Decreased plasma sPD-1 level correlates with disease activity in systemic juvenile idiopathic arthritis patients

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Abstract

Objectives: Soluble programmed death-1 (sPD-1) and its ligand (sPD-L1) take part in some autoimmune diseases. Little is known about its role in systemic idiopathic arthritis (sJIA). The study aimed to explore the sPD-1 and sPD-L1 levels in sJIA patients and elucidate their underlying immunomodulatory mechanisms.

Methods: Plasma levels of sPD-1, sPD-L1 and related cytokines were detected in sJIA patients and healthy controls (HCs) using an enzyme-linked immunosorbent assay (ELISA) and Luminex. The correlation of sPD-1/sPD-L1 with clinical characteristics, laboratory parameters and pro-inflammatory cytokines level of patients were analyzed. The effects of PD-1/PD-L1 signal on T cell differentiation and IL-6 secretion were measured using flow cytometry.

Results: The data revealed decreased levels of sPD-1 in active sJIA patients, and it negatively correlated with JADAS-27, PGA, PtGA and CRP. While the sPD-L1 level was positively correlated with Ferritin, S100A8, IL-6, IL-18, IL-1β and TNF-α level. Moreover, the sPD-1 and sPD-1/sPD-L1 could be sJIA diagnosis and IL-6R inhibitor treatment marker in patients. The vitro experiments showed that when blocking PD-1/PD-L1 signal, IFN-γ and IL-6 secretion were increased.

Conclusions: Our finding displayed decreased sPD-1 in active sJIA patients, which could be a new biomarker for differential diagnosis and critical to further elucidating the pathophysiological mechanism of sJIA.

1. Background

Systemic juvenile idiopathic arthritis (sJIA), featured with remitting fever for at least two weeks, evanescent erythematous rash, generalized lymph node enlargement, hepatomegaly, splenomegaly and serositis, is a systemic auto-inflammatory and autoimmune subset of juvenile idiopathic arthritis (JIA) [1]. Although sJIA takes up about 10% of JIA and the new treatments are identified, it accounts for more than two third of the mortality [2], which partly due to its fatal complication of macrophage activation syndrome (MAS) [3]. At present, sJIA patients are diagnosed according to clinical features and laboratory parameters [4]. However, this standard lacks of specificity for sJIA diagnosis and limits effective diagnosis and retard prompt treatment [5]. For this reason, a valid biomarker or indicator is a requirement for sJIA diagnosis.

Distinct from others JIA subsets, the cause and pathogenesis of sJIA are poorly understood. A biphasic model of sJIA was raised to reveal that sJIA not only an autoinflammatory but also autoimmune disease [6]. On the one hand, interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-18 (IL-18) are elevated and correlated with disease activity [1] and phagocyte-specific S100-proteins (S100A8, S100A9, S100A12) perpetuate inflammation [7], which support sJIA as a auto-inflammatory disease. On the other hand, the fact that chronic arthritis is mediated by autoreactive T cells support sJIA as autoimmune condition [8]. So a number of studies described the potential biomarkers of sJIA due to the pathogenesis of sJIA, such as IL-1β, S100A8, S100A9, S100A12. However, only part of them have been validated and used in clinical practice [9].

The programmed cell death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway plays a critical role in host immunosuppression mechanism, which inhibits the function and proliferation of T and B cells, and reduces interleukin-2 (IL-2), interleukin-10 (IL-10), and interferon-γ (IFN-γ) secretion [10]. Soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) are soluble form of PD-1 and PD-L1 in peripheral blood. sPD-1 is encoded by PD-1Δex3, which retains the extracellular domain but lacks the trans-membrane domain. And previous studies have shown that sPD-1 promotes T-cell responses by blocking the PD-1/PD-L1 pathway [11]. sPD-L1 (sPD-L1) is reported to be cleaved off the membrane by matrix metalloproteinases and its regulatory roles are not fully understood [12].

Plenty of studies have demonstrated that sPD-1 and sPD-L1 were involved in autoimmune disease, such as systemic lupus erythematosus (SLE) [13], autoimmune type 1 diabetes [14], Immune thrombocytopenia (ITP) [15] rheumatoid arthritis (RA) [16] and chronic hepatitis B [17], while few was reported on sJIA. Our previous
study have reported decreased PD-1 on CD4+T cell and reduced PD-L1 on mDC (myeloid dendritic cell) in active sJIA patients [18]. However, it remains unclear whether the sPD-1 and sPD-L1 plays a role in sJIA and its precise immune regulation on cells in sJIA still need to be elucidated. In this study, by examining the circulating plasma sPD-1 and sPD-L1 levels and its corresponding correlation with disease activity, we aimed to search for a new marker in sJIA, investigate their potential use in sJIA diagnosis and lay a clinical foundation for their immune modulation in sJIA.

2. Methods

2.1. Patients and clinical data collection

The clinical data and blood sample collection in this study were performed in accordance with the medical ethics committee of Shanghai Children’s Medical Center (SCMC). A total of 46 children fulfilled the diagnosis and classification of sJIA criteria of the International League of Associations for Rheumatology [4] were included in this study from 2016 to 2018 in SCMC. As our previous research [18], we divided our patients into 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 [19]. At the same time, 35 children having a healthy check were enrolled as healthy controls (HC).

Physical examination included presence of fever, rash, joint involvement, serositis, lymphadenopathy and hepatosplenomegaly. Laboratory findings consisted of WBC (white blood cell), Hb (hemoglobin), PLT (blood platelet), Ne (neutrophil), CRP (C-reactive protein) and ESR (erythrocyte sedimentation rate). Disease activity was measured by the juvenile arthritis disease activity score (JADAS) that was calculated by physician global assessment (PGA) of disease activity, parent’s and patient’s global assessments (PtGA) of well-being, active joint count and ESR [20]. Medications in one month before blood collection were recorded retrospectively.

2.2. Plasma preparation and detection

Blood were collected from the patients and sex- and age-matched HC. Plasma were isolated and stored at -80°C until use. The serum levels of IFN-γ, IL-4, IL-17A, TNF-α, IL-18, IL-6, IL-1β, Ferritin, S100A8, S100A12 were determined using the Human Premixed Multi-Analyte kit (Magnetic Luminex Screening Assay, R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. The serum levels of sPD-1 and sPD-L1 were determined using Human ELISA Kit (Dakewe, China) according to the manufacturer’s instructions.

2.3. Cell culture

Peripheral blood mononuclear cells (PBMCs) of HC were isolated by Ficoll-Hypaque gradient (Sigma-Aldrich, St. Louis MO). The CD4+T cells were isolated by positive selection using CD4+T cell microbeads (Miltenyi Biotec, Germany) according to the manufacturer’s instructions and adherent PBMCs were isolated after two hours culture. The adherent cells were seeded 2 × 10⁶ per well in 48-flat-plate with the presence of 100 ng/ml human granulocyte-macrophage colony-stimulating factor (GM-CSF, Peprotech, USA) and 50 ng/ml IL-4 (Peprotech, USA). Medium and cytokines were added on Day four and 100 ng/ml lipopolysaccharide (LPS, Sigma-Aldrich, USA) was added on Day six. The isolated CD4+T cells and induced DCs were cultured with 10 µg/ml anti-human PD-1 (eBioscience, USA), 10 µg/ml anti-human PD-L1 (eBioscience, USA) or 10 µg/ml Isotype (eBioscience, USA) for two hours before mixed cultured at the ratio of 5:1 with stimulation of 0.25 µg/ml anti-human CD3 Antibody (BD, USA) for three days. Cells were harvested and stained for cytokines with flow cytometry. PBMCs of HC were cultured with 10 µg/ml anti-human PD-L1 (eBioscience, USA) for 72 hours, and cells were harvested and stained for cytokines with flow cytometry.

2.4.
Flow cytometry

To detect IFN-γ and IL-17, cells were incubated in Cell stimulation cocktail (plus protein transport inhibitors, eBioscience, USA) for five hours, stained for surface marker with anti-human CD3 (eBioscience, USA), anti-human CD4 (eBioscience, USA), permeabilized with Cytofix/cytoperm solution (BD, USA), and harvested for intracellular staining of anti-human IFN-γ (eBioscience, USA) and anti-human IL-17 (eBioscience, USA). For IL-6 detection, cells were incubated in Cell stimulation cocktail (plus protein transport inhibitors, eBioscience, USA) for five hours, stained for surface marker with anti-human CD11c (eBioscience, USA), permeabilized with Cytofix/cytoperm solution (BD, USA), and harvested for intracellular staining of anti-human IL-6 antibodies (eBioscience, USA). Flow cytometric analysis was performed on BD FACS Cantoll plus (BD, USA) and Flowjo software statistical analysis.

2.5. Statistical analysis

Results were expressed as mean and standard deviation (SD) or median (min-max) and performed by IBM SPSS Statistics V22.0 (USA). Continuous variables 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 Krusal-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 categorical variables. The correlation between sPD-1/sPD-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. Receiver-operating characteristic (ROC) curves were performed to evaluate the diagnostic performance of sPD-1/sPD-L1 expression of sJIA patients and HCs. Sensitivity against 100% minus specificity was plotted at each cutoff threshold and the area under the curves (AUC) was calculated to indicate the possibility of correctly identifying patients with sJIA from HC.

3. Results

3.1. Characteristics of the study population

A total of 26 active sJIA patients (mean age ± SD, 7.96 ± 4.58 years; female/male, 9/17) and 20 inactive sJIA patients (mean age ± SD, 7.42 ± 3.99 years; female/male, 5/15) and 35 healthy donors (mean age ± SD, 7.42 ± 3.99 years; female/male, 18/17) were included in the study. No significant difference was observed between the groups with regard to age at disease onset, age at sample collection, gender ratio and treatment. The demographical, clinical and laboratory characteristics are summarized in Table 1.
### Table 1: The Clinical and laboratory profile of patients with sJIA

| Characteristics                        | Active sjIA       | Inactive sjIA      | Healthy control | P value |
|----------------------------------------|-------------------|--------------------|-----------------|---------|
| n                                      | 26                | 20                 | 35              |         |
| Female (n, %)                          | 9, 34.60%         | 5, 25.00%          | 18, 51.43%      | 0.132   |
| Mean age at disease onset (years)      | 4.27±.76          | 3.71±.60           | NA              | 0.348   |
| Mean age at sample collection (years)  | 7.96±.58          | 7.42±.99           | 7.78±.98        | 0.187   |
| Fever (n, %)                           | 21, 80.77%        | 0                  | 0               | 0.012   |
| Rash (n, %)                            | 5, 19.00%         | 0                  | 0               | 0.001   |
| Serositis (n, %)                       | 2, 7.70%          | 0                  | 0               | 0.001   |
| Lymphadenopathy (n, %)                 | 7, 26.90%         | 0                  | 0               | 0.001   |
| Hepatosplenomegaly (n, %)              | 5, 19.20%         | 1.50%              | 0               | 0.001   |
| Joint involvement (n, %)               | 22, 84.60%        | 7, 35.00%          | 0               |         |
| Number of affected joints Mean ± SD    | 3.12±.87          | 0.55±.80           | 0               | 0.001   |
| Number of affected joints Median (min-max) | 2 (0–13)      | 0 (0–2)            | 0 (0–0)         | 0.001   |
| WBC (x10^9/L)                          | 16.28±6.31        | 9.93±.98           | 7.86±.12        | 0.001   |
| Hb (g/L)                               | 116.85±14.29      | 124.45±24.45       | 126.18±2.17     | 0.023   |
| PLT (x10^9/L)                          | 404.27±31.68      | 362.45±105.61      | 300.21±08.81    | 0.005   |
| Ne (%)                                 | 76.72±6.72        | 56.19±61.13        | 46.89±60.27     | 0.001   |
| CRP (mg/L)                             | 53.08±3.08 m      | 4.30±.308          | NA              | 0.001   |
| ESR (mm/h)                             | 46.73±6.56        | 13.65±32.65        | NA              | 0.001   |
| JADAS-27                               | 18.34±8.45        | 1.81±.82           | NA              | 0.001   |
| PGA                                    | 5.92±.19          | 0.45±.67           | NA              | 0.001   |
| PtGA                                   | 6.46±.19          | 0.50±.74           | NA              | 0.001   |
| NSAIDs (n, %)                          | 2, 7.70%          | 4, 20.00%          | 0               | 0.224   |
| Prednisolone (n, %)                    | 16, 61.54%        | 13, 65.00%         | 0               | 0.812   |
| Methotrexate (n, %)                    | 11, 42.31%        | 4, 20.00%          | 0               | 0.114   |
| Sulfasalazine (n, %)                   | 4, 15.38%         | 5, 25.00%          | 0               | 0.420   |
| Cyclosporin A (n, %)                   | 3, 11.54%         | 2, 10.00%          | 0               | 0.869   |
| TNF-α inhibitor (n, %)                 | 1, 3.85%          | 1, 5.00%           | 0               | 0.851   |
| IL-6R inhibitor (n, %)                 | 4, 15.38%         | 2, 10.00%          | 0               | 0.595   |

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 ANOVA or Kruskal-Wallis was used for parametric analyses. #: p value was assessed among the active sjIA and inactive sjIA groups.

### 3.2. Decreased plasma level of sPD-1 in sjIA patients

Levels of sPD-1, sPD-L1, pro-inflammatory cytokines and the pro-inflammatory S100-proteins in sjIA patients were quantitated for every individual. As demonstrated in Fig. 1, the active and inactive sjIA patients had a significantly lower concentration of plasma sPD-1 compared with HCs (p < 0.001), whereas sPD-L1 showed no significant difference between any two groups. By screening the pro-inflammatory cytokines and S100-proteins...
related with sJIA pathogenesis, we found active sJIA patients had a markedly higher IL-18, IL-6, IL-1β, Ferritin, S100A8 and S100A12 level (p < 0.05) compared either with HCs (p < 0.05) or inactive sJIA patients (p < 0.05).

3.3. Association between sPD-1 and clinical features of sJIA patients

Next, Fig. 2 revealed the correlation of sPD-1 with the clinical parameters of sJIA patients. sPD-1 level negatively correlated with JADAS-27 (p < 0.05) as well as PGA (p < 0.01), PtGA (p < 0.05) and CRP (p < 0.05), which indicated plasma sPD-1 might be a potential marker for sJIA disease activity. At the same time, we found patients with fever had a relatively lower sPD-1 level than those without (p < 0.01). In parallel, patients with arthritis had a relatively lower sPD-1 level than those without (p < 0.01). However no association was observed between sPD-1 level with other pro-inflammatory cytokines (data not shown). These findings suggest that sPD-1 might play a critical role in clinical features occurrence.

3.4. Association between sPD-L1 and pro-inflammatory cytokines and proteins of sJIA patients

On the contrary, no association was observed between sPD-L1 level with either JADAS-27, PGA, PtGA or CRP (data not shown). Patients with and without fever or with and without arthritis varied no difference in PD-L1 level (data not shown). As shown in Fig. 3, sPD-L1 level was positively correlated with Ferritin (p < 0.01), S100A8 (p < 0.05), IL-6 (p < 0.05), IL-18 (p < 0.05), IL-1β (p < 0.01) and TNF-α (p < 0.01). These findings suggest that sPD-L1 might be related with inflammation mechanisms in sJIA.

3.5. Utility of sPD-1 and sPD-L1 level in sJIA diagnosis and treatment

To assess the diagnostic and treatment value of sPD-1 and sPD-L1 level in sJIA, we used ROC curve analysis. As shown in Fig. 4, the ROC curves of sPD-1 reflected that sPD-1 level was robust in separating patients with sJIA from HCs with an AUC of 0.824 (95% CI, 0.734–0.914). sPD-1/sPD-L1 was also effective in discriminating sJIA from HCs: the AUC was 0.868 (95% CI, 0.782–0.949), indicating plasma sPD-1 level and sPD-1/sPD-L1 represented a potential utility biomarker for sJIA. At the same time, we followed six patients using IL-6R inhibitor treatment. After using IL-6R inhibitor, the sPD-1 level (p < 0.05) and sPD-1/sPD-L1 (p < 0.01) was markedly unregulated, while the sPD-L1 (p < 0.05) level was significantly decreased.

3.6. The potential mechanism of PD-1/PD-L1 signal in sJIA

Given that PD-1/PD-L1 signal is crucial in immune regulation, we therefore explored its effect in sJIA. We have reported decreased CD4^+ T cell and increased mDC and corresponding reduced mPD-1/mPD-L1 expression in active sJIA patients [18]. But how the decreased mPD-1 and mPD-L1, and decreased sPD-1 take part in sJIA pathogenesis is still unknown. As is shown in Fig. 5, active sJIA patients had a markedly higher IFN-γ compared either with HCs (p < 0.001) or inactive sJIA patients (p < 0.001), while no difference of IL-4 and IL-17 was observed between active sJIA patients and healthy controls, indicating Th1 cell played a predominant role in sJIA. Then we explored the effect of PD-1/PD-L1 signal on T cell differentiation using DC and T cell co-culture system in vitro. The results showed that when blocking PD-1/PD-L1 signal, IFN-γ was significantly increased (p < 0.05). When blocking PD-L1, IL-6 secreted by DC was increased, this is consistent with findings of high IL-6 level in sJIA patients. Collectively, these data indicate that despite the opposite effect of mPD-1 and sPD-1 in PD-1/PD-L1 signal, they may contribute to unregulated IFN-γ and IL-6 secretion in sJIA pathogenesis.

4. Discussion

Although sJIA is well-defined by varied clinical features, laboratory tests and sometimes genetic backgrounds, it’s still a great challenge to differentiate sJIA from other arthritis, MAS and fever of unknown due to the lack of specific and sensitive biomarkers. So it’s imperative to search for new diagnostic markers for this complicated
We have reported decreased PD-1 on CD4+ T cells and decreased PD-L1 on mDC in active sJIA patients. Given the fact that sPD-1 and sPD-L1 were soluble part of mPD-1 and mPD-L1 in plasma, we reasoned that the levels would be informative. We thought sPD-1 as a potential biomarker for sJIA diagnosis and the reasons are as follows. First, sPD-1 is decreased in active sJIA patients compared to HCs and inactive patients, which is consistent with the decreased PD-1 expression on CD4+ T cells in active sJIA patients. Second, sPD-1 has a negative correlation with JADAS-27, PGA, PtGA, CRP, which is the recognized disease activity score specific for JIA. Third, as the most common symptoms in sJIA, patients with quotidian fever and arthritis have lower level of sPD-1, indicating its mechanism in sJIA symptoms. Last but not least, the ROC curve analysis indicated sPD-1 level might be biomarker to distinguish sJIA from HC.

We validated the same trend of pro-inflammatory cytokines and proteins in sJIA patients, which is in parallel with the reported studies [21]. Despite the fact that sPD-L1 does not vary among groups and no relationship was found between the sPD-1 level and pro-inflammatory cytokines and proteins, sPD-L1 had a positive correlation with ferritin, S100A8, IL-6, IL-18, IL-1β and TNF-α. And the ROC curve analysis indicated sPD-1/sPD-L1 might be biomarker for sJIA diagnosis. Collectively, they indicate a different role of sPD-L1 in sJIA pathogenesis.

sPD-1 and sPD-L1 was largely discussed in not only cancer but also immune disease. Du Y reported that serum sPD-1 and sPD-L1 were significantly higher in SLE patients and might take part in SLE pathogenesis via inhibiting the immune regulatory effects of the membrane-bound PD-1 and PD-L1 [13]. Birtas Atesoglu E reported that decreased sPD-1 levels may have a role in ITP pathogenesis as without the inhibitory regulation of PD-1, sustained activation of T cells may cause inflammatory responses which is the case in ITP [15]. Obviously, the role of sPD-1 is still under debate in autoimmune disease.

Though sPD-1 and sPD-L1 are soluble form of PD-1 and PD-L1 in peripheral blood, in this study we found no relationship between the PD-1 with sPD-1, neither between PD-L1 with sPD-L1 (data not shown). Co-stimulatory molecules can exist in both membrane and soluble forms. As to sPD-1, it is encoded by PD-1Dex3 with an IgV-type extracellular domain of membrane bound form, which is independent. sPD-L1 is released through proteolytic cleavage of membrane PD-L1, although any other source cannot be excluded [22]. The soluble protein factors can participate in blood circulation and play a regulatory role in the immune response like cytokines. Considering the distinct function of membrane and soluble forms, the mechanism of how the decreased PD-1/PD-L1 and sPD-1/sPD-L1 take part in sJIA need to be clarified. The fact that active sJIA patients had a markedly higher IFN-γ compared either with HCs or inactive sJIA patients indicate that Th1 cells played a predominant role in sJIA. In autoimmune and auto-inflammatory disorders, IFN-γ can either play a disease-enforcing role or act as protective agent [23]. New mouse model of systemic inflammation in IFN-γ-deficient BALB/c mice correspond well to the characteristics of sJIA [8]. The strong macrophage-activating potential of IFN-γ led to the consideration of the cytokine as pro-inflammatory agent in both sJIA and MAS. When we mimic the decreased PD-1/PD-L1 and sPD-1 level in sJIA patients with PD-1 and PD-L1 antibody in vitro, IFN-γ is increased, thus partly indicating in sJIA patients the decreased PD-1/PD-L1 and sPD-1 might increase IFN-γ, which in turn was involved in the arthritis and fever symptom. When we mimic the decreased sPD-1 level in sJIA patients with PD-L1 antibody in vitro, IL-6 is increased, thus partly indicating in sJIA patients the decreased sPD-1 level might increase IL-6.

5. Conclusions

In summary, for the first time we have observed decreased sPD-1 in active sJIA patients, which is a supplementary finding for our previous study. Additionally, sPD-1 is related with clinical indicators and could be a new biomarker for sJIA diagnosis. Exploring the membrane and soluble forms of PD-1/PD-L1 in sJIA will be helpful
in differential diagnosis and critical to further elucidating the pathophysiological mechanism of sJIA.

### Abbreviations

AUC: area under the curves; CRP: C-reactive protein; DCs: dendritic cells; ESR: erythrocyte sedimentation rate; GM-CSF: granulocyte-macrophage colony-stimulating factor; HCs: healthy controls; Hb: hemoglobin; ITP: Immune thrombocytopenia; IL-2: interleukin-2; IL-10: interleukin-10; IFN-γ: interferon-γ; IL-1β: interleukin-1β; IL-6: interleukin-6; IL-18: interleukin-18; JADAS-27: Juvenile Arthritis Disease Activity Score; JIA: juvenile idiopathic arthritis; LPS: lipopolysaccharide; MAS: macrophage activation syndrome; mDC: myeloid dendritic cell; Ne: neutrophil; sJIA: systemic juvenile idiopathic arthritis; sPD-1: Soluble PD-1; sPD-L1: Soluble PD-L1; PBMCs: peripheral blood mononuclear cells; PD-1: Programmed cell death 1; PD-L1: Programmed cell death ligand 1; PLT: blood platelet; PGA: physician global assessment; PtGA: parent’s and patient’s global assessments; ROC: receiver-operating characteristic; SD: standard deviation; WBC: white blood cell

### Declarations

#### Ethics approval and consent to participate

The clinical data and blood sample collection in this study were performed in accordance with the medical ethics committee of Shanghai Children’s Medical Center. The study was performed in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

#### Consent for publication

Not applicable.

#### Availability of data and materials

Not applicable.

#### Competing interests

The authors declare that they have no competing interest.

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#### Authors' contributions

LC carried out the ELISA experiment, analyzed the data, and drafted the manuscript. CXZ participated in the in vitro flow cytometry experiment and statistical analysis. JW collected the clinical data and revised the Methods section. WZ revised the Discussion section. TXC conceived the study, designed and participated in experiments, helped with coordination, analyzed data, and drafted, edited, and revised the manuscript. All authors read and approved the final manuscript.

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#### Authors' information

Not applicable.
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Figures
Levels of sPD-1, sPD-L1, pro-inflammatory cytokines and the pro-inflammatory S100-proteins in sJIA patients. Plasma from healthy controls (HC, \( n=35 \)), active sJIA \(( n=26 \)) and inactive sJIA \(( n=20 \)) patients were collected and examined to detect cytokine levels by ELISA and Luminex. (a) sPD-1 level, (b) sPD-L1 level, (c) TNF-\( \alpha \) level, (d) IL-18 level, (e) IL-6 level, (f) IL-1\( \beta \) level, (g) Ferritin level, (h) S100A8 level, (i) S100A12 level. Data are presented as mean \( \pm \) SD. * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \).
Figure 2

The correlation between plasma sPD-1 level and clinical parameters of patients with sJIA. Pearson’s correlation coefficient was applied for the correlation analysis and independent-sample t-test was applied to compare two independent groups respectively. (a) Correlation between sPD-1 level with JADAS-27, (b) Correlation between sPD-1 level with number of affected joints, (c) Correlation between sPD-1 level with PGA, (d) Correlation between sPD-1 level with PtGA, (e) Correlation between sPD-1 level with CRP, (f) sPD-1 level between patients with (n=21) and without (n=25) fever, (g) sPD-1 level between patients with (n=29) and without (n=17) arthritis.
Data are shown as mean ± SD. * p < 0.05, ** p < 0.01. JADAS-27: Juvenile Arthritis Disease Activity Score, PGA: physician global assessment, PtGA: parent’s and patient’s global assessments, CRP: C-reactive protein.
The correlation between sPD-L1 level and pro-inflammatory cytokines and the pro-inflammatory S100-proteins in sJIA patients. Pearson’s correlation coefficient was applied for the correlation analysis. (a) Correlation between sPD-L1 level with Ferritin, (b) Correlation between sPD-L1 level with S100A8, (c) Correlation between sPD-L1 level with IL-6, (d) Correlation between sPD-L1 level with IL-18, (e) Correlation between sPD-L1 level with IL-1β, (f) Correlation between sPD-1 level TNF-α.
sPD-1 and sPD-L1 value in sJIA diagnosis and treatment. (a) ROC analysis of the ability of sPD-1 to distinguish patients with sJIA from HC. (b) ROC analysis of the ability of sPD-1/sPD-L1 to distinguish patients with sJIA from HC. (c) The effect of tocilizumab treatment on sPD-1 level of sJIA patients (n=6). (d) The effect of tocilizumab treatment on sPD-L1 level of sJIA patients (n=6). (e) The effect of tocilizumab treatment on sPD-1/sPD-L1 level of sJIA patients (n=6). Independent-sample t-test was applied to compare two independent groups. Data are presented as mean ± SD. * p < 0.05, ** p < 0.01. ROC: receiver-operating characteristic.
Figure 5
Potential mechanisms of PD-1/PD-L1 signaling in sjIA patients. Plasma from HC (n=35), active sjIA (n=26) and
inactive sJIA (n=20) patients were collected and examined to detect cytokine levels by Luminex. (a) IFN-γ level, (b) IL-4 level, (c) IL-17A level. CD4+T cells and DCs were co-cultured with 10μg/ml αPD-1, 10μg/ml αPD-L1 or 10μg/ml Isotype for three days and stained for IL-17A and IFN-γ by flow cytometry. (d) Representative figures of IL-17A and IFN-γ levels, (e) Statistics of IFN-γ level of three experiments. PBMCs were cultured with 10μg/ml PD-L1 for 72 hours and IL-6 secretion was measured by flow cytometry. (f) Representative figures of IL-6, (g) Statistics of percentage of IL-6 of six experiments. One-way ANOVA were applied for group difference respectively. Data are presented as mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001. DCs: dendritic cells, PBMCs: peripheral blood mononuclear cells.