An increased expression profile of Th9/IL-9 correlated with Th17/IL-17 in patients with immune thrombocytopenia

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Abstract

Immune thrombocytopenia (ITP) is a heterogeneous autoimmune disease, characterized by dysregulation of cellular immunity. Th9 cells were recently identified as a new subtype of Th cells, characterized by preferential production of IL-9. Given the pleiotropic function of IL-9, Th9 cells are demonstrated to be involved in various autoimmune diseases. However, whether Th9 cells are involved in the pathogenesis of ITP remains unclear. In this study, 49 active ITP patients, 39 ITP with remission and 20 healthy controls were included. Peripheral blood mononuclear cells (PBMCs) were isolated from ITP and controls for measuring Th9 and Th17 cells by flow cytometry. Meanwhile, RNA was isolated from PBMCs for the measurement of the mRNA level of PU.1, IRF4, BATF, and RORγt by quantitative real-time PCR. Plasma levels of IL-9 and IL-17 were detected by ELISA. Our results showed that higher expressions of Th9, IL-9, and associated transcription factors (PU.1, IRF4, and BATF) were found in active ITP patients and restored to the normal level (except IL-9) in patients in remission. Meanwhile, Th9 cells and the IL-9 plasma level were positively correlated with Th17 cells and the IL-17 level in ITP patients, respectively. Moreover, a positive correlation of IRF4 or BATF with RORγt was found. In conclusion, an aberrant expression profile of Th9/IL-9 was associated with pathogenesis of ITP possibly through cooperatively working with Th17/IL-17 and therapeutically targeting Th9/IL-9 might be a novel approach in the treatment of ITP.

Keywords

IL-9, IL-17, immune thrombocytopenia, Th9, Th17

Introduction

Immune thrombocytopenia (ITP) is a heterogeneous autoimmune disease, characterized by anti-platelet autoantibody-mediated autoimmune response targeting megakaryocytes or platelets leading to accelerated platelet destruction and impaired platelet production, resulting in thrombocytopenia (lower platelet count), putting patients on a higher risk of bleeding [1,2]. The pathophysiology of ITP is very complicated with lots of factors involved. A large body of evidence demonstrated that ITP is primarily due to Fc receptor (FcR)-mediated phagocytosis and destruction by macrophages in the reticuloendothelial system in the spleen [3]. Furthermore, T cell-mediated megakaryocyte destruction and/or inhibition and platelet destruction both in vitro and in vivo have also been demonstrated to be involved in the pathogenesis of ITP [4].

ITP is an immune dysregulation-related disorder, characterized by loss of tolerance of the immune system to self-antigens expressed on the surface of platelets and megakaryocytes [5]. T cells are known to play a critical role in the regulation of immune response and platelet autoreactive T cells in ITP are revealed to be less apoptotic and more clonal expansion, leading to an imbalance of cytokine secretion [6]. Among T cell subtypes, T helper cells (Th cells) play an important role in the regulation of the immune system, particularly in the adaptive immune system, possibly through secretion of T cell-related cytokines [7]. In addition, Th cells are also essential in B cell antibody class switching [8]. Given critical roles in the regulation of immunity, imbalance of Th1/Th2 has been reported to be associated with the pathogenesis of ITP, with Th1-polarized immune response [9,10]. Moreover, in recent years, Th17 cells have also been reported to be involved in the pathogenesis of ITP [11,12]. Apart from Th1, Th2, and Th17 cells, Th9 cells were recently identified as a new subtype of CD4 Th cells, characterized by preferential production of IL-9 [13,14]. Th9 cells develop from naïve T cells upon stimulation with TGFβ and IL-4 in the presence of TCR signaling and co-stimulation [13,14]. Till now, several transcription factors have been identified to be required for Th9 cell differentiation, such as ETS-domain transcription factor PU.1 [15], IRF4 (Interferon regulatory factor 4) [16] and BATF (B cell, activating transcription factor–like) [17]. Given the pleiotropic functions of IL-9, increasing evidence demonstrated...
the important role of Th9 cells in the regulation of immune response and abnormal expression or function of Th9 cells and IL-9 were involved in pathogen immunity and various immune-mediated diseases \[18,19\]. Neutralization of IL-9 significantly reduced allergic symptoms in the mouse model of airway inflammation, and a similar outcome was found in mice with T cell specific deficiency of PU.1, which is required for Th9 differentiation \[15\]. In addition, transferring of specific Th cells differentiated under Th9-promoting conditions in vitro into mice led to the development of experimental autoimmune encephalomyelitis \[20\]. Given the critical roles in the regulation of immunity, whether Th9 cells are involved in the pathogenesis of ITP remains poorly understood.

In this study, we aimed to evaluate the role of Th9 cells in the pathogenesis of ITP through measuring the expression of Th9 cells, related transcript factors as well as the IL-9 plasma level in active ITP patients and ITP patients in remission. Given the closely relationship between Th9 and Th17 cells as they both require TGF-β and IRF4 during their development \[21\], profiles of Th17 cells and the IL-17 plasma level were also measured.

Materials and methods

Patients

From March 2015 to January 2016, 49 primary ITP patients (23 males and 26 females with a median age of 43, ranged from 19 to 84 years old) from Department of Hematology, the Affiliated Hospital of Xuzhou Medical University, Xuzhou China were recruited in this study (Table I). ITP was diagnosed based on the criteria from an international working group \[22,23\]. Patients with diabetes, hypertension, infection or cardiovascular diseases were excluded. These patients were defined as active ITP with a median platelet count of 10 × 10^9/L, ranged from 1 to 30 × 10^9/L (Table I). The samples were taken from active ITP patients who did not take any medication before the time of diagnosis. In addition, 39 ITP patients (19 males and 20 females with a median age of 41) (Table I) achieved remission after treated with glucocorticoid, prednisolone or intravenous immunoglobulin. These patients in remission were not on any of the drugs during sample collection and had a median platelet count of 133 × 10^9/L ranged from 60 to 388 × 10^9/L. Meanwhile, 20 healthy individuals, consisted of 10 males and 10 females with a median age of 39, were served as controls where age and sex distribution match those of the patients (Table I). The platelet count in healthy individuals ranged from 172 to 335 × 10^9/L with a median count of 237 × 10^9/L. Ethical approval for this study was obtained from the Medical Ethics Committee of the Affiliated Hospital of Xuzhou Medical University, Xuzhou China. Informed consent was obtained from all individual participants included in the study.

| Active ITP | ITP in remission | Controls |
|------------|------------------|----------|
| N          | 49               | 39       | 20       |
| Male/female| 23/26            | 19/20    | 10/10    |
| Age (range)| 43 (19–84)       | 41 (20–84)| 39 (20–72)|
| Platelet count (range) | 10 (1–30) | 133 (60–388) | 237 (172–335) |

Plasma isolation and preparation of peripheral blood mononuclear cells

Venous whole blood was drawn from patients or controls into tubes with EDTA as anti-coagulant followed by centrifugation at 800 g for 5 min to obtain plasma which was stored at –80°C until analysis. An equal amount of PBS (to isolated plasma) was added into the tube and mix. Then, peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation on Ficoll-Paque Plus (Sinopharm Chemical Reagent Ltd, China).

Flow cytometry

Isolated PBMCs were resuspended in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal calf serum (Gibco BRL) at a density of 1 × 10^6 cells/mL and then transferred to each well of 24-well plate. The cultured cells were stimulated with PMA (final concentration: 50 ng/mL) (Sigma-Aldrich, St. Louis, MO, USA), ionomycin (750 ng/mL) (Sigma-Aldrich) and BFA (10 μg/mL) (Invitrogen, Carlsbad, CA, USA) followed by moving the plates into a 37°C incubator with 5% CO₂ for 4 hours. At the end of culture, the cells were transferred into sterile tubes and centrifuged at 350 g for 5 min.

The cell suspensions were aliquoted into tubes followed by addition of a FITC-conjugated anti-human CD4 antibody (eBioscience, San Diego, CA, USA) and incubated for 15 min at room temperature under dark. After that, the cells were fixed and centrifuged followed by permeabilization. Then, PE-conjugated anti-human IL-9 (eBioscience) and APC-conjugated anti-human IL-17A (eBioscience) antibodies were added for intracellular staining for 30 min and analyzed in a FACSCalibur (Becton Dickinson, San Jose, CA, USA). Corresponding isotype antibodies (eBioscience) were used as control. CD4+ IL-9+ cells were defined as Th9 cells and CD4+ IL-17+ cells were Th17 cells.

RNA extraction

RNA was extracted using the TRIZOL reagent (Life Technology, Carlsbad, CA, USA) according to manufacturer’s instruction. RNA quantitation and quality was assessed by measuring the absorbance of RNA at 260 nm on a spectrophotometer and agarose gels, respectively.

Real-time quantitative PCR

Real-time quantitative PCR (RT-qPCR) was performed as previously described \[24,25\]. Extracted RNA was reversely transcribed into cDNA which was used for quantification of mRNA expression of PU.1, IRF4, BATF, and RORγt by RT-qPCR with GADPH as internal control. Amplification was performed in triplicate on LightCycler® 480 II (Roche Life Science) in a total volume of 20 μL, including 10 μL SYBR Green qPCR Super Mix, 0.5 μL forward primer (10 μM), 0.5 μL reverse primer (10 μM), 5 μL cDNA, and 4 μL sterile water. The primer sequences for PU.1, IRF4, BATF, RORγt, and GADPH are shown in supplemental table (Table S1). 

> The PCR reaction conditions were as follows: Initial denaturation (95°C for 5 min), 40 cycles of denaturation (95°C for 20 s), annealing (60°C for 15 s), and extension (72°C for 15 s). The melting curve analysis of the amplification products was performed at the end of each PCR reaction. The relative mRNA expression of target genes was calculated by comparative Ct method which is performed using the following formula: relative expression = 2^−ΔΔCt.
ELISA
Levels of IL-9 and IL-17 in plasma were measured using a commercial ELISA kit according to manufacturer’s instructions.

Statistical analysis
Data was presented as mean ± SE. By using the GraphPad Prism software, one-way ANOVA was used to perform the comparison of relevant parameters among different groups. For comparison of Th9/IL-9 or Th17/IL-17 in the same patients before and after treatment, the paired Student t-test was used. For correlation, linear regression analysis was applied. A p value less than 0.05 was considered to be statistically significant.

Results
Increased expression of Th9 cells in active ITP patients
To investigate the profile of Th9 cells, peripheral blood mononuclear cells (PBMCs) were isolated from healthy individuals, active ITP patients or ITP in remission followed by measurement of Th9 cells by flow cytometry. As seen in Figure 1, the expression level of Th9 cells was significantly higher in active ITP patients (0.712 ± 0.071%) than that in controls (0.339 ± 0.041%) (p < 0.01) (Figure 1). However, it was reduced in patients in remission (0.274 ± 0.016) with a significant difference to active ITP patients (p < 0.01) but no difference to controls (p > 0.05) (Figure 1). These data demonstrated abnormal expression of Th9 cells in active ITP patients which was restored to the normal level in patients in remission.

Elevated expressions of Th9 cells development-required transcription factors
Previous study demonstrated that Th9 cells development requires a transcription network involving lots of transcription factors, such as PU.1, IRF4, and BATF [15–17]. Given the aberrant expression of Th9 cells in active ITP patients, RNA was isolated from PBMCs to measure the expression of PU.1, IRF4 and BATF by RT-qPCR. Consistent with higher expression of Th9 cells in active ITP patients, significantly higher expressions of PU.1 (1.826 ± 0.239 vs. 1.056 ± 0.119, p < 0.05), IRF4 (2.064 ± 0.231 vs. 1.203 ± 0.244, p < 0.05), and BATF (1.807 ± 0.201 vs. 1.097 ± 0.154, p < 0.05) were observed in active ITP patients compared with controls (Figure 2). The expressions of these three transcription factors were significantly lower in patients in remission than that in active ITP patients (p < 0.05) (Figure 2). No significant difference of the expression of these transcription factors were observed between controls and ITP in remission (p > 0.05) (Figure 2). Taken together, these results showed abnormal expression of transcription factors might account for the higher expression of Th9 cells in active ITP patients.

Increased IL-9 plasma level in active ITP patients
Th9 cells is a new subtype of CD4+ T cells, which is characterized by preferential production of IL-9, a cytokine with pleiotropic functions especially in the regulation of immune response [13,14]. To investigate the IL-9 level in active ITP patients, plasma was isolated from controls or ITP patients for measurement of the IL-9 plasma level by ELISA. As seen in Figure 3, the
IL-9 plasma level was significantly higher in active ITP patients (18.36 ± 1.192 ng/L) than that in controls (5.627 ± 0.468 ng/L) \((p < 0.01)\). Although the IL-9 level was significantly lower in ITP patients in remission (14.62 ± 0.939 ng/L) than that in active ITP patients \((p < 0.05)\) \((\text{Figure 3})\), it was still significantly higher than controls \((p < 0.01)\).

### Figure 3. IL-9 level in plasma of ITP patients and controls

Peripheral whole blood was collected from controls or ITP patients followed by plasma isolation. The level of IL-9 in plasma was measured by a commercial ELISA kit according to manufacturer’s instructions.

IL-9 plasma level was significantly higher in active ITP patients \((18.36 ± 1.192 \text{ ng/L})\) than that in controls \((5.627 ± 0.468 \text{ ng/L})\) \((p < 0.01)\). Although the IL-9 level was significantly lower in ITP patients in remission \((14.62 ± 0.939 \text{ ng/L})\) than that in active ITP patients \((p < 0.05)\) \((\text{Figure 3})\), it was still significantly higher than controls \((p < 0.01)\).

### Abnormal Th17/IL-17 profile and associated transcription factor in active ITP

Given sharing some molecules required for the development of Th9 and Th17 cells \([21]\), profiles of Th17 cells and related transcription factor were also measured in ITP patients. As seen in Figure 4, expression of Th17 cells \((\text{Figure 4A})\) and related transcription factor RORγt \((\text{Figure 4B})\) were significantly higher in active ITP patients than that in controls \((p < 0.05)\), and significantly reduced in ITP patients in remission without difference to controls \((p > 0.05)\), further supporting the role of Th17 cells in the pathogenesis of ITP \([11,12]\). Consistent with the profiles of Th17 cells, the IL-17 plasma level was also significantly higher in active ITP patients \((5.248 ± 0.260 \text{ ng/L})\) than that in controls \((2.277 ± 0.160 \text{ ng/L})\) \((p < 0.01)\) \((\text{Figure 4C})\). Even the IL-17 level was reduced in ITP patients in remission \((4.141 ± 0.289 \text{ ng/L})\), it was still significantly higher than that in controls \((p < 0.01)\).

### Correlation analysis of Th9/IL-9 with Th17/IL-17 in ITP

Considering the same profile of Th9 and Th17 cells in ITP patients mentioned above, correlation analysis was performed to evaluate whether there was a correlation among them. As seen in Figure 5A, and 5B, a positive correlation between Th9 cells and Th17 cells was observed in both active ITP patients and ITP patients in remission. Similarly, the IL-9 plasma level was also positively correlated with the IL-17 level in both active ITP patients and ITP patients in remission \((\text{Figure 5C, and 5D})\). These data demonstrated an interaction between Th9 and Th17 cells in the development of ITP.

### Correlation analysis of transcription factors required for Th9/Th17 cell development

Previous studies demonstrated that the development of Th9 and Th17 cells require a transcription network \([21,26]\). Considering an association of Th9 with Th17 cells in ITP mentioned above, correlation analysis of their-related transcription factors were performed to evaluate their relationship in the development of ITP. Consistent with the correlation of Th9 cells with Th17 cells, a positive
Figure 4. Expression of Th17 cells, transcription factor ROR\(\gamma\)t, and the IL-9 plasma level in ITP patients and controls. Peripheral blood mononuclear cells were isolated from active ITP patients, ITP in remission or controls followed by measurement of Th9 cells by flow cytometry using FITC-CD4 antibody and APC-IL-17A antibody (A). Meanwhile RNA and plasma were extracted for measuring mRNA expression of ROR\(\gamma\)t by RT-qPCR (B) and the IL-17 plasma level by ELISA (C).

Figure 5. Correlation analysis of Th9 cells with Th17 cells and IL-9 with IL-17 in ITP patients. Expression of Th9 cells and Th17 cells in active ITP and ITP in remission were measured by flow cytometry. IL-9 and IL-17 plasma levels were detected by ELISA. Correlation analysis of Th9 with Th17 (A, B) and IL-9 with IL-17 (C, D) was performed by linear regression analysis.
Table II. Correlation analysis among PU.1, IRF4, BATF, and ROR-γt mRNA levels in active ITP patients (A) and ITP in remission (B) (*p < 0.05).

|                | Active ITP         | ITP in remission   |
|----------------|--------------------|--------------------|
| PU.1           | r                   | r                  |
|                | –                   | 0.23               |
|                | p                   | 0.26               |
|                | –                   | 0.23               |
|                | 0.263               | 0.268              |
| IRF4           | r                   | r                  |
|                | –                   | 0.26               |
|                | p                   | 0.26               |
|                | –                   | 0.35               |
|                | 0.249               | 0.35               |
| BATF           | r                   | r                  |
|                | –                   | 0.26               |
|                | p                   | 0.26               |
|                | –                   | 0.26               |
|                | 0.263               | 0.26               |
| ROR-γt         | r                   | r                  |
|                | –                   | 0.26               |
|                | p                   | 0.26               |
|                | –                   | 0.26               |
|                | 0.263               | 0.26               |

As seen in Table II, expression of IRF4 was also positively correlated with BATF, suggesting they might co-operatively regulate the development of Th9 cells. However, no correlation of PU.1 with IRF4, BATF or ROR-γt was observed (Table II).

Correlation analysis of Th9/IL-9 or Th17/IL-17 with platelet count

As abnormal profiles of Th9/IL-9 and Th17/IL-17 were observed in ITP patients, correlation analysis were performed to assess whether dysregulated Th9/IL-9 or Th17/IL-17 was correlated with platelet count in ITP. As seen in Table III, no correlations of Th9 cells, IL-9, Th17 cells or IL-17 with platelet count were found in both active ITP patients and ITP patients in remission (Table III).

Dynamic changes of profiles of Th9/IL-9 and Th17/IL-17 in ITP patients

In order to assess the dynamic changes of Th9/IL-9 and Th17/IL-17 before and after treatment, 20 ITP patients were selected, who achieved remission after treatment. Samples were collected from the same patients before and after treatment (4 weeks after achieving remission without taking any drugs during the sample collection) for analysis of the profile of Th9/IL-9 and Th17/IL-17.

As seen in Figure 6, expressions of Th9/IL-9 and Th17/IL-17 were significantly reduced in patients after treatment compared with patients before treatment, further demonstrating their role in the pathogenesis of ITP.

Discussion

Immune thrombocytopenia (ITP) is an autoimmune disease, characterized by accelerated platelet destruction and impaired platelet production, resulting in lower platelet count which may have a propensity to bleeding risk [1,2]. Just like other autoimmune diseases, ITP is an organ-specific disease and imbalance between immune attack and tolerance regulated by immune cells, such as T cells and B cells, has been reported to play an important role in the initiation and/or perpetuation of the disease [3]. Apart from Th1, Th2 and Th17, Th9 cells are a new subtype of CD4+ Th cells, characterized by preferential secretion of IL-9. Given the pleiotropic function of IL-9, Th9 cells have been shown to be involved in allergic, inflammatory and autoimmune diseases [18]. However, whether Th9 cells ARE involved in the pathogenesis of ITP remains poorly understood. In this study, through measuring the profile of Th9/IL-9 in ITP patients, we demonstrated aberrant expression of Th9 and IL-9 in ITP patients.

CD4+ Th cells are demonstrated to be critical in the regulation of immune responses, with distinct effector subsets characterized by their lineage-specific transcription factor expression, cytokines production and immune function [27]. Th9, a new effector of Th cells identified recently, are characterized by the potent production of IL-9. Th9 cells are pro-inflammatory, but appear to function in a broad spectrum of various autoimmune diseases. Previous study showed that the precise function of Th9 cells might depend on the organ or tissue microenvironment and other Th cell cytokines in the inflammatory sites [28]. Increasing evidence demonstrate the association of Th9 cells with many autoimmune diseases [18]. For example, Th9 cells could induce inflammation in a T cell transfer colitis model [13]. In addition, mice that received Th9 cells developed more severe EAE and lesions in the central nervous system [20]. Furthermore, increased numbers of Th9 cells have been observed in the peripheral blood mononuclear cells in allergic patients [29]. Consistent with the role in the regulation of autoimmun response, Th9 cells expression was demonstrated to be significantly higher in active ITP patients compared with controls and restored into the normal level in patients achieving remission, suggesting Th9 cells might be associated with the pathogenesis of ITP.

Th9 cells develop from naïve T cells after stimulation with TGF-β and IL-4 in the presence of TCR signaling and co-stimulation [13,14]. TGF-β and IL-4 can stimulate the expression of PU.1 and IRF4, respectively, which are two main transcription factors required for Th9 cell development as deficiency of either of them results in defective Th9 development and IL-9 production [27]. In recent years, through microarray analysis of genes expression in Th9 cells compared with other Th cells, BATF, the activator protein 1 family transcription factor, was among the

Table III. Correlative analysis of Th9, IL-9, Th17, or IL-17 expression with platelet count in active ITP patients (A) and ITP in remission (B).

|                | Active ITP         | ITP in remission   |
|----------------|--------------------|--------------------|
| Platelet count | r                   | r                  |
|                | –                   | 0.23               |
|                | p                   | 0.26               |
|                | –                   | 0.23               |
|                | 0.263               | 0.26               |
| Th9 (%)        | – 0.031             | – 0.047            |
| IL-9 (ng/L)    | 0.848 0.763         | 0.38 0.563         |
| Th17 (%)       | 0.141 0.088         | 0.134 0.420        |
| IL-17 (ng/L)   | 0.088 0.563         | – 0.139            |

Table III shows that Th9/IL-9 and Th17/IL-17 expression were not significantly correlated with platelet count in both active ITP patients and ITP patients in remission.
genes enriched in Th9 cells and required for the expression of IL-9 and other Th9-associated genes in both human and mouse T cells [17]. Consistent with the role in the differentiation of Th9 cells as transcription factors, a significantly higher expression of PU.1, IRF4, and BATF was observed in active ITP patients, which might account for the enhanced differentiation of Th9 cells. Interestingly, a positive correlation of IRF4 with BATF was found, suggesting they might co-operatively regulate the development of Th9 cells in the pathogenesis of ITP, consistent with a previous study revealing IRF4 cooperates with BATF to induce the transcriptional program of Th9 cells [17].

Although IL-9 was firstly identified as a Th2 cytokine, it is recently demonstrated to be produced by a new lineage of Th cells, termed Th9 cells. However, Th9 cells are not the only cells secreting IL-9 as multiple cells, such as Th17 cells, Treg cells, and NKT cells possess the ability to produce IL-9 [30]. IL-9 exerts pleiotropic biological effects on a number of distinct types of cells through binding to its receptor IL-9R [27]. Apart from its effects on the survival and proliferation of T cells and mast cells, IL-9 also affects other types of immune cells as well as resident tissue cells that contribute to the initiation of inflammation [31]. In addition, IL-9 plays important roles in the modulation of B cell responses as well as anti-apoptotic effects on neurons and the induction of chemokines in muscle and epithelial cells [30]. Transgenic expression of IL-9 results in increased numbers of peritoneal CD11b+ B1 B cells and can recover the B1 cell numbers in xid mice [32]. Furthermore, IL-9 could enhance IL-4-mediated IgE and IgG production from human B cells without affecting IgM production [33]. Consistent with its role in the regulation of immune cells, a significantly higher level of IL-9 was found in active ITP patients, supporting its role in the pathogenesis of ITP.

Several lines of evidence demonstrated the closely association of IL-9 with Th17 cells development. First, IL-9 could induce the differentiation of naïve CD4+ T cells into Th17 cells in the presence of TGF-β in vitro [34]. Second, IL-9 secreted by Th17 cells amplifies Th17 development in a positive autocrine loop [35]. Moreover, in the EAE mouse model, administration of the anti-IL-9 antibody significantly ameliorated EAE, which was possibly through down-regulating MOG-reactive Th17 cells [36]. Furthermore, IL-9 might be involved in the development of autoimmune disease through Th17-associated inflammation and angiogenesis in K5.hTGF-b1 transgenic mice with a psoriasis-like phenotype [37]. Consistent with the relationship between IL-9 and Th17 cell development, a positive correlation of Th9 cells with Th17 cells as well as IL-9 with IL-17 were observed in ITP patients, indicating Th9/IL-9 and Th17/IL-17 might be cooperatively involved in the pathogenesis of ITP. Furthermore, expression of IRF4 or BATF was positively correlated with RORγt, a master transcription factor required for Th17 cells development, consistent with a previous study revealing that early cooperative binding of BATF and IRF4 that governs chromatin accessibility results in the recruitment of RORγt to regulate a select set of Th17-relevant genes in Th17 cell differentiation [38]. However, the exact mechanism by how Th9 and Th17 regulate each other during the development of ITP remains unclear and requires further investigation.

In conclusion, our study demonstrated that increased expressions of Th9/IL-9 were observed in active ITP patients. In addition, positive correlations of Th9/IL-9 with Th17/IL-17 were also found, suggesting Th9/IL-9 might be involved in the pathogenesis of ITP through cooperatively working with Th17/IL-17 and targeting Th9/IL-9 might be a new therapeutic approach in the prophylaxis or treatment of ITP.

Declaration of interest
The authors have no conflict of interest to declare.

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Supplemental material

Supplemental data for this article can be accessed on the publisher’s website at tandfonline.com/ipt

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