PD-1/PD-L1 pathway activation restores the imbalance of Th1/Th2 and treg/Th17 cells subtypes in immune thrombocytopenic purpura patients

Die Wu, MD[a,b], Ying Liu, MM[a,b], Nannan Pang, PhD[a,b], Mingling Sun, MM[a,b], Xiujuan Wang, MM[a,b], Yasen Haridia, MM[a,b], Fang Zhao, MM[a,b], Yuting Qin, MM[a,b], Wenxia Fan, MM[a,b], Xinhong Guo, AP[a,b,*], Jianbing Ding, MM[c,d,e,*]

Abstract

This study aims to investigate the changes of cytokines and the effect of programmed death ligand 1 (PD-L1) signaling pathway on T cell function in patients with immune thrombocytopenic purpura (ITP).

Totally, 40 untreated ITP patients were recruited and 30 healthy people were recruited as the healthy control. Then whole blood of ITP patients and healthy control was collected, respectively. The sPD-L1/anti-PD-1 was used to activate or block the programmed death (PD-1)/PD-L1 signaling pathway. The expression of PD-1 and PD-L1 on peripheral blood mononuclear cells (PBMCs) were detected by flow cytometry. PBMCs were treated with cluster of differentiation (CD3), cluster of differentiation 28 (CD28), and phytohaemagglutinin (PHA) for 48 hours. Serum levels of sPD-1, sPD-L1, and cytokines were measured by enzyme-linked immunosorbent assay (ELISA).

Compared with the healthy control group, the percentages of PD-1+CD3+CD4+ T cells and PD-L1+HLA-DR+CD11c+ DC cells were increased in ITP patients. The levels of interferon-gamma (IFN-γ), interleukin-17 (IL-17), and sPD-1 in the serum of ITP patients were increased, while IL-4 and transforming growth factor-β (TGF-β) were decreased. Additionally, the level of sPD-1 was negatively correlated with the platelet count. Consistently, after treatment with CD3, CD28, and PHA, IFN-γ and IL-17 levels in culture supernatant of PBMCs from ITP patients were significantly higher than those from healthy controls whereas IL-4 and TGF-β levels were significantly lower. Furthermore, IFN-γ and IL-17 levels secreted by PBMCs from ITP patients decreased after sPD-L1 administration, however, IL-4 and TGF-β levels were increased. The level of IFN-γ in ITP group remained higher after anti-PD-1 blockage, but the levels of IL-4, TGF-β, and IL-17 were not significantly influenced.

sPD-1 may cause the dysfunction of PD-1/PD-L1 signaling pathway, and its level is related to the severity of ITP patients. Activation of PD-1/PD-L1 with sPD-L1 may restore the imbalance of Th1/Th2 and Treg/Th17 cell subtypes in ITP patients but anti-PD-1 may exacerbate disease by enhancing IFN-γ production.

Abbreviations: ITP = immune thrombocytopenic purpura, PBMC = peripheral blood mononuclear cell, PBS = phosphate buffer saline, PBST = phosphate buffer saline Tween-20, PD-L1 = programmed death ligand 1, PHA = phytohaemagglutinin.

Keywords: cytokine, immune thrombocytopenic purpura, programmed death, programmed death ligand 1

1. Introduction

Immune thrombocytopenic purpura (ITP) is a disease mediated by the acquired immune system. There are abnormalities of CD4+ T cell subsets in ITP patients. CD4+ T cells play an important role in the acquired immune system, including promoting the differentiation and maturation of B cells, assisting

the proliferation and differentiation of CD8+ T cells and other immune cells, as well as coordinating the interaction between different immune cells. Therefore, CD4+ T cells play a very important role in the pathogenesis of ITP.

T cells require dual signals for activation. The T cell antigen receptor specifically recognizes the antigen peptide presented by the antigen presenting cells and provides the first signal for T cell activation. Then, the corresponding receptors bind to the costimulatory molecules on the antigen presenting cells to provide the second activation signal. In addition, negative feedback regulation mechanisms exist in the activation process of T cells. Among them, the combination of programmed death (PD1) and programmed death ligand 1 (PD-L1) is included. Chronic stimulation of the PD-1/PD-L1 pathway can lead to effector T cell exhaustion and dysfunction, prevent excessive immune damage, and play an important regulatory role in maintaining immune homeostasis.[1]

Blockade of PD-1 and PD-L1 interactions in myeloma in mouse[2] enhanced specific immune responses. In another meta-analysis, patients with advanced non-small-cell lung cancer could benefit from anti-PD-1 or PD-L1 antibody therapies, which had higher safety compared with docetaxel chemotherapy.[3] PD-1/PD-L1 signaling pathway plays a role in the pathogenesis of autoimmune diseases such as multiple sclerosis,[4] systemic lupus erythematosus,[5] and rheumatoid arthritis.[6] However, therapy with anti-PD-1 and PD-L1 in autoimmune diseases is less studied. There are 2 forms of PD-1 and PD-L1, that is, secretory form (sPD-1 and sPD-L1) and membrane bound form.[7,8] The sPD-1 can block PD-1/PD-L signaling by specifically binding to PD-L, while sPD-L1 can stimulate PD-1 by retaining the IgV domain[9] and can promote the PD-1/PD-L1 signaling pathway, thus inhibiting T cell proliferation and activation.[10]

Here, we studied the expression of PD-1/PD-L1 in patients with ITP. At the same time, we used in vitro experiments to activate or block the PD-1/PD-L1 pathway, to observe the changes in cytokines secretion in CD4+ T cells and analyzed the effects of different states of PD-1/PD-L1 signaling pathway on T cells.

2. Materials and methods

2.1. Patients

A total of 40 patients with untreated ITP from the Hematology Center of the First Affiliated Hospital of Xinjiang Medical University from January 2016 to August 2017 were enrolled. All cases met the diagnostic criteria proposed by the Blood Branch of the Chinese Medical Association in 2016.[11] No patients received platelet transfusion and splenectomy within 1 month, and none had been treated with glucocorticoid or gamma globulin. Meanwhile, 30 healthy people examined at the physical examination center of the same hospital were selected as the healthy control group. The demographic and clinical characteristics of the study cohort are shown in Table 1. Informed consents were obtained from every patient and the study was approved by the ethics review board of the First Affiliated Hospital of Xinjiang Medical University.

2.2. Reagents

Murine anti-human monoclonal antibodies, including CD4-PerCP, CD8-FITC, PD-1-PE, CD3-APC, HLA-DR-PE-cy7, CD11c-PerCP, and PD-L1-PE were all purchased from BD Biosciences (Franklin Lakes, NY). Lymphocyte separation fluid was also purchased from BD Biosciences (Franklin Lakes, NY). ELISA kits for sPD-1, sPD-L1,

Table 1

The demographic and clinical characteristics of the study cohort.

	Healthy control	Patients	*P* value
Number	30	40	
Age, y	42.20 ± 1.71	41.68 ± 2.35	$P > .05$
Gender			$P > .05$
Male	22	16	
Female	8	24	
Platelet count ($\times 10^9$/L)	120.68 ± 10.30	17.25 ± 4.45	$P < .05$
Platelet count $\leq 10 \times 10^9$/L	0	3	
Platelet count $(10–20) \times 10^9$/L	0	24	
Platelet count $(20–30) \times 10^9$/L	0	13	
Platelet count $>30 \times 10^9$/L	30	0	

IFN-γ, IL-4, IL-17, and TGF-β were purchased from eBioscience (San Diego, CA). Soluble PD-L1 (sPD-L1) protein (26 μg/mL) was a gift from the Department of Immunology, Soochow University. Anti-human PD-1 Antibody (10 μg/mL) was purchased from GenScript (Nanjing, Jiangsu Province, China).

2.3. Flow cytometry

For T cell PD-1 detection, APC-labeled CD3, Percp-labeled CD4, FITC-labeled CD8, and PE-labeled PD-1 monoclonal antibodies were used. For DC PD-L1 detection, PerCp labeled CD11c, PE-labeled PD-L1, and PE-cy7-tagged HLA-DR monoclonal antibodies were used. The samples were incubated at room temperature in the dark for 15 minutes and then washed with PBS (phosphate buffer saline) for 2 times. Afterwards, the cells were resuspended in PBS to the concentration of 1×10^6/mL and analyzed on the MACSQuant flow cytometer (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). MACSQuant Software provided by the flow cytometer was used for data analysis.

2.4. Peripheral blood mononuclear cell separation

The whole blood was mixed with the lymphocyte separation solution and centrifuged at 2000 r/min for 20 minutes. The lymphocyte layer was collected gently with a pasteurized tube, and then mixed well with 4 mL PBS. After centrifugation at 1000 r/min for 5 minutes, the PBMCs were collected.

2.5. Cell grouping and treatment

The cells were divided into 4 groups, including blank group (RPMI1640 medium only), control group (RPMI1640 medium and cells), and experimental groups (RPMI1640 medium and cells, with 1 μg/mL CD3, 0.4 μg/mL CD28, 2.5 μg/mL phytohaemag-glutinin [PHA]), as well as the sPD-L1 stimulation group (RPMI1640 medium and cells, 1 μg/mL CD3, 0.4 μg/mL CD28, 2.5 μg/mL PHA, and sPD-L1) and the anti-PD1 blockage group (RPMI1640 medium and cells, 1 μg/mL CD3, 0.4 μg/mL CD28, 2.5 μg/mL PHA, and anti-PD-1 antibody). After incubation in a 37°C, 5% CO_2 incubator for 48 hours, the culture supernatant was collected to detect cytokines.

2.6. ELISA

Peripheral blood was collected from patients with ITP and healthy controls, respectively. Serum samples were collected at 2500 r/min for 10 minutes. Then the cytokines and sPD-1, sPD-L1 were

detected as per the instructions. Briefly, 50 μL of the diluted standard sample and the sample to be tested were sequentially added into each well, and then 50 μL of biotin-labeled antibody was added. After incubation at 37°C for 1 hour, the wells were washed 3 times with phosphate buffer saline Tween-20 (PBST). After that, 80 μL of affinity streptomycin-HRP was added and incubated at 37°C for 30 minutes. After washing 3 times with PBST, 50 μL each of substrate A and B were added to the reaction well and incubated at 37°C for 10 minutes. Then the reactions were stopped by adding 50 μL stop solution. The OD450 value was measured with a microplate reader.

The level of IFN-γ, IL-4, IL-17, and TGF-β in the culture supernatant was detected by ELISA. Briefly, IFN-γ, IL-4, IL-17, and TGF-β standards were serially diluted to prepare standard curve concentrations. The absorbance was measured with an automatic microplate reader at an OD450 nm value.

2.7. Statistical analysis

All data were analyzed by SPSS17.0 software. Measured results with a normal distribution are expressed as mean ± SD. After all clinical data was tested for homogeneity of variance (Levine test), an independent sample t test for measurement data was used, and a $P < .05$ was considered statistically significant. The correlation was analyzed by the Pearson linear correlation analysis.

3. Results

3.1. The expression of PD-1 and PD-L1 in ITP patients is higher than that in healthy individuals

To determine the percentages of PD-1+CD3+CD4+ T cells and PD-L1+HLA-DR+CD11c+ DC, flow cytometry was performed. The percentage of PD-1+CD3+CD4+ T cells in peripheral blood of ITP patients was (26.79 ± 8.91)%, higher than that in healthy controls (12.06 ± 2.84)% (Fig. 1A and B). The difference was statistically significant $(t = 8.715, P < .05)$. The percentage of PD-L1+HLA-DR+CD11c+ DC cells $(12.75 \pm 1.86$%) was also significantly higher than that of healthy controls $(4.90 \pm 0.80$%), $(t = 21.65, P < .05)$ (Fig. 1C and D). The results indicate that the expression of PD-1 and PD-L1 in patients with ITP is higher than that in normal subjects.

3.2. Serum levels of IFN-γ, IL-4, TGF-β, and IL-17 in patients with ITP

ELISA was used to determine the serum levels of IFN-γ, IL-4, TGF-β, and IL-17. Compared with the healthy controls, serum IFN-γ, IL-4, TGF-β, and IL-17 in ITP patients were statistically significantly different (Fig. 2). In detail, the serum concentrations of IFN-γ and IL-17 in ITP patients were higher than those in healthy subjects, respectively $(P < .05)$. And serum concentrations of IL-4 and TGF-β in ITP patients before treatment were
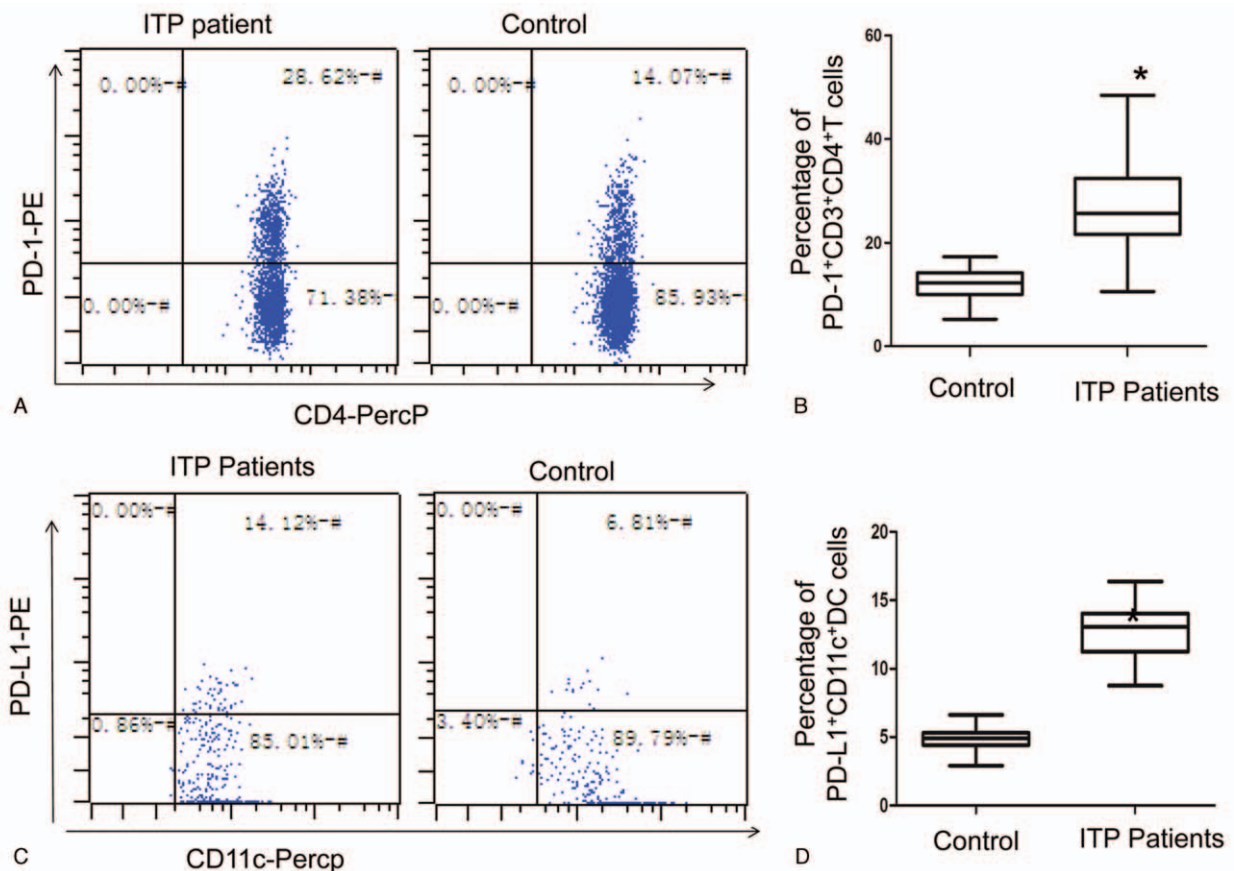

Figure 1. Percentages of PD-1+CD4+T cells and PD-L1+DCs in peripheral blood from ITP patients. (A) Flow cytometry of CD4-Percp lymphocytes. (B) Percentage of PD-1+CD3+CD4+T cells. *$P < .05$. (C) Flow cytometry of CD11c-Percp. (D) Percentage of PD-L1+CD11c+DC cells. *$P < .05$. ITP = immune thrombocytopenic purpura, PD-L1 = programmed death ligand 1.
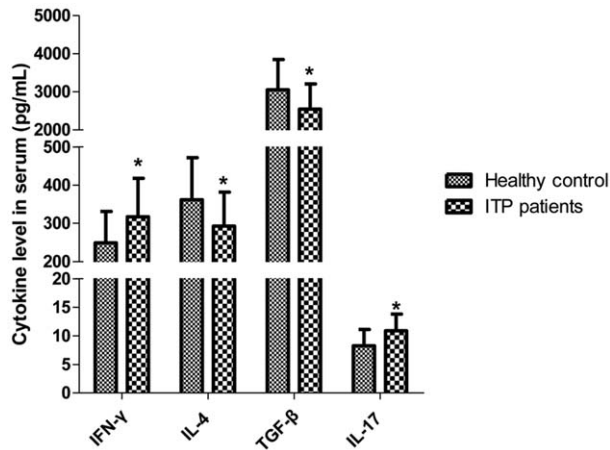
Figure 2. Serum levels of IFN-γ, IL-4, TGF-β, and IL-17. Peripheral blood was collected from patients with ITP and healthy controls, respectively. ELISA was used to determine the serum levels of IFN-γ, IL-4, TGF-β, and IL-17. Compared with healthy control, $*P < .05$. ITP = immune thrombocytopenic purpura.

lower than those in healthy subjects, respectively ($P < .05$). The results indicate an imbalance of Th1/Th2 and Th17/Treg immune cells in newly diagnosed ITP patients.

3.3. Comparison of sPD-1, SPD-L1, and platelet levels in healthy and ITP patients

To measure the secretion of sPD-1 and sPD-L1 by ITP patients, ELISA was performed. The level of sPD-1 in serum of ITP patients was significantly different from that in healthy controls.

However, there was no significant difference for sPD-L1 between patients and healthy controls ($P = .056$) (Fig. 3A). Pearson linear correlation analysis suggested that serum sPD-1 level was negatively correlated with platelet count in ITP patients ($r = -0.736$, $P < .05$) (Fig. 3B).

3.4. Cytokine secretion of PBMCs after stimulation of CD4 T cells

Cytokine changes in cultured PBMCs treated with CD3, CD28, and PHA for 48 hours in vitro are shown in Fig. 4. The levels of IFN-γ and IL-17 in culture supernatant were significantly higher than those in healthy controls ($P < .05$). Meanwhile, the levels of IL-4 and TGF-β in culture supernatant were significantly lower than those in healthy controls ($P < .05$). The results indicate that the trend of cytokine secretion by CD4+ T cells in the supernatant of cultured mononuclear cells was the same as that in ITP patients, showing an imbalance between Th1/Th2 and Th17/Treg immune cells.

3.5. Cytokine secretion by CD4 T cells after treatment with anti-PD-1 and sPD-L1

To detect the influence of cytokines secreted by CD4+ T cells after blocking the PD-1/PD-L1 pathway of the mononuclear cells in the healthy control group, ELISA was performed to analyze the cytokines secreted by PBMCs of healthy humans after in vitro culture with anti-PD-1 antibody for 48 hours. As shown in Fig. 5, the levels of IFN-γ were significantly higher than that before the treatment. However, there was no significant change for IL-4 ($P = .167$), TGF-β ($P = .948$), and IL-17 ($P = .393$). The results indicate that blocking the PD-1/PD-L1 pathway in healthy people would increase the activation and proliferation of Th1 cells and might induce autoimmune diseases.
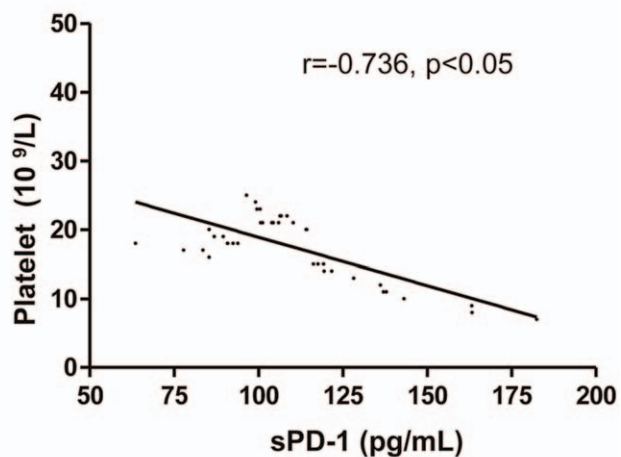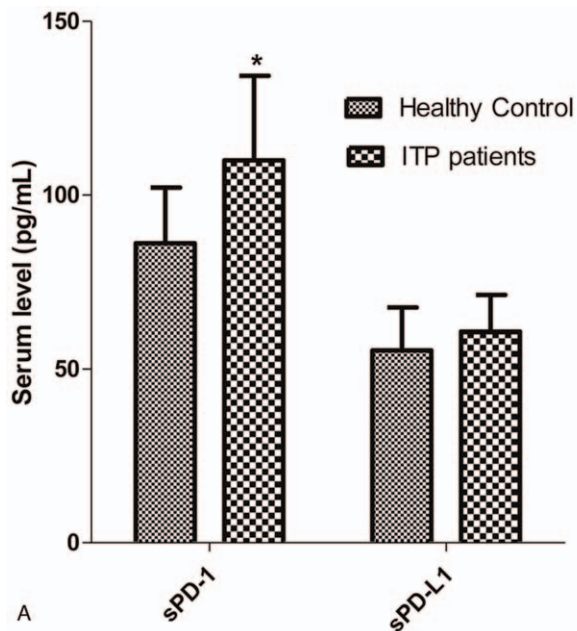
Figure 3. Analysis of sPD-1 and sPD-L1. (A) Peripheral blood was collected from patients with ITP and healthy controls, respectively. ELISA was used to determine the level of sPD-1 and sPD-L1. Compared with healthy control, $*P < .05$. (B) Correlation of platelet count with the serum sPD-1 level in ITP patients. Correlation was analyzed using Pearson correlation analysis. ITP = immune thrombocytopenic purpura, PD-L1 = programmed death ligand 1, sPD-L1 = soluble PD-L1.

Figure 4. Cytokine changes in cultured PBMCs treated with CD3, CD28, and PHA. PBMCs were collected from patients with ITP and healthy controls, respectively, and then treated with CD3, CD28, and P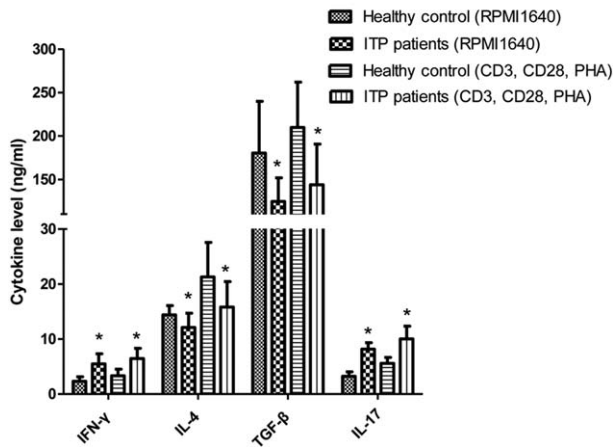HA in vitro for 48 hours. ELISA was used to determine the level of IFN-γ, IL-4, TGF-β, and IL-17 in the culture supernatant. Compared with the corresponding healthy control, *$P < .05$. ITP = immune thrombocytopenic purpura, PBMCs = peripheral blood mononuclear cells, PHA = phytohaemagglutinin.

To determine the effect of blocking the PD-1/PD-L1 pathway of mononuclear cells extracted from ITP patients on the cytokines secretion by CD4+ T cells, ELISA was performed. PBMCs extracted from ITP patients were cultured with anti-PD-1 antibody for 48 hours in vitro. The results showed that IFN-γ levels were higher than that before treatment, and the difference was statistically significant. However, there were no significant differences for IL-4 ($P = .076$), TGF-β ($P = .401$), and IL-17 ($P = .068$) when compared with that before the treatment (Fig. 5). The results showed that the imbalance of Th1/Th2 and Th17/Treg immune cells did not improve after blocking the PD-1/PD-L1 pathway.

Furthermore, we detected the changes in cytokine secretion by ELISA after 48 hours of treatment with sPD-L1. sPD-L1 was used

to activate the PD-1/PD-L1 pathway. In the healthy control group, none of the cytokines, including IFN-γ ($P = .636$), IL-4 levels ($P = .312$), TGF-β ($P = .745$), and IL-17 ($P = .220$) showed significant change compared with that before the treatment (Fig. 6). However, in ITP patients, the levels of IFN-γ and IL-17 were significantly decreased, whereas, the levels of IL-4 and TGF-β were significantly increased after activation of PD-1/PD-L1 pathway by sPD-L1 ($P < .05$) (Fig. 6). These results indicate that after activation of PD-1/PD-L1 pathway, CD4+ T cell activation may be inhibited in ITP patients, thus alleviating ITP.

4. Discussion and conclusion

ITP is an acquired autoimmune disease caused by immune imbalance in vivo. The activation of T cells and the cytokine production are main causes of immune damage. Generally, CD4+ T cell subsets are involved in the ITP immune response and pathogenesis. In this study, we found that serum levels of IFN-γ were higher in patients with ITP than in healthy controls, while IL-4 levels were lower, suggesting that Th1/Th2 cells are imbalanced in ITP patients. This was consistent with the previous studies, which showed that Th1/Th2 cell imbalance was also present in chronic ITP patients[12,13] and in children with ITP,[14] meanwhile the Th1/Th2 ratio was always negatively related to platelet counts.[13]

Treg cells can inhibit the activation and proliferation of CD4+ T cells or CD8+ T cells mainly through direct interaction between cells and through production of cytokines, such as IL-10 and TGF-beta.[15] IL-17, secreted by Th17 cells, binds to IL-17 receptor and acts on different target cells to trigger the release of inflammatory mediators, which mediates the occurrence of diseases such as inflammatory reactions, autoimmune diseases, tumors, and transplant rejection. In this study, it was found that compared with healthy controls, the level of TGF-β in ITP patients was lower whereas the level of IL-17 in ITP patients was higher, suggesting that ITP patients may have unbalanced Treg/Th17 cells. This is similar to the findings of other scholars.[16] Therefore, the above studies

Figure 5. The changes of cytokines secreted by CD4+ T cells after blockage of PD-1/PD-L1 pathway by anti-PD-1. PBMCs were collected from patients with ITP and healthy controls, respectively, and then treated with anti-PD-1 antibody in vitro for 48 hours. ELISA was used to determine the cytokine level of health control and ITP patients. Compared with the corresponding healthy control, *$P < .05$. ITP = immune thrombocytopenic purpura, PBMCs = peripheral blood mononuclear cells, PD-L1 = programmed death ligand 1.
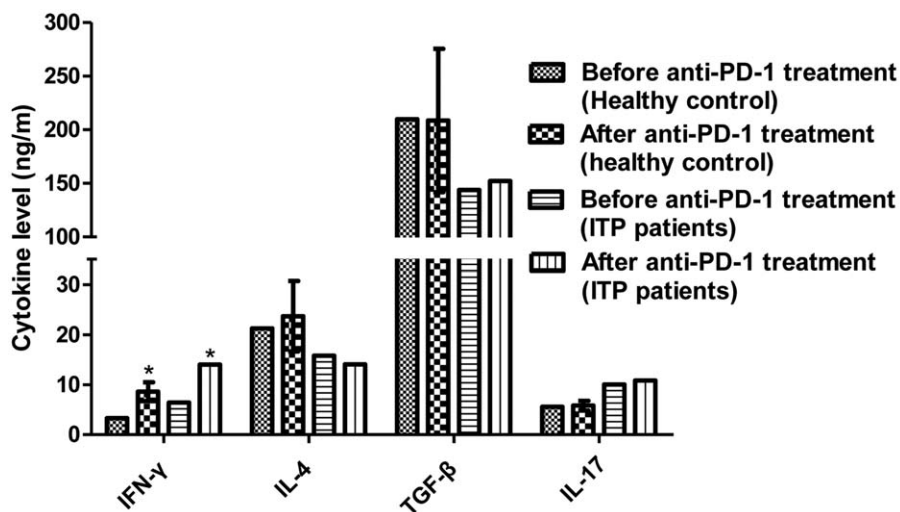
Figure 6. The changes of cytokines secreted by CD4+ T cells after activation of PD-1/PD-L1 pathway by sPD-L1. PBMCs were collected from patients with ITP and healthy controls, respectively, and then treated with sPD-L1 in vitro for 48 hours. ELISA was used to determine the cytokine level of health control and ITP patients. Compared with the corresponding healthy control, *$P < .05$. ITP = immune thrombocytopenic purpura, PBMCs = peripheral blood mononuclear cells, PD-L1 = programmed death ligand 1, sPD-L1 = soluble PD-L1.
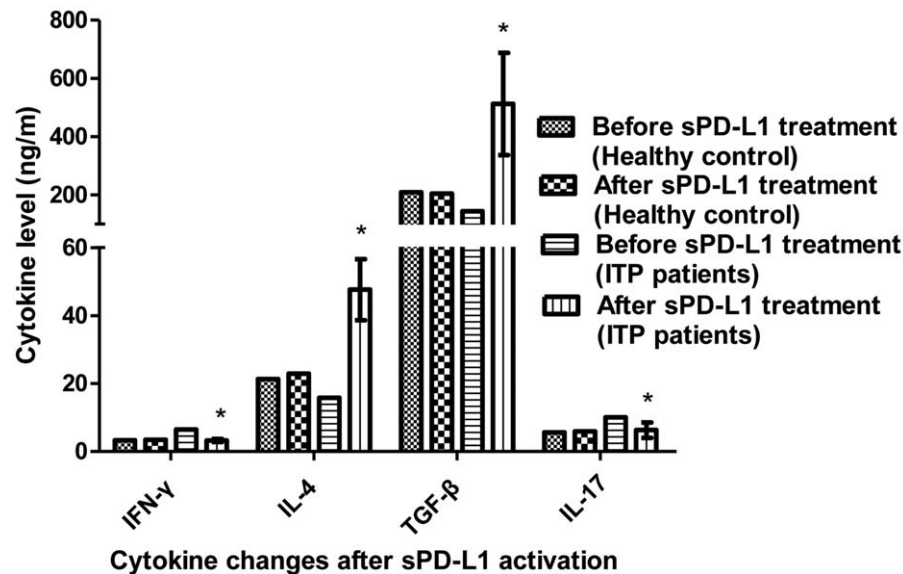
confirmed the presence of abnormalities of CD4+ T cell subsets in ITP patients.

In this study, we found that the proportions of PD-1+CD3 +CD4+ T cells and PD-L1+HLA-DR+CD11c+ DC cells in patients with ITP were higher than those in healthy controls. To further explore the role of PD-1/PD-L1 signaling pathway in ITP patients, we blocked or activated PD-1/PD-L1 signaling pathways in vitro to observe changes in cytokine production by PBMCs. The results showed that the levels of IFN-γ and Th17 secreted by ITP patients from PBMCs were significantly higher than those of healthy controls, while the levels of IL-4 and TGF-β were significantly lower. This is consistent with the trend of cytokine changes in the serum of ITP patients, showing polarization to type 1 cytokines and an imbalance of Treg/Th17 immune cells in vivo.

PD-1 and its ligands PD-L1 and PD-L2 have the effect of inducing and maintaining peripheral immune tolerance, and their mediating inhibitory signals can block the proliferation and function of effector T cells.[17] We found that when PBMCs from healthy controls were cultured for 48 hours in vitro with anti-PD-1, the PD-1/PD-L1 signaling pathway was blocked and IFN-γ production was increased. There was no significant change in the levels of IL-17, TGF-β, and IL-4. IFN-γ in the local microenvironment is an important factor in promoting the differentiation of Th0 cells into Th1 cells. IFN-γ can inhibit the differentiation and proliferation of Th2 cells, and the sustained response of Th1 cells is involved in the occurrence and development of autoimmune diseases. This experiment shows that blocking the PD-1/PD-L signaling pathway in healthy people will increase the activation and proliferation of Th1 cells and may induce autoimmune diseases. The PD-1/PD-L pathway is an important cause of immune escape from malignant tumors. It is reported that PD-L1 expression in patients with colorectal cancer suggested a poor prognosis.[18] After the patient was treated with anti-PD-1 antibody pembrolizumab, its anti-tumor activity was observed. In this study, we found that after 48 hours of in

vitro culture of PBMC from ITP patients with anti-PD-1, PD-1/PD-L1 signaling pathway was inhibited. The secretion of IFN-γ levels was significantly higher, and the levels of IL-4, TGF-β, and IL-17 did not change significantly, suggesting that the imbalance of Th1/Th2 cells is more serious and may worsen the condition of patients with ITP. Similarly, another study in mouse found that blocking the PD-1/PD-L1 pathway reduced the suppressive effects of T cells, induced the activation of islet autoreactive T cells, activated the proliferation of CD4+ and CD8+ T cells, and promoted IFN-γ, thus causing immune damage to the islet Beta cells and eventually leading to autoimmune diabetes.[19] In this study, we also found that after stimulation of PD-1/PD-L1 signaling pathway by sPD-L1 in PBMCs of healthy controls, there were no significant changes in the level of IFN-γ, IL-4, TGF-β, and IL-17 when compared before the treatment. However, in PBMCs of ITP patients, compared with before treatment, IFN-γ and IL-17 significantly decreased, while IL-4 and TGF-β significantly increased, indicating that the PBMC results suggest that treatment with sPD-L1 may be therapeutic for ITP patients. The results are similar to changes in cytokine levels in patients that are clinically treated with high-dose glucocorticoid therapy, suggesting that activation of the PD-1/PD-L1 pathway may have therapeutic effects on ITP patients.

The sPD-1 and sPD-L1 play opposite roles in PD-1/PD-L signaling. The sPD-1 can bind to PD-L and block PD-1/PD-L signaling whereas PD-L1 can stimulate PD-1 and PD-1/PD-L1 signaling.[9,10] In this study, we found that the percentage of PD-1 +CD3+CD4+ T cells and the percentage of PD-L1+HLA-DR +CD11c+ DC cells in patients with ITP were higher than those in healthy controls. However, in vitro experiments, when the PD-1/PD-L1 pathway was activated, the imbalance of CD4+ T cell subsets was restored in patients. We also found that the serum sPD-1 in ITP patients were significantly higher than in healthy controls, which suggests that the high levels of sPD-1 might inhibit the membranous PD-1/PD-L pathway, and thus negatively regulate T cells. Moreover, Pearson linear correlation analysis

showed that sPD-1 was negatively correlated with platelet counts in the patient's serum and could therefore be used to assess the severity of ITP. The in vitro results suggest that sPD-L1 activation of the PD-1/PD-L1 pathway may improve the imbalance of Th1/Th2 and Treg/Th17 immune cells in ITP patients, but this is an in vitro test and has certain limitations. The interaction of PD-1/PD-L1 signaling pathway with other factors in ITP patients needs to be further studied.

In conclusion, our results showed that PD-1/PD-L1 expression was increased in patients with ITP, but there was a significant imbalance of CD4+ T cells in ITP patients. And, we found higher levels of sPD-1 in ITP patients, which may inhibit the PD-1/PD-L1 and result in loss of negative regulation on T cell activation and proliferation. In vitro studies suggest that activation of the PD-1/PD-L1 pathway using sPD-L1 could improve the imbalance of Th1/Th2 and Treg/Th17 immune cells in ITP patients.

Author contributions

Conceptualization: Xinhong Guo, Jianbing Ding.
Data curation: Die Wu, Xinhong Guo.
Formal analysis: Die Wu, Mingling Sun, Yuting Qin, Wenxia Fan.
Funding acquisition: Jianbing Ding.
Investigation: Ying Liu, Fang Zhao.
Methodology: Die Wu, Ying Liu, Xiujuan Wang.
Project administration: Xinhong Guo.
Resources: Nannan Pang.
Software: Nannan Pang, Yasen Haridia.
Supervision: Jianbing Ding.
Writing – original draft: Die Wu.
Writing – review & editing: Xinhong Guo, Jianbing Ding.

References

[1] Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677–704.

[2] Iwai Y, Ishida M, Tanaka Y, et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA 2002;99:12293–7.

[3] Jiang Q, Xie M, He M, et al. Anti-PD-1/PD-L1 antibodies versus docetaxel in patients with previously treated non-small-cell lung cancer. Oncotarget 2018;9:7672–83.

[4] Ortler S, Leder C, Mittelbronn M, et al. B7-H1 restricts neuroantigen-specific T cell responses and confines inflammatory CNS damage: implications for the lesion pathogenesis of multiple sclerosis. Eur J Immunol 2008;38:1734–44.

[5] Nielsen C, Laustrup H, Voss A, et al. A putative regulatory polymorphism in PD-1 is associated with nephropathy in a population-based cohort of systemic lupus erythematosus patients. Lupus 2004;13:510–6.

[6] Li S, Liao W, Chen M, et al. Expression of programmed death-1 (PD-1) on CD4+ and CD8+ T cells in rheumatoid arthritis. Inflammation 2014;37:116–21.

[7] Sakthivel P, Shively V, Kakoulidou M, et al. The soluble forms of CD28, CD86 and CTLA-4 constitute possible immunological markers in patients with abdominal aortic aneurysm. J Intern Med 2007;261:399–407.

[8] Wilson JK, Zhao Y, Singer M, et al. Lymphocyte subset expression and serum concentrations of PD-1/PD-L1 in sepsis - pilot study. Crit Care 2018;22:95.

[9] Enninga EAL, Harrington SM, Creedon DJ, et al. Immune checkpoint molecules soluble program death ligand 1 and galectin-9 are increased in pregnancy. Am J Reprod Immunol 2018;79:

[10] Frigola X, Inman BA, Krco CJ, et al. Soluble B7-H1: differences in production between dendritic cells and T cells. Immunol Lett 2012;142:78–82.

[11] Thrombosis, Hemostasis Group, H.S., Chinese Medical, Association-Consensus of Chinese experts on diagnosis and treatment of adult primary immune thrombocytopenia (version 2016). Chin J Hematol 2016;37:89–93.

[12] Ogawara H, Handa H, Morita K, et al. High Th1/Th2 ratio in patients with chronic idiopathic thrombocytopenic purpura. Eur J Haematol 2003;71:283–8.

[13] Panitsas FP, Theodoropoulou M, Kouraklis A, et al. Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. Blood 2004;103:2645–7.

[14] Talaat RM, Elmaghraby AM, Barakat SS, et al. Alterations in immune cell subsets and their cytokine secretion profile in childhood idiopathic thrombocytopenic purpura (ITP). Clin Exp Immunol 2014;176:291–300.

[15] Benoist C, Mathis D. Treg cells, life history, and diversity. Cold Spring Harb Perspect Biol 2012;4:a007021.

[16] Ji L, Zhan Y, Hua F, et al. The ratio of Treg/Th17 cells correlates with the disease activity of primary immune thrombocytopenia. PLoS One 2012;7:e50909.

[17] Bardhan K, Anagnostou T, Boussiotis VA. The PD1:PD-L1/2 pathway from discovery to clinical implementation. Front Immunol 2016;7:550.

[18] O'Neil BH, Wallmark JM, Lorente D, et al. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with advanced colorectal carcinoma. PLoS One 2017;12:e0189848.

[19] Fife BT, Pauken KE, Eagar TN, et al. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol 2009;10:1185–92.