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Original article

Decreased PD-1 expression on circulating CD4⁺T cell and PD-L1 expression on myeloid dendritic cell correlate with clinical manifestations in systemic juvenile idiopathic arthritis

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ABSTRACT

Objectives: Programmed cell death-1 (PD-1) and its ligand (PD-L1) mediate negative signal in autoimmune diseases. While little is known about its role in juvenile idiopathic arthritis (JIA). The study aimed to reveal the circulating cell profile and the relative PD-1/PD-L1 expression of JIA subsets, elucidating their underlying immunomodulatory mechanisms.
Methods: We detected the circulating cells and the relative PD-1/PD-L1 signaling in 101 JIA patients and 50 controls by flow cytometry and analyzed their association with disease activity and clinical manifestations.
Results: Different from other JIA types, active systemic JIA (sJIA) patients had lower percentage and count of CD4⁺T cells and lower PD-1 expression on them compared with healthy controls ($P<0.05$), active polyarthritis ($P<0.05$) and enthesitis-related arthritis (ERA) patients ($P<0.05$). Also, they had higher percentage and count of myeloid dendritic cell (mDC) and lower PD-L1 expression on mDC compared with healthy controls ($P<0.05$). Both PD-1 on CD4⁺T cell and PD-L1 on mDC were negatively correlated with JADAS-27 in sJIA patients ($P<0.05$). In addition, PD-1 expression on CD4⁺T cell was negatively associated with the number of involved joints ($P<0.05$) and PD-L1 on mDC was lower in patients with fever ($P<0.01$), which could further divide patients into two groups of different manifestations.
Conclusions: Our finding displayed decreased CD4⁺T cell, increased mDC and reduced PD-1/PD-L1 signal in sJIA PBMC comparing with other JIA subsets, which might be helpful in JIA differential diagnosis and responsible for distinct clinical manifestations via different mechanisms.

1. Introduction

Juvenile idiopathic arthritis (JIA), featured with chronic arthritis and associated with multiple system involvement before sixteen years old, is the most typical rheumatic disease in children [1]. It is not a single disease but an umbrella term comprising seven subsets: systemic JIA (sJIA), oligoarthrtitis, RF (rheumatoid factor)-negative polyarthritis, RF-positive polyarthritis, psoriatic arthritis, enthesitis-related arthritis (ERA) and undifferentiated arthritis [2]. Most rheumatologists classified JIA patients into these subtypes according to the related symptoms and the number of involved joints [3]. There is no golden standard for JIA diagnosis so far [4]. Due to the clinical heterogeneity characteristic and the lack of sensitive biomarkers, which limit effective diagnosis and retard prompt treatment, a valid biomarker or indicator is a requirement for JIA diagnosis and detailed pathogenesis mechanism of JIA need to be better understood.

Apart from oligoarthrtitis which accounts for almost half of JIA patients yet with the best prognosis and outcome [5], polyarthritis, sJIA and ERA are most common seen in clinic practice [6]. Polyarthritis, similar with rheumatoid arthritis (RA) in adults, can affect over four joints during the first six month and could be further classified into RF^+ and RF^- types [7]. ERA, belonged to spondyloarthropathies, is marked by the involvement of enthesitis and arthritis [8]. Unlike other JIA patients whose arthritis is the most predominantly observed manifestation, sJIA is a systemic

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autoinflammatory disease characterized by a combination of arthritis and systemic features, including remitting fever for at least two weeks, evanescent erythematous rash, generalized lymph node enlargement, hepatomegaly, splenomegaly and serositis [9]. Although sJIA takes up about 10% of JIA, it accounts for more than two third of the mortality [10]. That's not only because the exclusive diagnosis of sJIA is difficult, but also because some children may be complicated by macrophage activation syndrome (MAS), which can result in multiple organ damage, high mortality and poor prognosis [11].

Increasing evidences revealed distinct pathogenesis between polyarthritis and sJIA, the former was reported as abnormal antigen-driven adaptive immune response while the latter an autoinflammatory disease with innate immune abnormality [12]. Meanwhile, studies on ERA focused mainly on microbiome, NK cell and γδT cell recently [13,14]. By summing up these findings, we hypothesized that cell profile in JIA were diverse and might be an indicator to differentiate the subsets from each other.

Programmed cell death 1 (PD-1 or CD279) is a member of CD28 superfamily and expressed on $CD4^+$ and $CD8^+$ T cells, NK cells, B cells and activated monocytes. Binding to the ligands PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273) on T cells, B cells, dendritic cells (DCs) and macrophages, PD-1 conducts its inhibitory function to regulate T cell activation, tolerance, and immune-mediated tissue damage [15]. Plenty of studies have demonstrated that PD-1/PD-L1 pathway was involved in autoimmune disease mediated by T cells [16], such as autoimmune type 1 diabetes [17], experimental autoimmune encephalomyelitis (EAE) [18], RA [19] and systemic lupus erythematosus (SLE) [20], while few was reported on JIA. Decreased PD-L1 expression on myeloid cells in sJIA was reported compared with other febrile patients [21]. However, it remains unclear whether the PD-1/PD-L1 pathway plays a role in all JIA subsets and its precise immune regulation on cells in JIA still needs to be elucidated.

In this study, by examining the circulating cell phenotype and its corresponding PD-1/PD-L1 expression in polyarthritis, ERA and sJIA patients, we aimed to search for special circulating cell phenotype in different JIA subgroups, investigate their potential use in JIA diagnosis and lay a clinical foundation for their immune modulation in JIA.

2. Methods

2.1. Subject population and clinical data collection

The medical ethics committee of Shanghai Children's Medical Center (SCMC) approved the study before clinical data and blood sample collection. A total of 101 children diagnosed with JIA and 50 healthy controls were enrolled in this study from 2016 to 2017 in SCMC. All the patients fulfilled the diagnosis and classification of JIA criteria of the International League of Associations for Rheumatology [9]. According to American Rheumatism Association (ACR) criteria, the state of JIA could be divided into clinical remission, inactive disease, low, moderate and high activity disease [22,23]. We classified our patients into two catalogs, including active disease (moderate or high disease activity) and inactive disease (mild disease activity or inactive disease) based on a newly proposed core set of items for measuring sJIA disease activity [24]. The demographical, clinical and laboratory characteristics are summarized in Table 1.

The clinical information was recorded at patients' visit. Physical examination included presence of fever, rash, and joint involvement. Laboratory values consisted of WBC (white blood cell), Hb (hemoglobin), PLT (blood platelet), Ne (neutrophil), CRP (C-reactive protein), ESR (erythrocyte sedimentation rate), RF, Anti-CCP (anti-cyclic peptide containing citrulline), ANA (antinuclear antibody) and HLA-B27. The Juvenile Arthritis Disease Activity Score (JADAS) is a tool developed in 2009 that includes physician global assessment (PGA) of disease activity, parent's and patient's global assessments (PtGA) of well-being, active joint count, and ESR [25]. Medications in one month before blood collection were recorded retrospectively.

2.2. Sample processing of cell

Due to cell number limitation in children, different samples were used in different experiments. Two to three milliliter venous blood from patients and donors were collected after informed consent. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque gradient (Sigma-Aldrich, St. Louis MO).

2.3. PBMC phenotyping by flow cytometry

PBMC from both patients and healthy controls were stained with anti-human CD3 (Clone: OKT3), CD4 (Clone: RPA-T4), CD14 (Clone:61D3), CD11c (Clone:3.9), PD-1 (Clone: eBioJ105), PD-L1 (Clone:MIH1) and PD-L2 (Clone:MIH18) fluorescence conjugated antibodies (eBioscience, USA) at 4 °C for 30 min. 7AAD (BD Pharmingen, USA) was added before flow cytometry was performed on BD-FACS CantoII plus (BD, USA) and data were analyzed on Flowjo software (USA). The gating methods were refereed in Appendix A, Fig. S1 [see the supplementary material associated with this article online].

2.4. Statistical analyses

Data were expressed as the mean and standard deviation (SD) or median (min–max) and performed by IBM SPSS Statistics V22.0 (USA). Continuous data were tested for normal distribution by the Kolmogorov–Smirnov test. Multiple group comparisons were made using one-way-ANOVA with least significant difference (LSD) comparison or Kruscal–Wallis test. An independent-sample *t*-test was applied to compare two independent groups. Data with non-normal distribution were implemented non-normal transformation for further ANOVA test with appropriate post hoc comparisons. The Fisher's exact test and chi-square test was used for differences between non-parametric analyses. The correlation between PD-1/PD-L1 expression and disease activity were analyzed with a Pearson's correlation coefficient. A two-sided *P* value of $P < 0.05$ was considered as statistically significant.

2.5. Role of funding source

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3. Results

3.1. Cell count and phonotype of PBMC in different forms of JIA and healthy controls

Owing to the heterogeneity nature of JIA, the age of polyarthritis, ERA and sJIA patients varied from each other ($P < 0.001$). Our findings displayed female predominance in polyarthritis and male predominance in both ERA and sJIA patients, as shown in Table 1. To elucidate the cell phenotype in different JIA subsets, PBMC were

Table 1
Clinical and laboratory profile of patients with JIA.

Characteristics	Active polyarticular	Inactive polyarticular	Active ERA	Inactive ERA	Active sJIA	Inactive sJIA	Healthy control	*P* value
n	27	14	17	13	17	13	50	
Female (*n*, %)	21, 77.7%	11, 78.5%	2, 11.7%	2, 15.3%	4, 23.5%	3, 23.0%	24, 4%	<0.001
Mean age at disease onset (years)	7.0 ± 3.8	6.2 ± 3.7	9.9 ± 2.6	10.2 ± 2.2	4.8 ± 2.2	3.0 ± 1.7	NA	<0.001
Mean age at sample collection (years)	10.1 ± 4.2	8.5 ± 4.6	11.0 ± 2.7	13.4 ± 3.0	10.7 ± 4.0	6.1 ± 3.6	6.7 ± 2.8	<0.001
Fever (*n*, %)	0	0	2, 11.7%	0	12, 70.5%	0	0	<0.001
Rash (*n*, %)	1, 3.7%	0	0	0	3, 17.6%	0	0	<0.05
Joint involvement (*n*, %)	26, 96.3%	2, 14.2%	16, 94.1%	2, 15.3%	15, 88.2%	3, 23.0%		<0.001
Number of affected joints Mean ± SD	3.4 ± 2.6	0.1 ± 0.3	2.2 ± 1.6	0.1 ± 0.3	3.8 ± 3.0	0.3 ± 0.6	0	<0.001
Number of affected joints Median (min–max)	3 (0–11)	0 (0–1)	2 (0–7)	0 (0–1)	2 (0–13)	0 (0–2)	0	<0.001
WBC ($\times 10^9$/L)	7.85 ± 3.37	7.75 ± 2.19	7.05 ± 2.32	6.99 ± 1.48	11.01 ± 6.03	9.75 ± 2.90	7.88 ± 2.04	<0.01
Hb (g/L)	122.7 ± 17.7	125.8 ± 7.5	119.1 ± 16.1	131.0 ± 18.3	117.5 ± 14.9	125.8 ± 11.9	126.2 ± 7.8	0.104
PLT ($\times 10^9$/L)	346.1 ± 120.9	332.7 ± 87.8	369.3 ± 114.6	282.1 ± 100.4	314.2 ± 111.5	360.6 ± 81.6	301.0 ± 51.4	0.086
Ne (%)	59.7 ± 11.4	54.0 ± 10.4	54.0 ± 12.4	53.6 ± 11.6	65.0 ± 21.6	51.3 ± 12.3	47.0 ± 10.8	<0.001
CRP (mg/L)	16.2 ± 19.4	3.7 ± 3.8	14.1 ± 1.4	2.9 ± 4.0	53.9 ± 65.1	4.9 ± 4.9	NA	<0.01
ESR (mm/h)	25.7 ± 20.8	16.0 ± 7.6	33.5 ± 24.2	9.9 ± 13.1	39.1 ± 40.6	7.1 ± 4.8	NA	0.001
Positive RF (*n*, %)	11, 40.7%	4, 28.5%	0	0	0	0	NA	<0.001
Positive Anti-CCP (*n*, %)	11, 40.7%	5, 35.7%	0	0	0	0	NA	<0.001
Positive ANA (*n*, %)	10, 37.0%	4, 28.5%	1, 5.8%	0	0	0	NA	<0.001
Positive HLA-B27 (*n*, %)	0	0	12, 70.5%	8, 61.5%	0	0	NA	<0.001
JADAS-27	NA	NA	NA	NA	18.3 ± 5.5	0.9 ± 1.3	NA	<0.001
PGA	NA	NA	NA	NA	6.2 ± 1.8	0.8 ± 1.6	NA	<0.001
PtGA	NA	NA	NA	NA	6.5 ± 2.7	0.92 ± 1.9	NA	<0.001
NSAIDs (*n*, %)	1, 3.7%	0	11, 64.7%	4, 30.7%	1, 5.5%	3, 23.0%	0	<0.001[#]
Prednisolone (*n*, %)	8, 29.6%	0	1, 5.8%	0	11, 64.7%	7, 53.8%	0	0.001[#]
Methotrexate (*n*, %)	21, 77.7%	8, 57.1%	3, 17.6%	1, 7.6%	6, 35.2%	4, 30.7%	0	<0.001[#]
Sulfasalazine (*n*, %)	11, 40.7%	2, 14.2%	9, 52.9%	8, 61.5%	5, 29.4%	4, 30.7%	0	0.520[#]
TNF-α inhibitor (*n*, %)	5, 18.5%	2, 14.2%	3, 17.6%	3, 23.0%	0	1, 7.6%	0	0.268[#]
IL-6R inhibitor (*n*, %)	0	0	0	0	3, 17.6%	3, 23.0%	0	0.010[#]

JADAS-27: 27-joints Juvenile Arthritis Disease Activity Score; PGA: physician global assessment (range 0–10, visual analog scale); PtGA: parent or patient global assessment of overall well-being (range 0–10, visual analog scale); NSAIDs: non-steroidal anti-inflammatory drugs; NA: not applicable.
The Fisher's exact test was used for differences between non-parametric analyses and one-way ANONVA or Kruskal–Wallis was used for parametric analyses.
[#] *P* value was assessed among the active polyarthritis, active ERA, active sJIA and inactive sJIA groups.

screened for $CD4^+$T, $CD8^+$T, $CD19^+$B, $CD14^+$ and NK cell. The percentage of $CD8^+$T, $CD19^+$B and NK cell in total PBMC showed no difference among these patients and healthy controls (Data not shown). As demonstrated in Fig. 1A, the active sJIA patients had a significantly lower proportion of $CD4^+$T cell in total PBMC compared with healthy controls ($P < 0.05$), active polyarthritis ($P < 0.05$) and ERA patients ($P < 0.05$). In parallel, the absolute number of $CD4^+$T cell showed the same tendency compared with healthy controls ($P < 0.01$), active polyarthritis ($P < 0.05$) and ERA patients ($P < 0.001$). Besides, smaller amount of $CD4^+$T cell number was observed in active sJIA patients than inactive patients ($P < 0.01$). As for $CD14^+$cell shown in Fig. 1B, the active sJIA patients had a markedly higher $CD14^+$ cell both in proportion in total PBMC ($P < 0.05$) and absolute number ($P < 0.05$) compared with healthy controls. Next, we identified $CD14^+$ cell into myeloid DC (mDC) and Mo (monocyte) as shown in Fig. 1C. mDC expressed a significantly higher proportion in active sJIA patients than healthy controls ($P < 0.01$), active polyarthritis ($P < 0.01$) and active ERA patients ($P < 0.01$) and normalized in inactive sJIA patients ($P < 0.01$). In parallel, the proportion of mDC in PBMC showed the same trend as was shown in Fig. 1D. In all, specific low $CD4^+$T cell and high mDC in active sJIA suggested their possible role in JIA differentiation and pathogenesis in sJIA.

3.2. Identification of PD-1/PD-L1 expression in different forms of JIA and healthy controls

Since PD-1 could be inducibly expressed on peripheral $CD4^+$T cell and PD-L1/PD-L2 could be constitutively expressed on mDC [15], we detected the PD-1 expression on $CD4^+$T cell and PD-L1/PD-L2 expression on mDC respectively. As Fig. 2A showed, the active sJIA patients had a statistically lower percentage and MFI (mean fluorescent intensity) of PD-1 on $CD4^+$T cell compared with healthy controls ($P < 0.05$), active polyarthritis ($P < 0.05$) and ERA patients ($P < 0.05$) and went back to normal in inactive sJIA patients in PD-1 percentage ($P < 0.05$). As for the PD-L1 expression demonstrated in Fig. 2B, the percentage and MFI of PD-L1 on mDC in active sJIA patients was less than healthy controls ($P < 0.05$) and went back to normal in inactive sJIA patients ($P < 0.05$). However, no remarkable difference was observed on PD-L2 expression among the groups shown in Fig. 2C. The decreased expression of PD-1 on $CD4^+$T cells and PD-L1 on mDC in active sJIA patients implied that the PD-1/PD-L1 pathway involved in $CD4^+$T/mDC interactions might participate in the pathogenesis of sJIA.

3.3. The association between PD-1/PD-L1 with sJIA disease activity

To assess the potential utility of PD-1/PD-L1 expression as indicated biomarker for sJIA activity, we examined the correlation between the PD-1/PD-L1 expression with clinical variables. Neither the $CD4^+$T cell nor $CD14^+$ cell percentage had any association with JADAS-27 in sJIA (Appendix A, Fig. S2). However, as is displayed in Fig. 3A and B, the percentage and MFI of PD-1 on $CD4^+$T cell negatively correlated with JADAS-27 ($P < 0.05$) as well as number of involved joints ($P < 0.05$). Patients with and without fever showed no difference in PD-1 level. As for the PD-L1 expression on mDC shown in Fig. 3C and D, the percentage and MFI negatively correlated with JADAS-27 ($P < 0.05$), but not with the number of joints. However, patients with fever had a relatively lower PD-1 level than those without ($P < 0.01$). These results indicated that PD-1 on $CD4^+$T cell might be related with joints

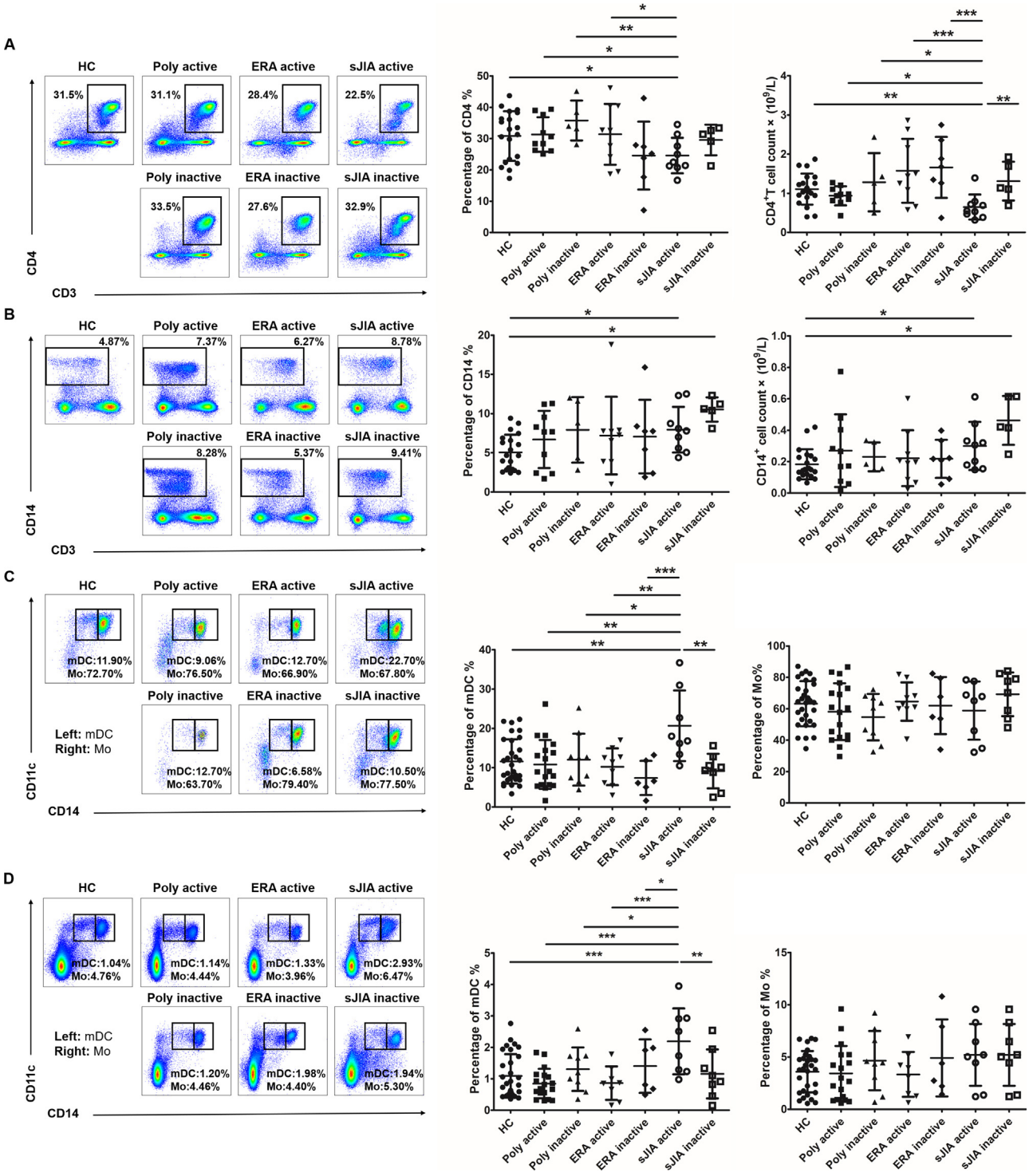

Fig. 1. Phenotype of JIA patients' PBMC. (A)&(B) Representative example, the cell distribution and absolute number of $CD4^+$T and $CD14^+$ cell in PBMC. HC ($n=20$); active polyarthritis ($n=10$); inactive polyarthritis ($n=5$); active ERA ($n=9$); inactive ERA ($n=7$); active sJIA ($n=9$); inactive sJIA ($n=5$). (C) & (D) Representative example and cell distribution of mDC and Mo in monocyte and PBMC gating. HC ($n=30$); active polyarthritis ($n=18$); inactive polyarthritis ($n=9$); active ERA ($n=9$); inactive ERA ($n=6$); active sJIA ($n=8$); inactive sJIA ($n=8$). Data are shown as mean ± SD. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

symptom while PD-L1 on mDC might be related with systemic symptom.

3.4. Two sJIA subsets based on PD-1 and PD-L1 level

Eleven sJIA patients were detected for both PD-1 and PD-L1 expression at the same time. As is shown in Fig. 4, there were two subsets of patients based on PD-1 level on $CD4^+$T cell and PD-L1 level on mDC: PD-1 shortage dominant group (group A) and PD-L1 shortage dominant group (group B). As listed in Table 2, PD-L1 expression on mDC in group A were significantly higher than that in group B ($P<0.01$). Though PD-1 level on $CD4^+$T cell showed no difference between the two groups, the ratio of PD-L1/PD-1 exhibited an obvious difference ($P<0.001$). Besides, other clinical parameters including WBC, CRP, ESR and JADAS-27 showed no difference between the two groups. Notablye, there were relativelye

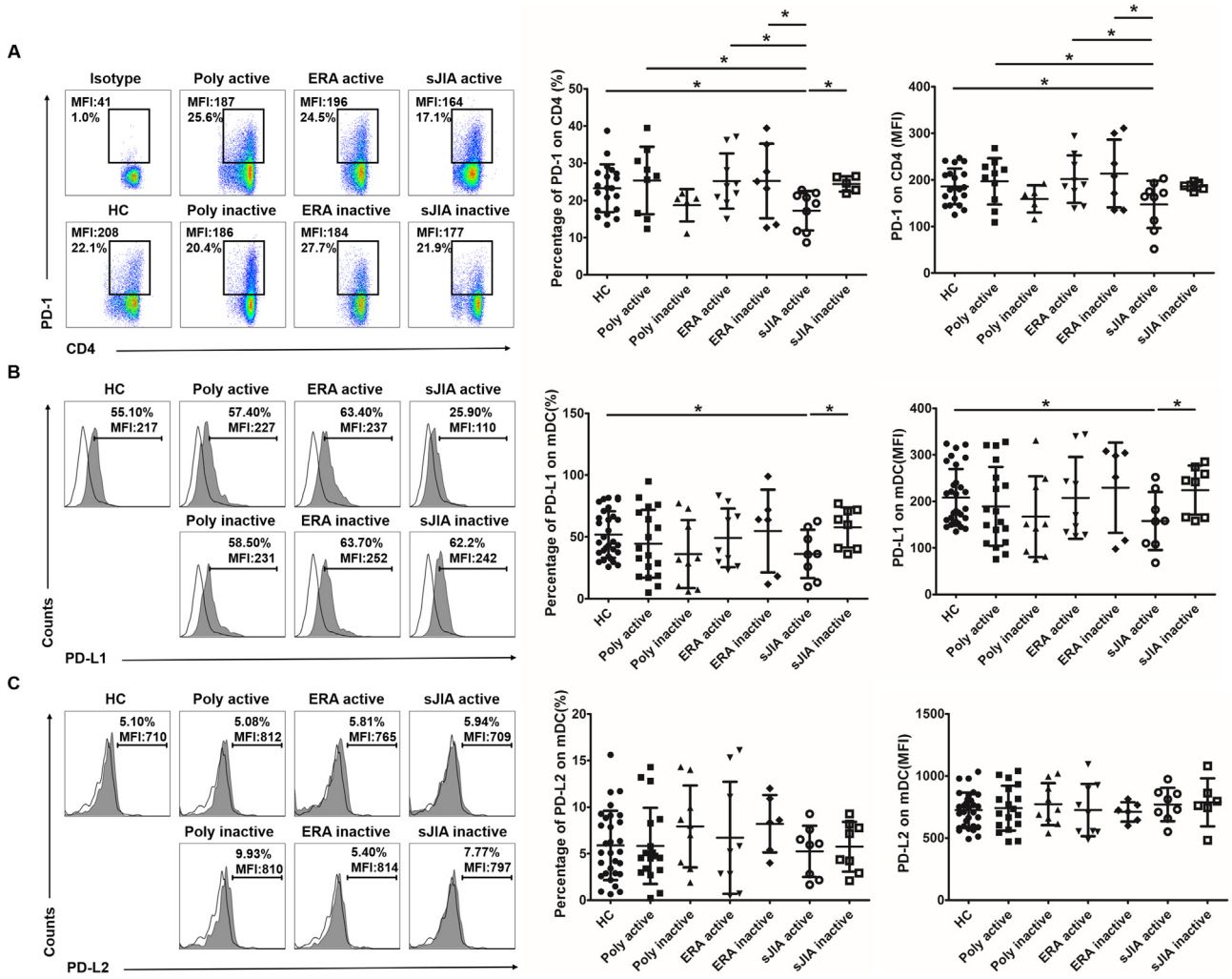

Fig. 2. PD-1/PD-L1 expression on CD4⁺T and mDC cell of JIA. (A) Representative example, the percentage and MFI of PD-1 expression on CD4⁺T cell for studied individuals. HC ($n = 20$); active polyarthritis ($n = 10$); inactive polyarthritis ($n = 5$); active ERA ($n = 9$); inactive ERA ($n = 7$); active sJIA ($n = 9$); inactive sJIA ($n = 5$). (B) and (C) Representative example, the percentage and MFI of PD-L1 and PD-L2 expression on mDC for studied individuals. HC ($n = 30$); active polyarthritis ($n = 18$); inactive polyarthritis ($n = 9$); active ERA ($n = 9$); inactive ERA ($n = 6$); active sJIA ($n = 8$); inactive sJIA ($n = 8$). Data are shown as mean ± SD. * $P < 0.05$.

more joints involvements in group A ($P < 0.05$) and more fever symptoms in group B ($P < 0.05$), which implied that PD-1 and PD-L1 could divided patients into two subsets: one with joints symptom predominance and one with systemic symptom predominance.

4. Discussion

JIA is a kind of prominent crippling disease with unknown etiology. Even though it is a group of well-defined arthritis disease marked by varied clinical features, laboratory tests and sometimes genetic backgrounds [26], it is still hard to differentiate JIA subsets with each other, some other disease of arthritis presentation and some fever of unknown. At the same time the pathophysiological nature of JIA is complicated and remains largely unknown [12]. The PD-1/PD-L1 signaling has been largely explored in autoimmune diseases except for JIA [27]. In this study, for the first time, we completely screened the phenotype of peripheral blood obtained from children with polyarthritis, ERA and sJIA, their corresponding PD-1/PD-L1 expression, and their relationship with disease activity.

To minimize the deviation bias caused by disease activity, patients need to be grouped. According to ACR criterion, patients with JIA can be classified into clinical remission, inactive disease, low, moderate and high activity disease [23]. Due to the limited patients number which were not appropriate for so many groups, we separated our patients into two categories, namely active and inactive one. Meanwhile, for some low activity patients only presented with no more than two articular symptoms, we classified them into inactive disease to divide all the patients into two roughly equal groups, just as the new set of items for measuring disease activity in sJIA went [24].

Given the special clinical and pathogenesis feature of sJIA [10], we reasoned that the analyses of PBMC cellular composition would be informative. The few existing studies focused on the cell phenotype in JIA were inconsistent or even controversial [28–30]. In our study, the fact that the percentage and absolute number of CD4⁺T cell decreased in active sJIA patients compared to healthy controls, active polyarthritis and ERA patients, while that of CD14⁺ cell increased compared with healthy controls, indicated CD4⁺T cell could be an indicator of sJIA and helpful in differential diagnosis from other JIA types. Though one kind of cell percentage change might be attributed to relative changes in other populations, the changes of absolute CD4⁺T and CD14⁺ cell numbers confirmed the circulating cell proportion alteration in active sJIA patients. It is well known that CD4⁺T cell is a key member in adaptive immune response and its expression in sJIA varied in published studies. Some groups found similar percentages of CD4⁺T cells in sJIA and healthy controls [28], while others found increased CD4⁺T cells in flared sJIA patients compared to quiescent ones [31]. However,

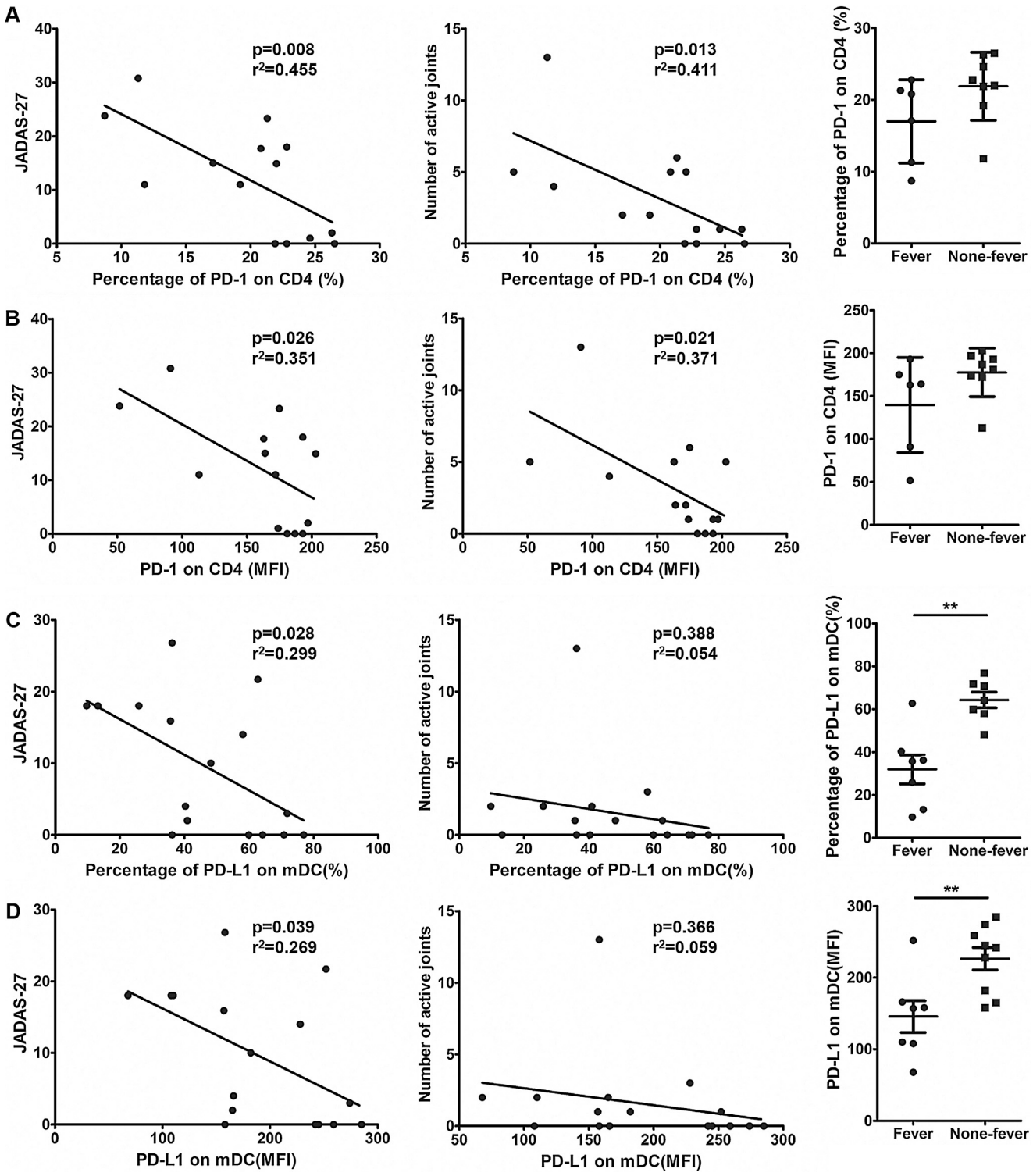

Fig. 3. The correlation between PD-1/PD-L1 and disease activity of sJIA patients. (A) and (B) Correlation between percentage/MFI of PD-1 on $CD4^+$T cell with JADAS-27. Number of joints and fever. (C) and (D) Correlation between percentage/MFI of PD-L1 on mDC with JADAS-27, number of joints and fever. ** $P < 0.01$. Pearson's correlation coefficient and one-way ANOVA were applied for the correlation analysis and group difference respectively.

these studies did not use healthy controls or distinguish samples based on disease activity, neither did they compare all the three subsets at the same time. In line with reported findings of $CD14^+$ cell, our study showed coincident rising trend of $CD14^+$ cell in sJIA patients [28,29]. CD14 protein is a surface marker expressed on Mo, macrophage and DC [32], and can further segregated the cells into $CD14^{lo}CD11c^+$mDC and $CD14^{hi}CD11c^+$Mo [33], among which mDC is a kind of professional antigen-presenting cells (APC) and responsible for capturing, processing and presenting antigens to T cells, serving as a bridge linking adaptive and innate immune responses [34]. Our findings showed that it was mDC rather than Mo that increased in active sJIA patients, which accounted for the

Table 2
Clinical features of sJIA subsets based on PD-1 and PD-L1 levels.

Characteristics	PD-1 shortage dominant group (group A)	PD-L1 shortage dominant group (group B)	*P* value
n	6	5	
Male (*n*, %)	0	2, 40.0%	0.182
Fever (*n*, %)	2, 33.3%	5, 100%	<0.05
Rash (*n*, %)	0	0	
Number of affected joints Mean ± SD	7.8 ± 3.7	2.6 ± 1.7	<0.05
Number of affected joints Median (min–max)	6 (4–13)	2 (0–5)	<0.05
WBC ($\times 10^9$/L)	10.47 ± 2.00	9.58 ± 3.83	0.668
CRP (mg/L)	73.1 ± 66.5	10.6 ± 11.4	0.093
ESR (mm/h)	25.17 ± 16.88	15.4 ± 12.3	0.356
JADAS-27	19.6 ± 6.9	14.5 ± 3.1	0.206
PGA	6.50 ± 1.50	6.00 ± 2.19	0.695
PtGA	7.00 ± 1.63	5.60 ± 2.42	0.329
PD-1 on $CD4^+$T cell (%)	15.9 ± 6.2	18.8 ± 4.9	0.624
PD-L1 on mDC (%)	36.6 ± 8.6	16.4 ± 5.0	<0.01
PD-L1/PD-1	2.16 ± 0.53	0.88 ± 0.13	<0.001

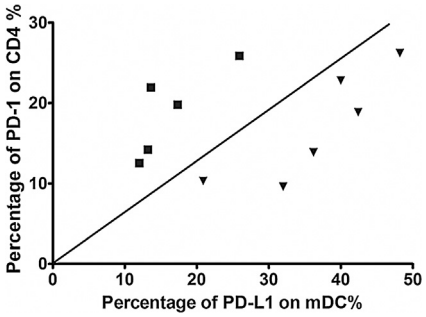

Fig. 4. Two sJIA subsets based on PD-1 and PD-L1 levels. ■: PD-L1 shortage dominant group; ▼: PD-1 shortage dominant group.

accumulation of $CD14^+$ cell. And the increased proportion of mDC in sJIA PBMC confirmed the cell profile change. The hallmark of cell patterns in JIA revealed the downregulated $CD4^+$T cell and upregulated mDC were typical in active sJIA patients, suggesting $CD4^+$T cell, mDC or their interaction might be involved in the pathogenesis.

As a negative regulator in T cell activation, proliferation and cytokine production, PD-1/PD-L1 signaling pathway is well-known for maintaining this critical balance and plays an important role in a variety of autoimmune diseases [16]. However, up to now there was only one study demonstrating reduced PD-L1 on mDC in sJIA patients comparing to other febrile patients [21]. It is still unclear whether the inhibitory signals participate in JIA, which also belongs to autoimmune diseases. Our results implicated that PD-1 on $CD4^+$T cell and PD-L1 on mDC might take part in sJIA pathogenesis. Firstly, they were statistically lower in active sJIA patients than healthy controls and normalized in inactive patients, which was specific for sJIA patients. Secondly, the PD-L1 protein on mDC showed an upregulation delay in sJIA patients, both the active and inactive ones, which indicated the functional disability of PD-L1 in sJIA patients (data not shown). Third, the negative correlation of PD-1 on $CD4^+$T cells and PD-L1 on mDC with JADAS-27 implied them as potential biomarkers in disease activity.

As the most common symptoms in sJIA [35], the presentation of quotidian fever and arthritis were usually associated with sJIA by clinicians. JADAS-27 is a validated disease activity score specific for JIA and can be calculated by sum of active joints counts of 27 indicated joints, PGA, PtGA and normalized ESR [25]. No relationship was found between the percentage of $CD4^+$T and $CD14^+$ cell with JADAS-27 and number of joints, partly because the circulating cells phenotype could not represent pathogenic cells in joints. But both PD-1 on $CD4^+$T cell and PD-L1 on mDC had a negative correlation with JADAS-27. What's more, it was noteworthy that PD-1 and PD-L1 was related with number of joints and fever respectively, indicating their different mechanism in sJIA. Considering the distinct relationship of PD-1 and PD-L1 with clinical symptoms, sJIA patients could be divided into two catalogs, one with fever predominant and the other with active joint predominant, which further approved different role of PD-1 on $CD4^+$T cell and PD-L1 on mDC in sJIA.

A biphasic sJIA model divided sJIA into acute febrile and chronic arthritis phase, the first stage mainly conducted by activation of endothelium, leukocytes and resident tissue lineages and the second one conducted by $CD4^+$T subsets such as Th1, Th17 and Treg [36]. Regarding to the underlying mechanisms how PD-1 on $CD4^+$T cell an PD-L1 on mDC modulates sJIA pathogenesis, we speculate as follows. To begin with, we propose the downregulation of PD-1/PD-L1 may weaken the inhibitory signaling, thus enhancing the number of DC, as was reported that blocking PD-1/PD-L1 signaling could promote DC maturation and proliferation [37]. Then, the accumulated mDC increases secretion of cytokines such as IL-6, IL-18 and TNF-α, which exert a fundamental role in sJIA pathogenesis and cause fever manifestation [38]. Elevated $CD4^+$T cell number was widespread reported when blocking PD-1 signal [39]. However, the contradictory results of reduced PD-1 expression and decreased instead of increased $CD4^+$T cell in sJIA patients remains to be solved. As circulating cells could migrate from the bloodstream to the synovial tissue and therefore take part in inflammatory response, the downregulation of $CD4^+$T cell in PBMC might resulted from its accumulation and influx into joints [40], which may partly explain the observed discrepancy. Of course, more investigations need to be done to clarify the interesting phenomenon.

In summary, for the first time we have observed decreased $CD4^+$T cell and increased mDC and corresponding reduced PD-1/PD-L1 expression in active sJIA patients. Additionally, the number of joints related PD-1 expression on $CD4^+$T cell and fever-associated PD-L1 on mDC could divided sJIA patients in two groups with different predominant symptoms. Exploring the phenotype of the immune response in JIA will be helpful in differential diagnosis and critical to further elucidating the pathophysiological mechanism of sJIA.

Declaration of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data (Figs. S1 and S2) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbspin.2018.03.003.

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