Original article

Characteristics of circulating natural killer cells and their interferon-γ production in active adult-onset Still's disease

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Conflicts of Interest

The authors declare that they have no financial or personal conflicts of interest.

Abstract

Objective. To investigate the characteristics of circulating NK cells and their IFN-γ-producing ability in adult-onset Still's disease (AOSD).

Methods. Peripheral blood mononuclear cells were obtained from 22 patients in the acute phase of AOSD (acute AOSD); 7 of the 22 patients after treatment (remission AOSD), and 11 healthy controls (HC). NK cells and their IFN-γ expression levels were analyzed by flow cytometry. Additionally, the cytokine receptors of interleukin (IL)-12, IL-15, and IL-18 on NK cells were also evaluated.

Results. The frequency of NK cells was significantly lower in acute AOSD than in HC. NK cell counts significantly increased in remission AOSD. Expression of IL-12 and IL-15 receptors on NK cells was significantly increased in acute AOSD, whereas that of IL-18 receptor indicated no significant difference among three groups. IFN-γ expression in NK cells was significantly higher in acute AOSD than in HC, and significantly decreased in remission AOSD. The absolute number of NK cells and IFN-γ-expressing NK cells revealed an inverse correlation with serum ferritin levels in acute AOSD. In two distinct subsets of NK cells, $CD56^{dim}$ NK cells significantly exhibited higher IFN-γ expression than $CD56^{bright}$ NK cells in acute AOSD.

Conclusion. In acute AOSD, NK cells displayed lower proportion, whereas they had higher ability of IFN-γ production than in HC; moreover, upregulation of IL-12 and IL-15 receptors on NK cells may

contribute to promoting IFN-γ production. Besides, a disease activity may be implicated in regulating the number of NK cells and IFN-γ-expressing NK cells in AOSD.

Introduction

Adult-onset Still's disease (AOSD) is a systemic autoinflammatory disease characterized by daily spike fevers, polyarthritis, evanescent rash, pharyngitis, lymphadenopathy, and hepatosplenomegaly. Furthermore, AOSD sometimes indicates life-threatening involvements such as aseptic meningitis, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, and macrophage activation syndrome (MAS) as a reactive hemophagocytic lymphohistiocytosis. The hallmarks of AOSD are hyperferritinemia, increased levels of inflammatory mediators including C-reactive protein (CRP), and increased white blood cell counts (1, 2). In addition, elevated levels of proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, IL-12, IL-18, interferon-γ (IFN-γ), and tumor necrosis factor-α, are found in the acute phase of AOSD (3-6). These cytokines activate macrophages and neutrophils, which play a pivotal role in the pathogenesis (2, 7, 8), suggesting that innate immunity contributes to the development of AOSD. Immune impairment of natural killer (NK) cells is also associated with the pathogenesis of AOSD or systemic juvenile idiopathic arthritis (sJIA), which have been regarded as the adult or juvenile spectrum of same disease, respectively (9); and moreover, lower expression and defective cytotoxicity of NK cells were demonstrated in an active phase of disease (10-13).

NK cells are identified as cytotoxic cells in the category of innate lymphoid cells capable of immune response without antigen-specific cross-linking. To defend the host against invasive and neoplastic

pathogens, they stimulate macrophages, upregulate MHC class Ⅰ on antigen-presenting cells, and promote effecter function in T cell lineage by IFN-γ release; or alternatively, they perform direct cytotoxicity (14, 15). Besides, a functional response of NK cells can be promoted by cytokine combination signals with IL-12, IL-15, and IL-18; notably, their relevant cytokine receptors are found on NK cells (14-17). Accordingly, a specific immune response of NK cells in AOSD is supposed to be affected by the exposure of proinflammatory cytokines related to the disease. On the other hand, the IFN-γ-producing ability of NK cells remains unclear in AOSD.

In this study, we investigated the characteristics of circulating NK cells including their IFN-γ-producing ability as well as the relevant cytokine receptors expression in patients with AOSD.

Material and Methods

Patients and samples

Twenty-two patients with AOSD were employed in this study (mean age: 51 ± 16 years [range 25–80 years], 5 men and 17 women). They were definitely diagnosed according to the criteria proposed by Yamaguchi (18) in Shinshu University hospital. The clinical characteristics of the diagnosis and other features related to the disease are shown in Table 1. The complication of MAS and the activity score were also investigated according to the proposal diagnostic criteria (19) and the systemic score proposed by Pouchot, *et al* (20), respectively. Blood samples were obtained from them

prior to the immunosuppressive treatments. As the healthy controls (HC), blood samples from 11 healthy individuals (mean age: 47 ± 12 years, 6 men and 5 women) were also provided. No significant differences in the mean age and distribution of sex were shown between AOSD patients and HC.

For evaluating the results in the remission phase of AOSD, blood samples were provided from 7 of 22 patients at the mean period of 39 ± 43 months after starting immunosuppressive therapy. Remission was defined by fulfilling both assignments as follows; the Pouchot's score was achieved in 0, and no physical findings concerning disease activity shown in Table 1 were found. When the blood samples were provided, five patients had received maintenance therapy including prednisolone (n = 4), cyclosporine (n = 1), methotrexate (n = 2), golimumab (n = 1), and tocilizumab (n = 2). Their laboratory findings were also significantly improved as follows (Table 1): the number of white blood cells ($P = 0.032$), neutrophils ($P = 0.014$), serum levels of aspartate transaminase ($P = 0.0004$), alanine transaminase ($P = 0.022$), lactate dehydrogenase ($P = 0.0003$), CRP ($P < 0.0001$), erythrocyte sedimentation ($P = 0.0004$), and ferritin ($P < 0.0001$).

This study was approved by the Local Ethics Committee in Shinshu University (approval number: 601). All individual participants provided informed consent.

Sample preparation and flow cytometry

Whole blood samples were collected into EDTA-coated tubes. Peripheral blood mononuclear cells (PBMCs) from whole blood samples were isolated by gradient centrifugation with Ficoll-Hypaque

PLUS (GE Healthcare, Pittsburgh, PA, USA). To define NK cells in flow cytometric analysis, unstimulated PBMCs were stained with Pacific blue-conjugated anti-CD3 (BioLegend, San Diego, CA, USA), fluorescein isothiocyanate (FITC)-conjugated anti-CD16, and phycoerythrin (PE)-conjugated anti-CD56 (both from Beckman coulter, Brea, CA, USA). NK cells were phenotypically defined as CD3−CD16+CD56+ cells in the population gated on total lymphocytes. NK cells were additionally stained with allophycocyanin (APC)-conjugated anti-CD212 (IL-12Rβ1) (Miltenyi Biotec, Bergisch Gladbach, Germany), alternatively APC-conjugated anti-CD215 (IL-15Rα) (BioLegend), or APC-conjugated anti-CD218 (IL-18Rα) (Miltenyi Biotec). To explore intracellular IFN-γ expression in NK cells, PBMCs were stimulated with 0.5 μg/mL of ionomycin, 0.04 μg/mL of phorbol myristate acetate (both from Sigma-Aldrich, St.Louis, MO, USA), and 2 μM monensin (BD Bioscience, San Diego, CA, USA) at 37°C for 4 hours. Stimulated PBMCs were permeabilized with Cytofix/Cytoperm (BD Bioscience) after being stained with above-described cell-surface makers including CD3, CD16, and CD56. Permeabilized cells were subsequently stained with APC-conjugated anti-IFN-γ (BioLegend). IFN-γ expression was detected in the population gated on NK cells. Stained cells were acquired on a FACSCanto II flow cytometer (BD Bioscience), and the acquired data were analyzed by FlowJo version 7.6.5 software (Tree Star Inc., Ashland, OR, USA).

Serum IL-18 measurement

Serum samples were stored at -80°C until use with enzyme-linked immunosorbent assay (ELISA).

The serum concentration of IL-18 was measured using commercially available ELISA kit (Medical and Biological Laboratories, Nagoya, Japan). The minimal detectable concentration of IL-18 was 12.5 pg/mL.

Statistical analysis

The clinical findings shown in Table 1 were represented as the median [interquartile range (IQR)]. The analyzed results were shown as the mean ± S.D. Statistical significance was defined as 2-side *P* values of less than 0.05. For comparing the analyzed findings between patients with AOSD and HC, the Mann-Whitney U test was employed. The Wilcoxon signed-ranked test was performed to compare data before and after treatment in patients with AOSD. Correlation coefficient test was used for evaluating a significant relationship between the analyzed data and clinical findings.

Results

Circulating NK cell proportion and counts in AOSD

To determine the proportion of NK cells in the peripheral blood, we compared the frequency of NK cells in the population of peripheral lymphocytes between 22 patients with AOSD prior to the treatment (acute AOSD), 7 of those in the remission phase of AOSD (remission AOSD), and HC. The proportion of NK cells was significantly lower in acute AOSD than in HC (mean 8.2% vs. 19.2%; *P* = 0.002) (Figure 1A). Meanwhile, no significant difference was demonstrated between remission

AOSD and HC (mean 12.6%, P = 0.063). Of 7 patients in remission AOSD, 6 patients indicated increased counts of NK cells, ultimately demonstrating statistical significance (P = 0.042) (Figure 1B).

Expression of cytokine receptors on NK cells in AOSD

Since IL-12, IL-15, and IL-18 are pivotal cytokines promoting NK cell lineage, we investigated the expression of cytokine receptors responsive to IL-12, IL-15, and IL-18 (IL-12Rβ1, IL-15Rα, and IL-18Rα, respectively) on NK cells (Supplementary Figure 1A). IL-12Rβ1 expression was significantly higher in acute AOSD than in HC (mean 94.0% vs. 87.2%; %, P = 0.004; median fluorescence intensity (MFI), P = 0.002) (Figure 2A, 2B). No significant difference was shown between remission AOSD and HC (mean 90.2%; %, P = 0.717; MFI, P = 0.497) (Supplementary Figure 2A, 2B). A significant decrease of IL-12Rβ1-MFI was demonstrated in a remission phase (P = 0.017) (Figure 3A), whereas IL-12Rβ1+ NK cell counts were not significantly different between an acute and remission phase (P = 0.062) (Figure 3B). IL-15Rα proportion was significantly higher in acute and remission AOSD than in HC (mean 21.4%, 9.4%, 4.6%, respectively; acute AOSD vs. HC, P < 0.0001; remission AOSD vs. HC, P = 0.016) (Figure 2C) (Supplementary Figure 2C), and IL-15Rα-MFI was significantly higher in acute AOSD than in HC (P < 0.0001) whilst being not significantly different between remission AOSD and HC (P = 0.205) (Figure 2D) (Supplementary Figure 2D). In comparison between an acute and remission phase, a decrease in IL-15Rα-MFI was significantly demonstrated (P = 0.028) despite no statistical significance in IL-15Rα+ NK cell counts (P = 0.225) (Figure 3C, 3D).

Percent frequency of IL-18Rα was around 80% in acute, remission AOSD, and HC (mean 80.4%, 77.7%, and 82.7%, respectively), showing no significant difference (acute vs. HC, P = 0.516; remission vs. HC, P = 0.441) (Figure 2E) (Supplementary Figure 2E). Neither the comparison of IL-18Rα-MFI (acute vs. HC, P = 0.169; remission vs. HC, P = 0.556; acute vs. remission, P = 0.612) nor that of IL-18Rα+ NK cell counts between an acute and remission phase (P = 0.063) indicated statistical significances (Figure 2F, 3E, 3F) (Supplementary Figure 2F).

Kinetic evaluation of IFN-γ-expressing NK cells in AOSD

IFN-γ production is a crucial function in NK cell machinery. Therefore, we additionally investigated IFN-γ expression in NK cells. In acute AOSD, IFN-γ expression in NK cells was significantly higher than in HC (mean 53.2% vs. 24.1%; %, P = 0.0001; MFI, P = 0.0001) (Figure 4A, 4B) (Supplementary Figure 1B). In remission AOSD, that was significantly lower than in HC (mean 13.1%; %, P = 0.008; MFI, P = 0.040) (Figure 4C, 4D). IFN-γ-MFI decreased in 7 patients at a remission phase, showing statistical significance in remission AOSD (P = 0.018) (Figure 4E). Meanwhile, no significant difference was shown in the comparison of IFN-γ-expressing NK cells counts (P = 0.398) (Figure 4F); even in three patients who had treated with biologics, only one patient revealed decrease of IFN-γ-expressing NK cells counts (data not shown).

Relationship between IFN-γ-expressing NK cells and serum ferritin levels in AOSD

We analyzed the relationship between NK cells and clinical findings. It has been found that serum

IL-18 was strongly associated with the disease activity and/or the clinical features in AOSD (2-5, 21, 22). Therefore, serum IL-18 levels were additionally measured. Higher serum levels of IL-18 were expectedly revealed in acute AOSD than in HC (mean 2212.4 pg/mL vs. 71.6 pg/mL, $P < 0.0001$) (Supplementary Figure 3A). They were still higher in remission AOSD than in HC (mean 109.2 pg/mL, $P = 0.008$), while a significant decrease was demonstrated in a remission phase ($P = 0.018$) (Supplementary Figure 3B). However, serum IL-18 levels had significant correlations with neither clinical findings described in Table 1, including MAS complication and the Pouchot's score, nor any data analyzed by flow cytometry (data not shown). MAS complication or the Pouchot's score had no significant correlation with any data analyzed by flow cytometry (data not shown). On the other hand, serum ferritin levels significantly demonstrated inverse correlations with the absolute number of NK cells and IFN-γ-expressing NK cells in acute AOSD ($P = 0.017$ and $P = 0.003$, respectively) despite no correlation with IFN-γ-MFI in NK cells ($P = 0.202$) (Figure 5A, 5B, 5C).

Next, we re-analyzed the property of NK cell related to IFN-γ production by subdividing CD3−CD16+CD56+ population into high- and low-density expression of CD56 ($CD56^{bright}$ and $CD56^{low}$, respectively) (Supplementary Figure 4A). The proportion of $CD56^{bright}$ in NK cells was almost equal between acute AOSD and HC (mean 6.7% vs. 7.4%, $P = 0.268$) whilst being lower in remission AOSD than in HC (mean 3.9%, $P = 0.042$) (Figure 5D) (Supplementary Figure 4B). IFN-γ-MFI in $CD56^{bright}$ was significantly lower in acute AOSD than in HC ($P = 0.014$), whereas that in

CD56^{dim} was significantly higher in acute AOSD than in HC ($P = 0.012$) (Figure 5E) (Supplementary Figure 5A). In acute AOSD, IFN-γ-MFI was significantly lower in CD56^{bright} than in CD56^{dim} ($P = 0.0003$). IFN-γ-MFI in CD56^{dim} significantly decreased in a remission phase ($P = 0.018$) (Figure 5F). Although IFN-γ-MFI in CD56^{brigh} showed no significant difference in a remission phase ($P = 0.310$), increased expression of that was shown in three patients who had treated with biologics (Supplementary Figure 5C).

Discussion

The proportion of NK cells was found to be significantly lower in acute AOSD than in HC, supporting previous studies, which demonstrated impairment of NK cells based on disease activity of AOSD (10, 11). MAS may be attributed to defective cytotoxic function of NK cells as a hallmark of AOSD pathogenesis (7, 13). A high amount of serum IL-18, which is strongly associated with the disease activity in AOSD (2-5, 21, 22), reduces functional NK cells, and is implicated in MAS induction (21, 23), suggesting that the dysfunction of NK cell is a fundamental immune disorder in AOSD development. Although it was insufficient to indicate direct relationship between NK cell reduction and MAS or serum IL-18 levels in this study, NK cell reduction was significantly related to high levels of serum ferritin. Meanwhile, high elevation of serum ferritin is found to be associated with MAS and/or serum IL-18 elevation in AOSD (5, 24); and moreover, serum ferritin is a valuable

serological marker of disease activity (2, 25, 26). Accordingly, this result may suggest that the higher AOSD disease activity develops, the more the reduction of NK cells may be substantially promoted. In fact, NK cell counts significantly increased in remission AOSD.

IFN-γ, which is known to activate macrophages, is primarily produced by activated NK cells and effector T cells (27). Previous studies have shown that serum levels of IFN-γ significantly increase in the acute phase of AOSD (4-6). Indeed, the present study demonstrated that IFN-γ expression in NK cells was significantly higher in acute AOSD than HC. In the activating cascade of NK cells, IL-12, IL-15, and IL-18 are potential inducers of IFN-γ secretion (15, 28). Therefore, upregulation of proinflammatory cytokines including IL-18 and IL-12, which are shown in an acute phase of AOSD (4), is supposed to enhance NK cell activation. Elevated expression of serum IL-15 was also demonstrated in patients with sJIA (29). In a series of this study, we also focused on the relevant cytokine receptors on NK cell. IL-18 receptor expression on NK cells in acute AOSD was not significantly different from that in both remission AOSD and HC. In NK cells, IFN-γ can be predominantly produced based on IL-18 participation in the presence of IL-12 or IL-15 under physiological conditions (28, 30). When extracellular IL-18 binds to IL-18 receptor α (IL-18Rα), IL-18 receptor β (IL-18Rβ) is recruited as the co-receptor for forming a high-affinity receptor complex in the process of intracellular IL-18 signal transduction (28, 31-33). However, defective phosphorylation of IL-18Rβ impaired NK cell function and reduced IFN-γ secretion even after IL-18

stimulation in sJIA (32). Accordingly, IL-18 signaling within NK cells from patients with AOSD may be impaired even though IL-18Rα expression remains. This theory may explain the causal attribution of our result that no significant correlation was shown between serum IL-18 levels and IFN-γ-expressing NK cells in acute AOSD. On the other hand, expression of IL-12 and IL-15 receptors on NK cells was significantly higher in acute AOSD than in both remission AOSD and HC. The ability to drive IFN-γ secretion by IL-12 or IL-15 seems to be restricted under deficient IL-18 signaling (15, 30). However, NK cells may exert the compensatory mechanism mediating IFN-γ production by inducing intracellular IL-12 and IL-15 signals via upregulated their affiliate receptors in AOSD.

In acute AOSD, IFN-γ-expressing NK cell counts was inversely correlated with serum ferritin levels. Given the evaluation of NK cell features divided into two subsets based on CD56 antigen in this study, IFN-γ expression in $CD56^{bright}$ was significantly lower in acute AOSD than in HC; and conversely, increased IFN-γ expression in $CD56^{dim}$ was significantly demonstrated. This result is supposed to be paradoxical as the physiological phenomenon because $CD56^{bright}$ NK cells are recognized as the main producer of IFN-γ (16, 34, 35). However, $CD56^{dim}$ NK cells can more prominently produce proinflammatory cytokine after K562 cell interaction as a target cell than $CD56^{bright}$ NK cells (36). In fact, K562 cell interaction was found to reduce the proportion and/or function of NK cells in sJIA and AOSD (10, 13). Besides, cell-cell interaction with dendritic cells may also affect NK cell function in which IFN-γ production can be induced (15). Therefore, $CD56^{dim}$ NK cells may develop their IFN-γ-

producing ability via cross-talk with immunopathogenic cells related to AOSD development. Furthermore, it is hypothesized that the absolute number of IFN-γ-expressing NK cells may be changed during IFN-γ-producing dominancy being shifted from $CD56^{bright}$ to $CD56^{dim}$ in acute AOSD, allowing that the inverse correlation with serum ferritin levels might be ultimately determined as the result of reducing the absolute number of IFN-γ-expressing NK cells depending on high disease activity.

Meanwhile, IFN-γ expression in NK cells significantly decreased in remission AOSD compared with that in HC. Previous studies demonstrated that NK cell function including IFN-γ production is suppressed by the treatment with immunosuppressive agents (37, 38). Given the above-mentioned results as well as the relevant citations, it should be considered that defective NK cell function may contribute to not only onset of AOSD, but also insufficient host immunity against infectious microbes during the treatment. However, only 7 patients in remission AOSD could be sequentially analyzed in our study, thus the number of samples might be insufficient for obtaining a complete determination.

In conclusion, the proportion of NK cells were significantly lower in acute AOSD than in HC, whereas NK cells revealed higher expression of IFN-γ as well as IL-12 and IL-15 receptors in acute AOSD than in HC. It was suggested that upregulation of IL-12 and IL-15 receptors may be implicated in compensating increased intracellular IFN-γ production in NK cells from patients with acute AOSD despite no significant expression of IL-18 receptor. Meanwhile, the number of NK cells and IFN-γ-

expressing NK cells were correlatively reduced in accordance with elevated serum levels of ferritin. Besides, $CD56^{dim}$ NK cells prominently produced IFN-γ compared with $CD56^{bright}$ NK cell in acute AOSD. It was assumed that the ability of IFN-γ production in NK cells may be affected depending on a disease activity in AOSD. On the other hand, NK cells are also induced in response to certain viruses and haptens via a different mechanism (14, 17, 39). Some inhibitory receptors binding to host-MHC class I regulate NK cell activity including cytotoxicity and cytokine production (15, 40). Moreover, the experimental system with multiple-proinflammatory cytokines impacting AOSD development may be needed for clarifying more precise IFN-γ-producing machinery in NK cells, because the activation with PMA/ionomycin was solely used in our study. Therefore, further investigation requires evaluating a wide variety of immune reactions in NK cell lineage together with recruiting more patients.

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Figure legends

Figure 1. NK cell counts in patients with AOSD. Frequency of NK cells in peripheral blood lymphocytes were compared between 22 patients with acute AOSD, 7 with remission AOSD, and 11 healthy controls (HC) (**A**). NK cell counts were sequentially evaluated in 7 patients between acute and remission phase (**B**). Values are shown as the mean ± SD. Statistically significant difference was evaluated by the Mann-Whitney U test (**A**) or Wilcoxon signed-ranked test (**B**), indicating as follow: $^{*}P < 0.05$ and $^{**}P < 0.005$.

Figure 2. The comparison of interleukin (IL)-12Rβ1, IL-15Rα, and IL-18Rα expression on NK cells between acute AOSD and HC. Percentage of IL-12Rβ1, IL-15Rα, or IL-18Rα (**A, C, E**) and median fluorescence intensity (MFI) of them (**B, D, F**) in NK cells were compared between patients with acute AOSD and HC. Values are shown as the mean ± SD. The Mann-Whitney U test was employed in the comparison. Statistically significant differences are indicated as follows: $^{**}P < 0.005$ and $^{****}P < 0.0001$.

Figure 3. The evaluation of interleukin (IL)-12Rβ1, IL-15Rα, and IL-18Rα expression on NK cells in a remission phase. IL-12Rβ1, IL-15Rα, or IL-18Rα-MFI in NK cells (**A, C, E**) and their expressing NK cell counts (**B, D, F**) were sequentially evaluated in 7 patients between acute and remission phase. The Wilcoxon signed-ranked test was used in the comparison. Statistically significant difference is indicated as $^{*}P < 0.05$.

Figure 4. IFN-γ production in NK cells from patients with AOSD. Percentage and MFI of IFN-γ in NK cells were compared between patients with acute AOSD and HC (**A, B**), or between those with remission AOSD and HC (**C, D**). IFN-γ-MFI in NK cells (**E**) and IFN-γ-expressing NK cell counts (**F**) were sequentially evaluated in 7 patients between acute and remission phase. Values are shown as the mean ± SD. The Mann-Whitney U test was employed in the comparison between three groups. The Wilcoxon signed-ranked test was used in that between an acute and remission phase. Statistically significant differences are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P = 0.0001$.

Figure 5. Inverse correlation with serum ferritin levels and IFN-γ-producing ability in two distinct NK cell subsets. With regards to the absolute number of NK cells, IFN-γ-expressing NK cells, and IFN-γ-MFI in NK cells, the correlations with serum ferritin levels were evaluated in patients with acute AOSD by the correlation coefficient test (**A, B, C**). In the population of NK cells (CD3−CD16+CD56+ cells), $CD56^{bright}$ and $CD56^{dim}$ subsets were divided. The proportion of $CD56^{bright}$ in NK cells was compared between patients with acute AOSD and HC (**D**). In the population of $CD56^{bright}$ or $CD56^{dim}$ NK cells, IFN-γ-producing ability was evaluated. IFN-γ-MFI in each subset was compared between patients with acute AOSD and HC (**E**). In addition, IFN-γ-MFI in $CD56^{dim}$ NK cell population was compared between an acute and remission phase (**F**). Values are shown as the mean ± SD. The Mann-Whitney U test was employed in the comparison between patients with acute AOSD and HC. The Wilcoxon signed-ranked test was used for the comparison between $CD56^{bright}$ and

$CD56^{dim}$ in acute AOSD, or for that between an acute and remission phase. Statistically significant differences are indicated as follows: $^{*}P < 0.05$ and $^{***}P < 0.0005$.

Table 1. Clinical characteristics of patients with adult-onset Still's disease in the acute and remission phases

	Acute (total) (n = 22)	Consecutive patients (n = 7)		HC (n = 11)	*p* value	
		Acute*	Rem			
Patients					vs. HC	
					Acute (total)	Acute*
Age, yrs [range]	51 [25–80]	49 [34–74]		47 [36–69]	n.s.	n.s.
Sex (M/F)	5/17	2/5		6/5	n.s.	n.s.
Physical findings, n (%)					Consecutive patients Acute* vs. Rem	
Fever	22 (100)	7 (100)	0		0.0003	
Rash	21 (95)	7 (100)	0		0.0003	
Sore throat/Pharyngitis	16 (73)	5 (71)	0		0.010	
Lymphadenopathy	11 (50)	4 (57)	0		0.035	
Arthritis	18 (82)	5 (71)	0		0.010	
Myalgia	6 (27)	1 (14)	0		n.s.	
Pleuritis	5 (23)	1 (14)	0		n.s.	
Pericarditis	2 (9)	0	0		n.s.	
Hepatomegaly	9 (41)	4 (57)	0		0.035	
Splenomegaly	11 (50)	4 (57)	0		0.035	
MAS criteria	7 (32)	2 (29)	0		n.s.	
Pouchot's score, median [IQR]	6 [4.25–6]	6 [5–7.5]	0		0.018	
Laboratory findings, median [IQR]					vs. Rem	
					Acute (total)	Acute*
White blood cells, /μL	14770 [9633–20820]	10400 [9485–22735]	7120 [6940–8670]		0.032	n.s.
Neutrophils, /μL	12721 [7640–18397]	9412 [7627–20834]	1582 [1169–2443]		0.014	n.s.
Platelet, 10^4/μL	29.2 [19.5–34.5]	26.5 [20.1–32.2]	24.0 [18.7–26.3]		n.s.	n.s.

AST, U/L	63 [40–109]	49 [40–64]	22 [16–26]	0.0004	0.018
ALT, U/L	55 [30–138]	34 [31–48]	20 [16–21]	0.022	0.034
LDH, U/L	516 [175–906]	449 [375–651]	219 [182–223]	0.0003	0.018
C-reactive protein, mg/dL	9.0 [3.8–12.9]	11.8 [9.6–19.5]	0.02 [0–0.04]	<0.0001	0.018
ESR, mm/h	42.0 [21.0–91.0]	65.0 [34.0–94.0]	6.0 [3.0–15.0]	0.0004	0.028
Ferritin, ng/mL	8392 [1462–15456]	8706 [2995–17627]	24 [16–79]	<0.0001	0.018

Rem, remission; HC, healthy controls; MAS, macrophage activation syndrome; IQR, interquartile range; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; ESR, erythrocyte sedimentation rate; n.s., not significant. *Seven patients in the acute phase of disease who were consecutively followed.

Figure 1

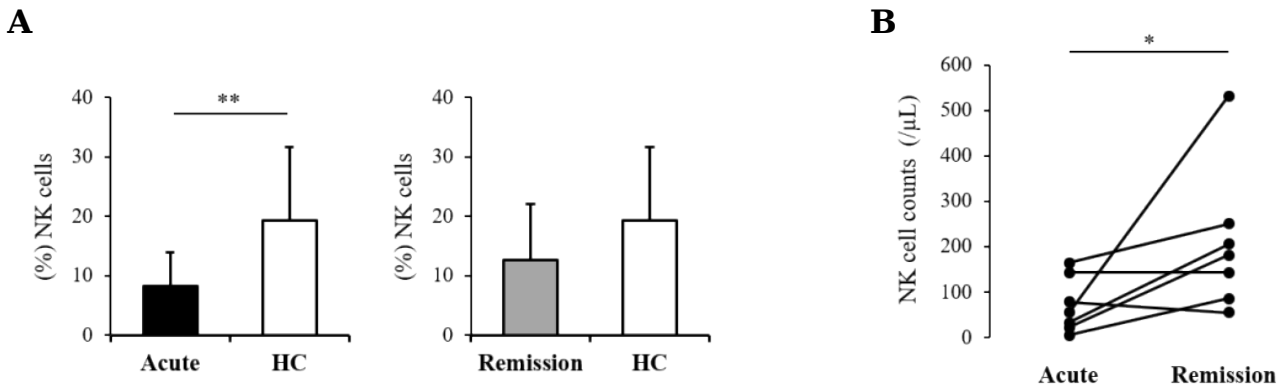

Figure 2

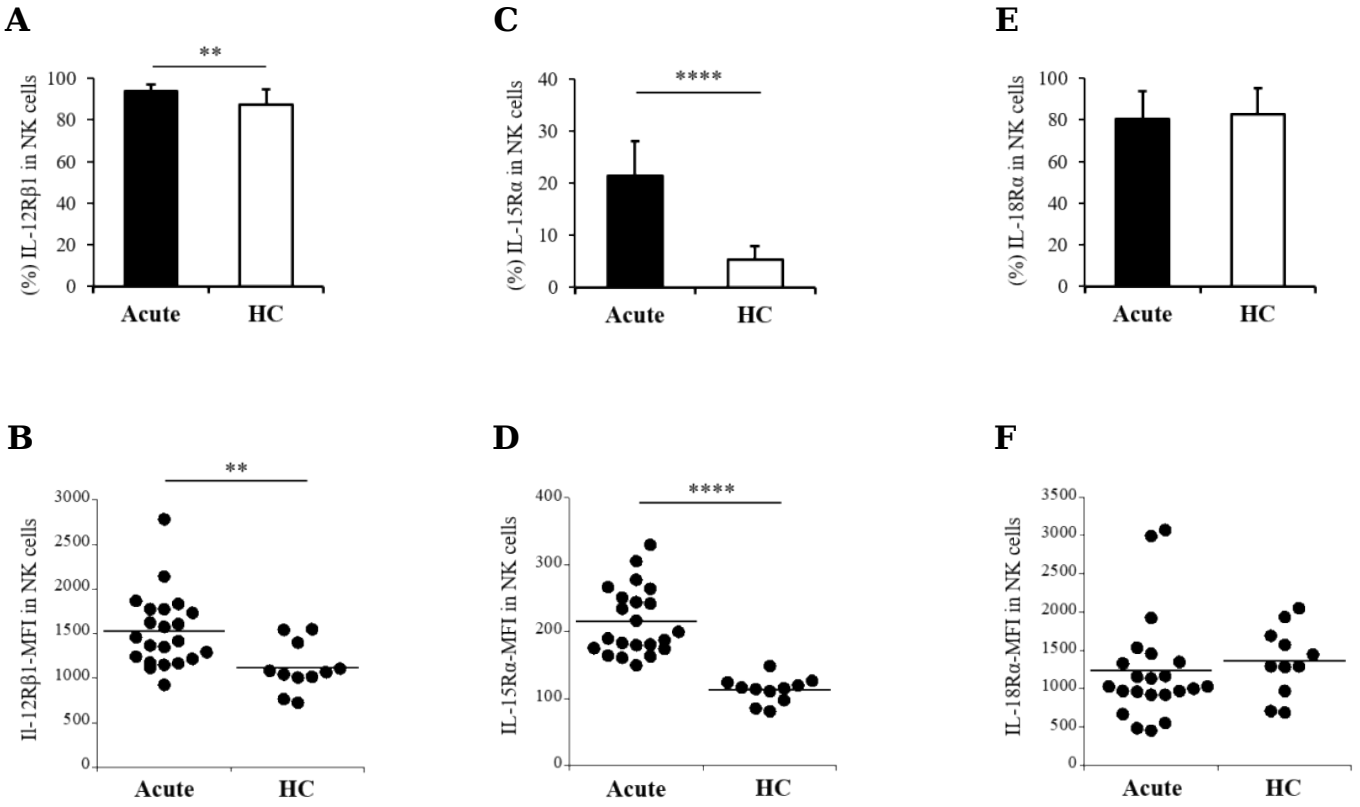

Figure 3

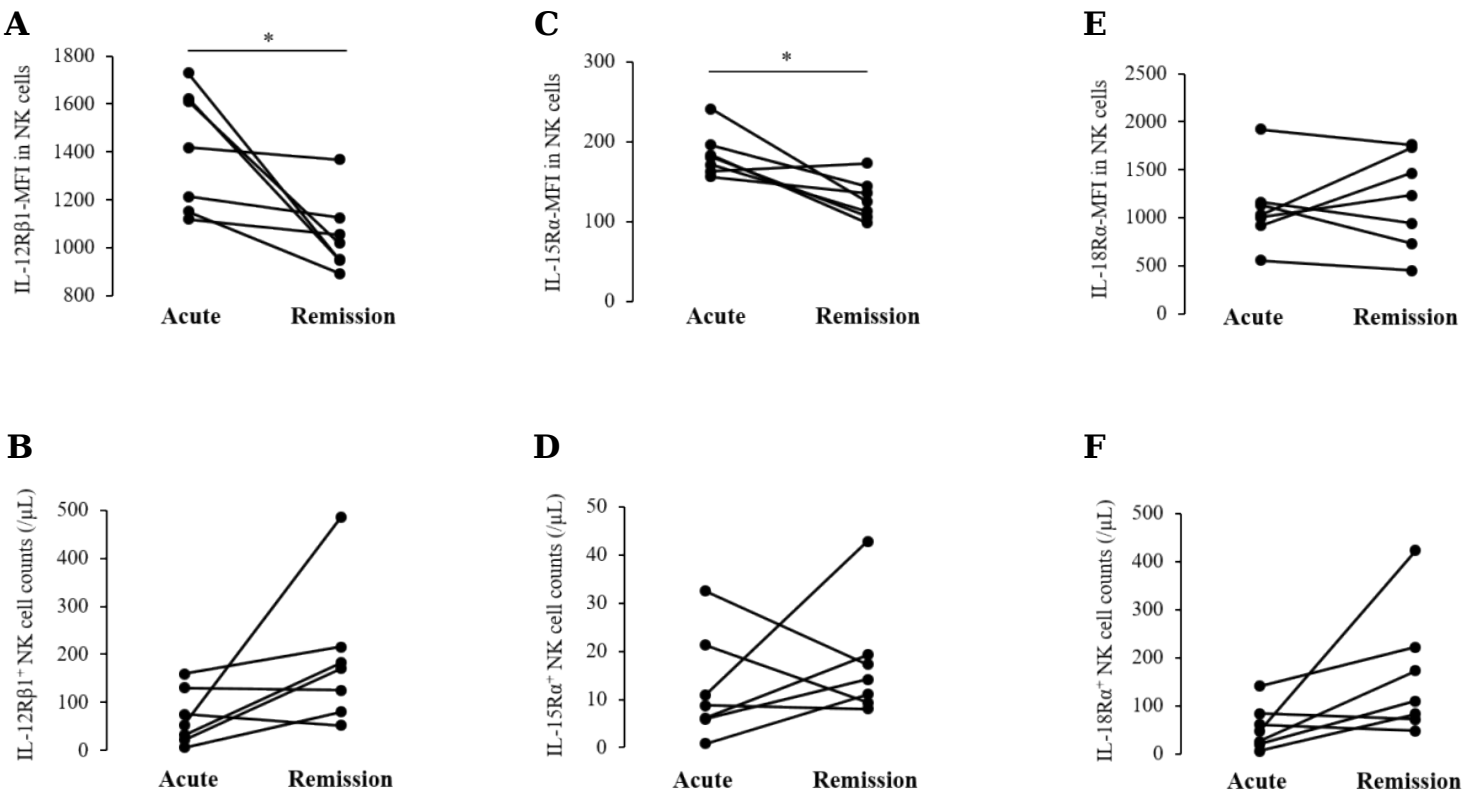

Figure 4

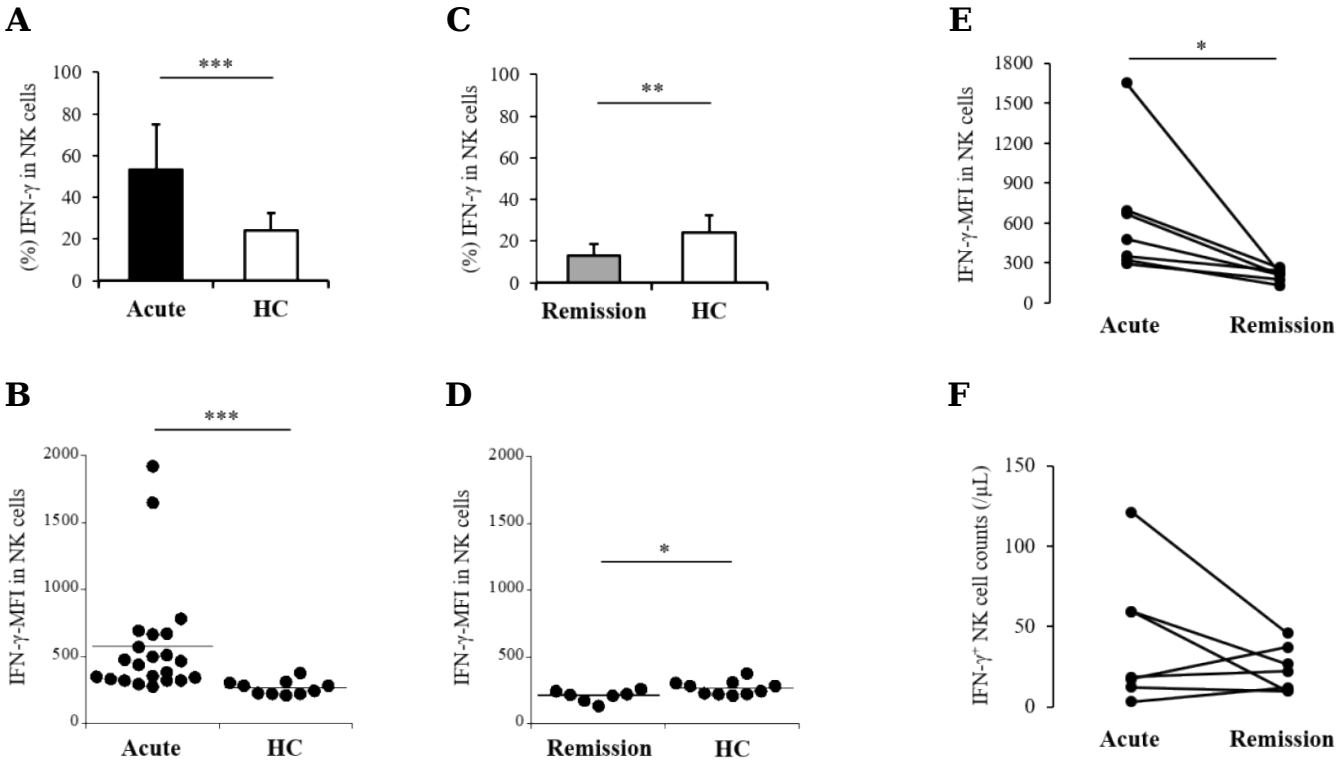

Figure 5