaminase (AST; r=0.551, $P=0.041$; Table 1). HLA-DQ expression in whole blood was significantly associated with leukocytes (r=–0.563, $P=0.036$) and hemoglobin (r=–0.581, $P=0.029$), where HLA-DQ was correlated negatively with leukocytes (r=–0.64, $P=0.014$), hemoglobin (r=–0.559, $P=0.038$), and IL-17 (r=–0.445, $P=0.049$). HLA-DR expression correlated with IL-23 (r=0.588, $P=0.035$) in whole blood, AST (r=0.724, $P=0.003$) in monocytes, and erythrocyte sedimentation rate (ESR; r=–0.566, $P=0.035$). The frequency of granulocytes presenting HLA-DR was associated with IL-23 (r=0.681, $P=0.01$) and IL-18 (r=0.599, $P=0.031$).

Correlations of the frequencies of differentiated T cells in PBLs and several markers for disease activity in patients with AOSD

The correlations between the differentiated T cell populations and the disease activity markers in patients with AOSD were shown in Supplement

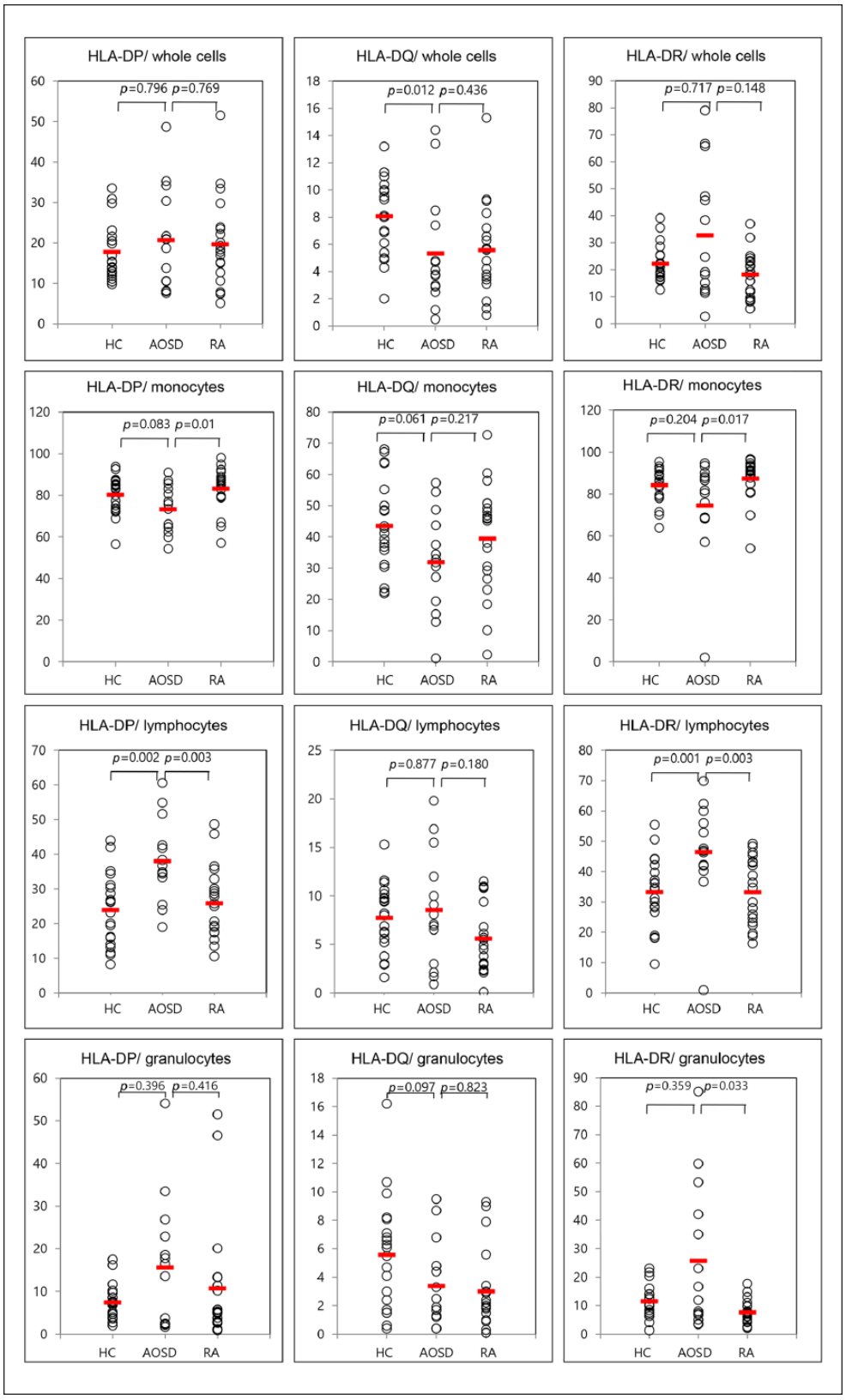

Figure 1. Flow cytometric results: the percentage of surface-stained cells presenting HLA-DP, DR, and DQ in AOSD patients, RA patients, and HCs. Results were obtained from 14 patients with adult-onset Still's disease (AOSD), 20 with rheumatoid arthritis (RA), and 20 healthy controls (HC). The horizontal line indicates the mean value for each group. *P* values were determined by the Mann–Whitney U test.

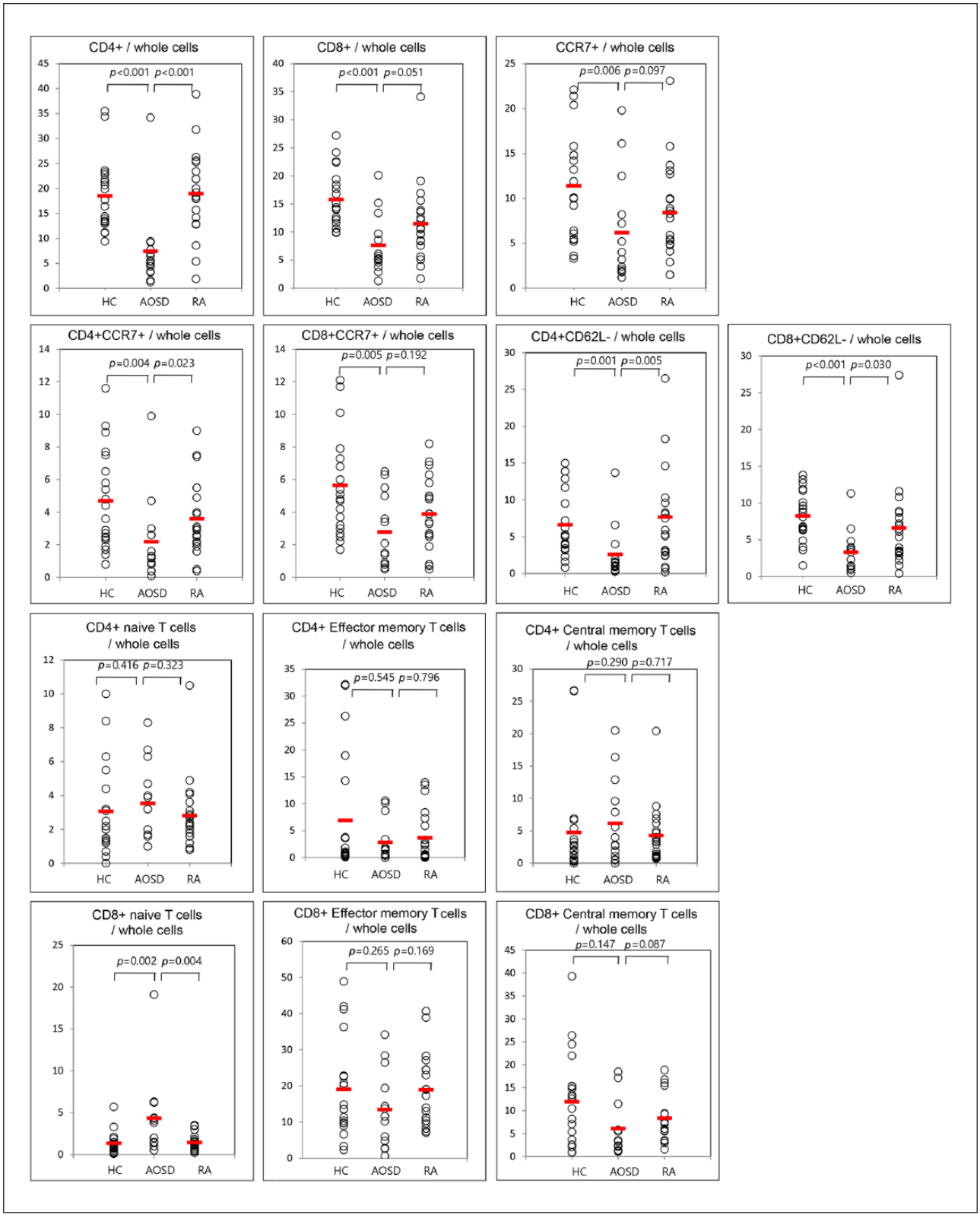

Figure 2. Percentage of surface-stained cells presenting CD4+, CD8+, CCR7+, CD4+CCR7+, CD8+CCR7+, CD4+CD62L–, CD8+CD62L–, CD4+naïve T cell, CD4+ effector memory T cell, CD4+ central memory T cells, CD8+ naïve T cells, CD8+ effector memory T cells, and CD8+ central memory T cells in patients with AOSD, a patient with rheumatoid arthritis (RA), and a healthy control (HC). Results were obtained from 14 patients with AOSD, 20 RA patients, and 20 HCs. The horizontal line indicates the mean value for each group. The *P* value was determined by the Mann–Whitney U test.

Table 1. Correlations between expression levels of cells and disease activity markers in patients with adult-onset Still's disease.

Disease activity markers	Correlation coefficient, r (*P* value)						
	HLA-DP whole	HLA-DP monocyte	HLA-DP lymphocyte	HLA-DQ whole	HLA-DQ lymphocyte	HLA-DR lymphocyte	HLA-DR granulocyte
Systemic score	0.237 (0.414)	0.279 (0.334)	0.011 (0.97)	−0.177 (0.546)	−0.277 (0.337)	0.154 (0.598)	0.47 (0.09)
Leukocyte	−0.073 (0.805)	0.118 (0.688)	−0.101 (0.731)	**–0.563 (0.036)**	**–0.64 (0.014)**	−0.172 (0.557)	0.062 (0.834)
Hemoglobin	0.055 (0.852)	−0.279 (0.335)	0.033 (0.911)	**–0.581 (0.029)**	**–0.559 (0.038)**	0.218 (0.454)	−0.156 (0.594)
Platelet	−0.152 (0.605)	−0.114 (0.697)	−0.253 (0.383)	−0.279 (0.334)	−0.2 (0.493)	−0.437 (0.118)	−0.169 (0.563)
ESR	0.117 (0.691)	−0.116 (0.694)	−0.187 (0.522)	0.053 (0.858)	0.084 (0.776)	**–0.566 (0.035)**	0.253 (0.382)
CRP	−0.2 (0.493)	0.172 (0.557	−0.182 (0.533)	0.305 (0.288)	0.275 (0.342)	−0.169 (0.563)	0.007 (0.982)
Ferritin	0.473 (0.088)	0.473 (0.088)	0.292 (0.311)	0.147 (0.615)	−0.116 (0.692)	−0.147 (0.615)	0.459 (0.098)
LDH	**0.56 (0.037)**	**0.695 (0.006)**	**0.6 (0.023)**	0.262 (0.366)	0.244 (0.401)	0.033 (0.911)	0.486 (0.078)
Bilirubin	−0.058 (0.845)	−0.281 (0.33)	−0.024 (0.934)	0.084 (0.775)	−0.133 (0.651)	−0.223 (0.443)	−0.409 (0.146)
AST	0.363 (0.202)	**0.551 (0.041)**	0.464 (0.094)	0.035 (0.905)	0.095 (0.748)	0.264 (0.362)	0.231 (0.427)
ALT	0.055 (0.852)	0.143 (0.626)	0.178 (0.543)	0.077 (0.794)	0.218 (0.455)	0.147 (0.615)	0.042 (0.887)
IL-17	0.281 (0.353)	−0.245 (0.419)	−0.325 (0.279)	−0.19 (0.535)	**–0.445 (0.049)**	0.248 (0.415)	−0.025 (0.936)
IL-1β	0.091 (0.768)	0.278 (0.357)	−0.113 (0.714)	−0.036 (0.908)	−0.245 (0.42)	0.352 (0.238)	0.404 (0.171)
IL-23	0.489 (0.09)	0.242 (0.426)	0.038 (0.901)	0.066 (0.831)	−0.236 (0.437)	0.505 (0.078)	**0.681 (0.01)**
IL-1	0.473 (0.103)	0.209 (0.493)	0.11 (0.721)	0.17 (0.578)	−0.187 (0.541)	0.516 (0.071)	**0.599 (0.031)**

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; LDH: lactate dehydrogenase; AST: aspartate transaminase; ALT: alanine transaminase; IL: interleukin.
Spearman's correlation coefficients were calculated. Bold means significant P value ($P \leq 0.05$).

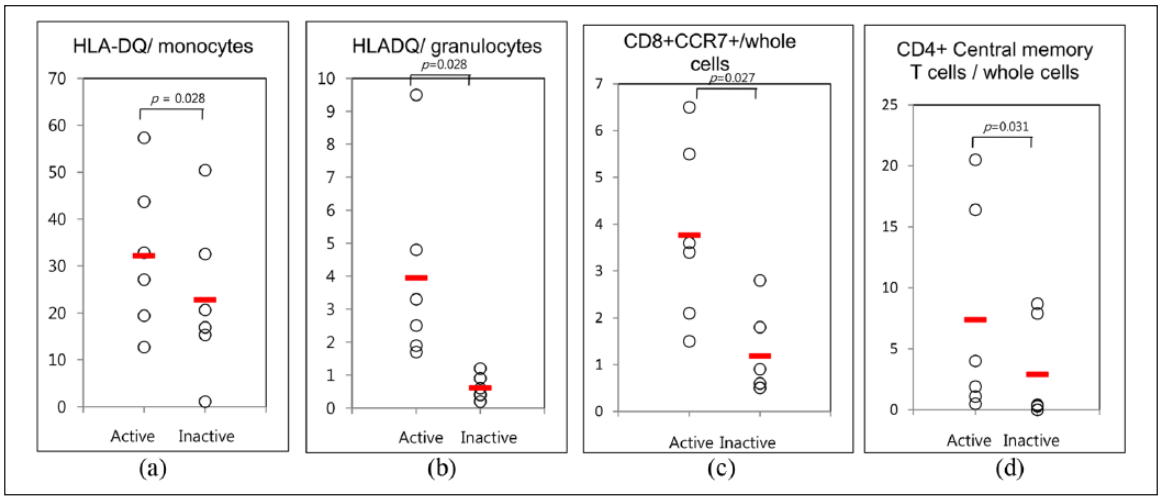

Figure 3. Percentage of surface-stained cells presenting HLA-DQ on (a) monocytes, (b) granulocytes, (c) CD8+CCR7+T cells, and (d) CD4+ central memory T cells according to disease activity in patients with AOSD. The results were obtained from six samples from AOSD patients when disease are active, untreated, and after their disease activity resolved. The horizontal line indicates the mean value for each group. The *P* value was determined with the Wilcoxon signed-rank test.

Table 2. The frequencies of CD4+, CD8+, CCR7+, CD4+CCR7+, CD8+CCR7+, CD4+CD62L–, and CD8+CD62L– cells in whole blood were not correlated with any disease activity marker. Within whole blood, CD4+naïve T cells levels were significantly associated with AST (r=0.698, P=0.006) and alanine transaminase (ALT; r=0.681, P=0.007), while CD4+ effector memory cells correlated negatively with the systemic score (r=–0.719, P=0.004), AST (r=–0.705, P=0.005), ALT (r=–0.659, P=0.01), and IL-23 (r=–0.614, P=0.026). CD4+ central memory T cell levels were associated with AST (r=0.552, P=0.041) and ALT (r=0.635, P=0.015). Inverse associations were seen for the CD8+ populations, with CD8+ naïve T cells positively associated with the systemic score (r=0.700, P=0.008) and IL-23 (r=0.608, P=0.036), while CD8+ effector memory T cells correlated negatively with the systemic score (r=–0.593, P=0.033) and IL-23 (r=–0.636, P=0.026). The frequencies of CD8+ central memory T cells in peripheral blood were correlated with ALT (r=–0.692, P=0.009), IL-23 (r=0.634, P=0.027), and IL-18 (r=0.595, P=0.041).

Significant associations were also evident among the lymphocyte populations. CD4+ lymphocytes correlated with IL-17 (r=–0.79, P=0.001), while CCR7+ cells were positively correlated with AST (r=0.572, P=0.033) and ALT (r=0.705, P=0.005). Double-positive CD4+CCR7+ lymphocytes correlated positively with platelets (r=0.565, P=0.035), AST (r=0.563, P=0.036), and ALT (r=0.757, P=0.002), while CD8+CD62L– lymphocytes correlated with hemoglobin (r=0.558, P=0.038). As seen in whole blood, naïve and effector populations show inverse correlations, with CD4+ naïve T lymphocytes associated with C-reactive protein (CRP; r=0.57, P=0.033), AST (r=0.855, $P<0.001$), and ALT (r=0.726, P=0.003), while CD4+ effector memory T lymphocyte levels correlated negatively with the systemic score (r=–0.682, P=0.007), CRP (r=–0.589, P=0.027), bilirubin (r=0.61, P=0.021), AST (r=–0.837, $P<0.001$), and ALT (r=–0.811, $P<0.001$). CD4+ central memory T lymphocytes were associated with AST (r=0.533, P=0.05) and ALT (r=0.634, P=0.015), CD8+ naïve T lymphocytes correlated with LDH (r=0.597, P=0.024) and AST (r=0.567, P=0.034), and CD8+ central memory T lymphocytes correlated negatively with ALT (r=–0.578, P=0.03; data not shown).

Frequencies of HLA allele presentation and differentiated T cell populations in active and quiescent disease

Six patients provided blood samples both while they had active disease and after disease resolution (Figure 3). Their mean corticosteroid dose was 3.75 ± 2.09 mg/d prednisolone equivalent. Three patients were treated with methotrexate and two patients were treated with cyclosporine-A. The percentages of cells presenting HLA-DP and HLA-DR in whole blood were similar for the active and inactive phases; however, the numbers of monocytes and granulocytes presenting

HLA-DR both decreased after symptom resolution (both $P=0.031$). The frequencies of CD8+CCR7+ and CD4+ central memory T cells of whole blood were higher in active AOSD patients compared in inactive patients ($P=0.031$ for both).

Discussion

AOSD and JIA patients have been reported to exhibit distinct patterns of HLA allele variants. HLA-DRB1*12 and 15 were observed more frequently in Korean patients with AOSD, while HLA-DRB1*15:01 and DR5 were associated with susceptibility to AOSD in Japanese patients.[9] We observed higher proportions of lymphocytes presenting HLA-DP and DR, with HLA-DP levels significantly associated with LDH levels. Furthermore, the proportion of granulocytes expressing HLA-DR was correlated with IL-23 and IL-18. LDH is one of DAMP provoking the immune response and a biomarker for hematologic malignancies. HLA-DP mismatch leads to direct allocative T cell response after hematopoietic cell transplantation.[10] Altered HLA-DP expression in peripheral blood mononuclear cells (PBMC) could affect immunopathology of AOSD with LDH elevation.

The frequencies of CD8+ naïve T cells in PBLs of AOSD patients were elevated, with the frequencies of CD8+ naïve T cells mirroring the systemic score and IL-23 expression. From a mechanistic standpoint, an increase in circulating CD8+ naive T cells may be related with a significant death of effector T cells through underlying AOSD pathogenesis. The frequencies of CD4+ naïve and CD4+ central memory T cells in whole blood and lymphocytes were correlated with both AST and ALT. In autoimmune hepatitis, secreted pro-inflammatory cytokines drive the differentiation of CD4+ T cells by recognizing a self-antigen peptide through HLA class II molecules and provoke an aggressive immune attack on liver tissue.[11] This showed a strong correlation between the differentiated CD4+T cells in PBMC and the liver damage in AOSD. A deficiency of CD62L in immune cell was related with reduced immune responsiveness, and its population can be changed depend on the presence of antigen.[12] The decreased frequencies of CD4+CD62L– and CD8+CD62L– cells observed in this study represent an inappropriate autoreactivity in AOSD. When T cells mature or differentiate surrounded by antigens from active inflammatory status, loss of CD62L might occur less than other condition in AOSD.

The ability to extrapolate these results to AOSD is limited. The sample size was small, so the differences in HLA expression profiles and frequencies of immune cell subtypes observed may not fully represent their roles in AOSD pathology. And the associations with typical manifestations or clinical outcome including mortality could not be analyzed.

This study found that the proportions of lymphocytes expressing HLA-DP and DR were increased in AOSD patients, while the proportion of whole blood cells presenting HLA-DP was correlated with LDH. The percentages of CD4+, CD8+, CCR7+, CD4+CCR7+, CD8+CCR7, CD4+CD62L–, and CD8+CD62L– cells were decreased in PBLs of AOSD patients, while the percentages of CD8+ naïve T cell levels in PBLs were increased in PBLs of AOSD patients, with the compositions of these cells being correlated with both systemic score and IL-23 expression. CD4+ naïve, CD4+ effector memory, and CD4+ central memory cell levels in PBLs or lymphocytes were strongly correlated with liver enzyme levels. Further studies with more samples and functional studies examining the effect of these markers on immune function will be necessary to understand AOSD pathogenesis more.

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Hyoun-Ah Kim and Seonghyang Sohn contributed equally to this work.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplement Table 1. Clinical characteristics.

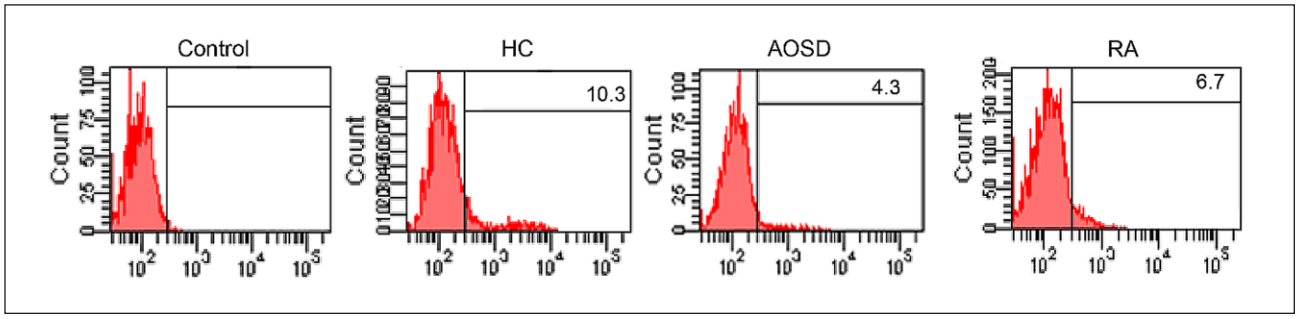
	AOSD patients ($n = 14$)	RA patients ($n = 20$)	HC ($n = 20$)
Age (years)	52.5 ± 20.4	48.0 ± 11.3	41.0 ± 13.0
Gender (F/M)	11/3	16/4	15/5
Fever	13 (92.9)		
Sore throat	9 (64.3)		
Skin rash	10 (71.4)		
Lymphadenopathy	4 (28.6)		
Splenomegaly	2 (14.3)		
Hepatomegaly	2 (14.3)		
Pleuritis	2 (14.3)		
Arthritis	11 (78.6)		
Hemoglobin, g/dL	11.4 ± 2.1	12.8 ± 1.5	
Leukocytes, μL	12,371 ± 4,831	6,030 ± 1,873	
Platelets, $\times c10^3/\mu L$	305.0 ± 95.4	227.6 ± 54.9	
Ferritin, ng/mL	4,965.0 ± 9,404.1		
LDH, U/L	383.1 ± 227.0		
ESR, mm/h	58.2 ± 24.8	12.5 ± 10.9	
CRP, mg/dL	10.40 ± 7.30	0.28 ± 0.60	
AST, mg/dL	66.3 ± 56.1	26.3 ± 8.3	
ALT, mg/dL	69.1 ± 84.9	25.0 ± 19.4	
Bilirubin, mg/dL	0.84 ± 0.53	0.76 ± 0.21	
Albumin, g/dL	4.14 ± 0.52	4.4 ± 0.18	
ANA positivity	3 (21.4)	4 (20)	
RF positivity	1 (7.1)	15 (75.0)	
Systemic score	4.79 ± 1.85		
DAS-28		2.83 ± 1.23	

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. DAS-28, disease activity score including 28 joints. All values are presented as numbers (with percentages) or as means ± standard deviation. The systemic scoring system of Pouchot *et al.*[2] 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 ($\geqslant 15{,}000/mm^2$), sore throat, myalgia, and abdominal pain.

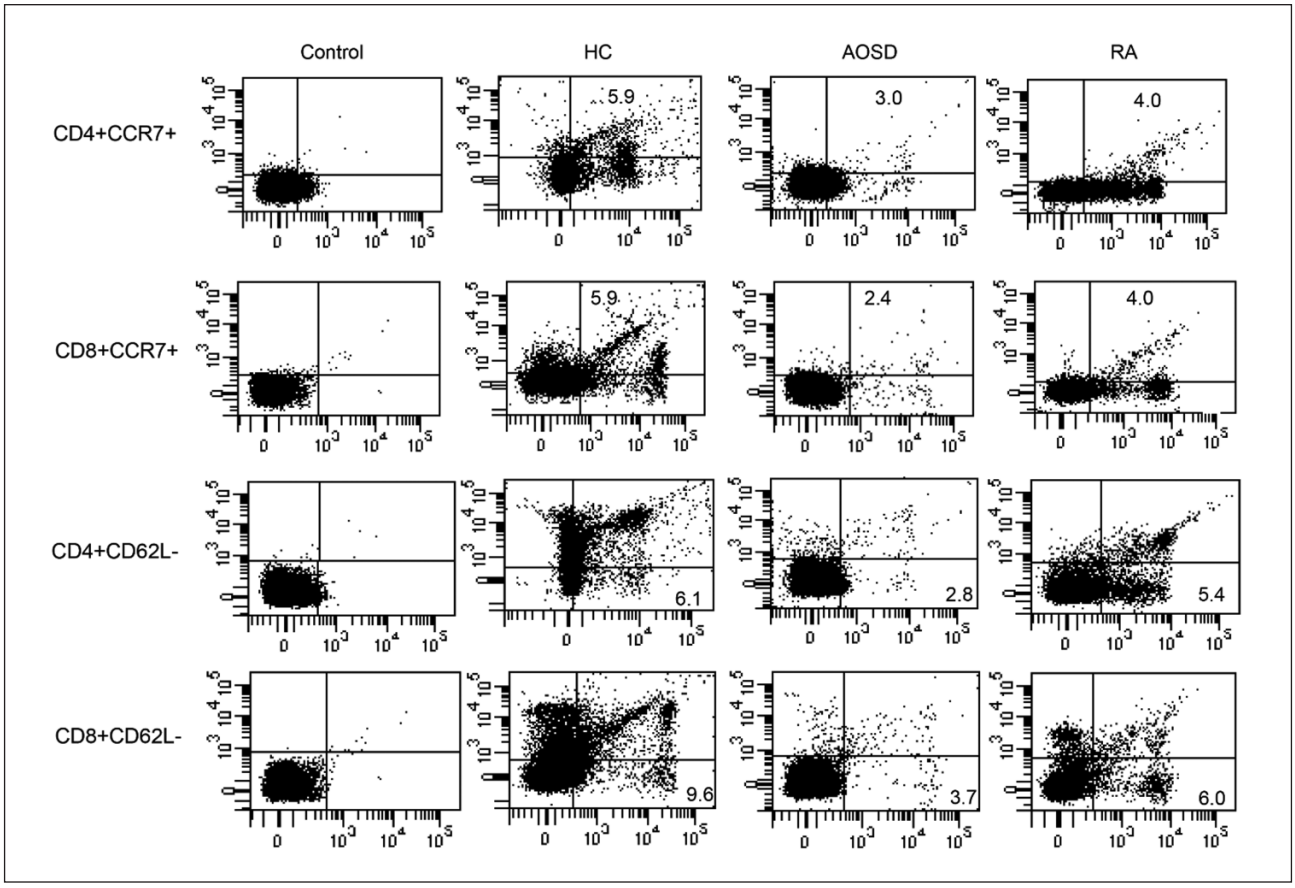
Supplement Table 2. Correlations between T cell populations and disease activity markers in peripheral blood leukocytes of patients with adult-onset Still's disease.

Disease activity markers	Correlation coefficient, r (*p*-value)					
	CD4+ naïve T cells	CD4+effector memory T cells	CD4+ central memory T cells	CD8+ naïve T cells	CD8+effector memory T cells	CD8+ central memory T cells
Systemic score	0.351 (0.218)	**-0.719 (0.004)**	0.371 (0.191)	**0.700 (0.008)**	**-0.593 (0.033)**	0.469 (0.106)
Leukocyte	0.065 (0.825)	-0.166 (0.571)	0.029 (0.923)	0.408 (0.167)	-0.162 (0.596)	-0.366 (0.218)
Hemoglobin	-0.259 (0.371)	0.268 (0.355)	-0.356 (0.211)	-0.110 (0.72)	0.415 (0.158)	-0.460 (0.114)
Platelet	0.483 (0.080)	-0.267 (0.355)	0.473 (0.088)	0.239 (0.431)	-0.324 (0.280)	-0.063 (0.837)
ESR	0.198 (0.498)	-0.471 (0.089)	0.304 (0.291)	0.526 (0.065)	-0.543 (0.055)	0.272 (0.369)
CRP	0.417 (0.138)	-0.440 (0.116)	0.297 (0.303)	-0.039 (0.901)	-0.286 (0.344)	0.503 (0.079)
Ferritin	0.284 (0.324)	-0.212 (0.467)	0.244 (0.401)	0.336 (0.262)	-0.407 (0.168)	0.008 (0.979)
LDH	0.368 (0.195)	-0.369 (0.194)	0.349 (0.221)	0.380 (0.201)	-0.467 (0.108)	0.143 (0.641)
Bilirubin	-0.335 (0.241)	0.515 (0.059)	-0.241 (0.406)	-0.390 (0.188)	0.416 (0.158)	-0.429 (0.144)
AST	**0.698 (0.006)**	**-0.705 (0.005)**	**0.552 (0.041)**	0.318 (0.289)	-0.465 (0.109)	0.410 (0.164)
ALT	**0.681 (0.007)**	**-0.659 (0.010)**	**0.635 (0.015)**	0.311 (0.301)	-0.484 (0.094)	**0.691 (0.009)**
IL-17	0.017 (0.957)	-0.206 (0.499)	-0.226 (0.459)	-0.102 (0.753)	-0.109 (0.737)	-0.067 (0.837)
IL-1β	0.004 (0.989)	-0.188 (0.538)	0.151 (0.622)	0.105 (0.745)	-0.182 (0.571)	0.316 (0.317)
IL-23	-0.044 (0.886)	**-0.614 (0.026)**	0.132 (0.668)	**0.608 (0.036)**	**-0.636 (0.026)**	**0.634 (0.027)**
IL-18	-0.165 (0.589)	-0.501 (0.081)	-0.033 (0.915)	0.531 (0.075)	-0.538 (0.071)	**0.595 (0.041)**

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL, interleukin; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine transaminase. The systemic scoring system of Pouchot *et al.* [2] 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 ($\geqslant$ 15,000/mm^2), sore throat, myalgia, and abdominal pain.



Supplement Figure 1. Representative examples of flow cytometric histograms of surface-stained cells presenting human leukocyte antigen (HLA)-DQ from the peripheral blood of one patient with adult-onset Still's disease (AOSD), a patient with rheumatoid arthritis (RA), and a healthy control (HC).



Supplement Figure 2. Representative examples of flow cytometric dot plots of surface-stained cells presenting CD4+CCR7+, CD8+CCR7+, CD4+CD62L–, and CD8+CD62L– from the peripheral blood of one patient with adult-onset Still's disease (AOSD), a patient with rheumatoid arthritis (RA), and a healthy control (HC).