Role of IL-10 and IL-22 cytokines in patients with primary immune thrombocytopenia and their clinical significance

Qifeng Liu | Yan Liu

Abstract

Background: Immune thrombocytopenia purpura (ITP) is an autoimmune disease that leads to accelerated platelet clearance. The objective of this study was to examine the clinical role of cytokines in ITP patients and to correlate them with disease stages.

Materials and Methods: A total of 110 ITP patients were enrolled, including 55 with active ITP, 55 with remission ITP, and 55 with healthy controls. The enzyme-linked immunosorbent assay technique was used to examine IL-10 and IL-22 serum levels in all subjects. Real-time quantitative PCR was used to assess the mRNA expression of IL-10 and IL-22 in PBMC. The clinical significance of both cytokines was assessed using ROC analysis.

Results: IL-10 serum levels in active ITP patients were significantly lower than in control and remission ITP subjects (p < 0.05). IL-22 serum levels were elevated in active ITP patients compared to the control and remission group (p < 0.05). mRNA expressions of IL-10 and IL-22 in active ITP patients were also having a significant difference from than control and remission ITP group (p < 0.05). ROC analysis showed that IL-10 and IL-22 can differentiate the ITP patients from controls. A positive correlation between serum IL-10 and PBMC IL-10 with statistical significance was observed. Similarly, the serum IL-22 and PBMC IL-22 were correlated positively with statistical significance.

Conclusion: IL-10 and IL-22 seem to predict the clinical course of ITP, as a significant imbalance of these cytokines was detected in active ITP patients.

KEYWORDS
interleukin-10, interleukin-22, thrombocytopenia

1 | INTRODUCTION

Primary immune thrombocytopenia (ITP) is an autoimmune disorder that causes immune-mediated platelet loss in the peripheral blood and insufficient platelet synthesis in the bone marrow. Antiplatelet autoantibodies and specific T-cell responses are involved in the development of ITP. This drop in the platelets can cause bleeding episodes such as bruising, petechiae, and even cerebral hemorrhage. ITP pathophysiology is diverse and complicated. Recent research indicates that a complicated imbalance of the immune system plays a role in the etiology of this illness. Recent years have seen a gradual improvement in our understanding of ITP pathogenesis, from the discovery of platelet autoantibodies in the initial destruction of platelets in the spleen to the
inhibition/destruction of megakaryocytes in the bone marrow to the study of autoimmune mechanism. Platelet antibodies against glycoproteins (GPs) IIb/IIIa and Ib/IX are the most common forms.

Platelets have been linked to inflammatory processes involving both the innate and adaptive immune systems. Platelets have been proven to have anti-inflammatory as well as pro-inflammatory effects. Platelets attack invading pathogens and are activated by them, releasing a variety of immune-modulating chemokines contained in their α-granules. As a result, platelets play a role in the first (innate) host defense. Platelets have been demonstrated in vitro to decrease CD4 T-cell proliferation in response to antigen stimulation, showing that platelets may also operate as an anti-inflammatory agent by reducing the immune response. There is a scarcity of data on the immunomodulatory effects of platelets or megakaryocytes in the genesis of ITP. Many studies have reported that only approximately 60% of ITP patients showed positive platelet antibody detection with T helper 1/T helper 2 (Th1/Th2) cell subsets disequilibrium, suggesting that T cell abnormality plays a vital role in the occurrence of ITP.

Numerous findings on serum cytokines in ITP have indicated a distinct activation of T helper (Th)1 cell.

Studies have shown that T helper 17 (Th17) cells, a pro-inflammatory CD4+ T lymphocyte subset that secretes many kinds of cytokines, including interleukin (IL) 17, IL-17F, IL-21, tumor necrosis factor (TNF), and IL-22, can also mediate inflammatory responses and autoimmune diseases, for example, allergic-specific responses, rheumatoid arthritis (RA), and experimental autoimmune encephalomyelitis (EAE). IL-22, a family of IL-10, exhibits specific biological activities of tissue repair and wound healing. Research shows that Th17 can secret IL-22 and Th22 lymphocyte subsets and natural killer (NK) cells can also secrete IL-22. Interleukin 10 (IL-10) is a cytokine that acts as an anti-inflammatory and suppresses immunological responses. Macrophages, Th2 cells, and mast cells all release IL-10. Cytotoxic T cells also produce IL-10 to suppress the function of NK cells induced by viral infection. IL-10 inhibits the synthesis of several pro-inflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor, interferon γ (IFN-γ), TNF-α, IL-3, and IL-2. It has been shown to stimulate mast cells, B cells, and some T cells.

Guo et al. demonstrated that the proportion of Th17 cells and the secretion of the pro-inflammatory cytokines IL-17A and IL-22 were elevated in ITP, whereas the proportion of Treg cells and the production of the anti-inflammatory cytokine IL-10 were decreased. Recent research has reported that bleeding risk models based on IL-10, IL-17A, IL-22, TGF-β, PLT counts, and ITP duration conveniently predict ITP for bleeding risk. Based on the autoimmune nature of ITP and the significance of platelets in immune response, we examined the role of IL-10 and IL-20 in ITP patients in our study.

2 METHODS

2.1 Participants

We selected ITP patients who were either active or in remission who came to HOSPITAL sequentially between 2019, 4 and 2020, 4. Inclusion criteria: (1) Meet the criteria for ITP diagnosis and treatment criteria; (2) Do not receive immune or cytotoxic drugs; (3) Received blood transfusion in the last 1 month. The study group had received blood transfusion therapy in the past 1 month. Additionally, we chose healthy individuals who underwent routine health check-ups throughout the same period as age- and gender-matched controls. Participants were excluded with (1) tumor, infection and other diseases; (2) combined with cardiac, hepatic and renal insufficiency; (3) psychiatric disease; (4) combined with connective tissue disease, lymphatic system Connective tissue diseases, lymphatic system proliferative diseases, myelodysplasia, malignant secondary thrombocytopenia due to combination of connective tissue diseases, lymphatic system proliferative diseases, myelodysplasia, malignant blood diseases, hypersplenism, etc. For the detection of IL-10 and IL-22, three separate groups of subjects were used: control (n = 55), active ITP (n = 55), and ITP remission (n = 55). The differences between the three groups were not statistically significant (p > 0.05) and were comparable.

| TABLE 1 Clinical information of the patients and healthy controls |
|---------------------------------------------------------------|
| **Age (years)** | **Control (n = 55)** | **Active ITP (n = 55)** | **Remission ITP (n = 55)** | **p value** |
|-----------------|----------------------|------------------------|---------------------------|-------------|
| 0–3             | 25                   | 21                     | 23                        | 0.7416      |
| >3              | 30                   | 34                     | 32                        |             |
| **Gender**      |                      |                        |                           |             |
| Male            | 30                   | 34                     | 31                        | 0.7243      |
| Female          | 25                   | 21                     | 24                        |             |
| **Platelet count** |                    |                        |                           |             |
| >30 × 10^9/L    | 55                   | 0                      | 1                         | <0.0001     |
| ≤30 × 10^9/L    | 0                    | 55                     | 54                        |             |
2.2 | Blood samples

After 2 h at room temperature, blood was centrifuged at 3000 rpm for 10 min. Following that, the supernatant was collected and kept at 80°C. We employed density gradient centrifugation with Ficoll-Hypaque (1.077 g/ml, GE Healthcare) to separate peripheral blood mononuclear cells (PBMCs) from EDTA-anticoagulated whole blood.

2.3 | Enzyme-linked immunosorbent assay (ELISA)

The concentrations of IL-10 and IL-22 in serum were assessed by ELISA with Human IL-10 ELISA Kit and Human IL-22 ELISA kit (Beyotime Biotechnology), as directed by the manufacturer.

2.4 | RT-qPCR

To measure the mRNA expression of IL-10 and IL-22 in PBMCs samples of patients with ITP. The total RNA in the PBMC and plasma samples after transfection was extracted using Trizol reagent (Trizol RNA Preparation kit) according to instructions. RNA concentration evaluated by NanoDrop spectrophotometer ND1000 (NanoDrop Technologies Inc). Following that, using the reverse transcription kit, total RNA was reverse-transcribed into cDNA (Shanghai Sangon Biological Engineering Co., LTD). The following criteria were considered in the PCR reaction: (A): 10 min of pre-denaturation at 95°C; (B): 15 s of denaturation at 95°C; 15 s of annealing at 60°C; and 20 s of elongation at 72°C, for a total of 40 cycles. (C): 72°C for 15 min. The process was stopped at 4°C. The sequence for the qPCR assays is as follows: IL-10 forward: 5′-TTGCTGGAGGACTTTAAGGGT-3′, reverse: 5′-CTTGATGTCTGGGTCTTGGTT-3′; IL-22 forward: 5′-TTTCTGACCAAACACTAGCA-3′, reverse: 5′-CTGGATGTCTGGGTCTTGGTT-3′; GAPDH forward: 5′-TATGA TGATATCAAGAGGATG-3′, reverse: 5′-TGATACGC AAACCTAATTGTCATAC-3′. Three replicates were established for each sample, and the results were analyzed quantitatively using 2−ΔΔCt.

2.5 | Statistical and data sets analysis

SPSS 20.0 version (SPSS Inc.) was used to conduct all statistical analyses in this study, and data were represented as mean ± SD. Power analysis has been conducted using the G*Power software as previously described.32 The t-test was used to compare the two groups. Multiple groups were compared using a one-way ANOVA analysis. Correlation analysis was done using Pearson correlation. The sample size was determined by power analysis. The clinical importance of IL-10 and IL-22 was determined using the receiver operating curve (ROC) and the area under the curve in control and active ITP subjects (AUC), p < 0.05 was deemed statistically significant.

3 | RESULTS

3.1 | Clinical characteristics

Patients with ITP were assigned to two groups: those who had active ITP and those who were in remission. Active ITP was considered as a platelet count of less than <100×10^9/L with or without recent bleeding, whereas remission was considered as a platelet count of more than >100×10^9/L with no recent bleeding episodes.31,33 At the time of blood collection, patients in remission were not getting any therapy. The patients in the active ITP group was having significantly lower platelet counts compared to control and remission ITP groups (p < 0.001) (Figure 1).

3.2 | IL-10 and IL-22 serum levels

Serum IL-10 levels were considerably lower in active ITP patients than in remission patients (enter value pg/ml) (p < 0.05) and healthy subjects (p < 0.001). (See Figure 2A). Additionally, remission patients had lower serum IL-10 levels than healthy subjects (p < 0.05). Serum IL-10 level was not correlated with the age of the patients (Figure S1).

Serum IL-22 levels were substantially higher in patients with active ITP than in remission patients (p < 0.05) and healthy subjects (p < 0.001). (Figure 2B). Moreover, remission patients exhibited substantially higher blood IL-22 levels than healthy subjects (p < 0.05). Serum IL-22 level was not correlated with the age of the patients (Figure S1).

3.3 | mRNA expression of IL-10 and IL-22 in PBMCs

Active ITP patients exhibited considerably decreased IL-10 mRNA expression than healthy subjects and remission patients’ IL-22
serum levels were highly elevated IL-10 serum levels were significantly lower than control and remission ITP subjects \( p < 0.001 \) \( (p < 0.05) \) \( \text{Figure 3A} \). Patients in remission had reduced levels of IL-10 in their PBMCs than healthy controls \( p < 0.05 \) \( \text{Figure 3A} \). The active ITP patients had significantly higher IL-22 mRNA expression than the control participants \( p < 0.001 \), and the remission ITP group \( p < 0.05 \) \( \text{Figure 3B} \). Patients in remission showed lower IL-22 levels in their PBMCs than healthy controls \( p < 0.05 \) \( \text{Figure 3B} \).
3.4 | Correlation of serum IL-10 and IL-22 levels with platelet and PBMC IL-10 and IL-22 levels

Correlation of serum IL-10 and IL-22 levels with platelet and PBMC IL-10 and IL-22 levels were analyzed. For the active ITP group, positive correlation between serum IL-10 and PBMC IL-10 with statistical significance was observed ($r = 0.5056, p < 0.0001$) (Figure 4A). Similarly, the serum IL-22 and PBMC IL-22 were positively correlated with statistical significance ($r = 0.5447, p < 0.0001$) (Figure 4B). Moreover, the serum IL-10 levels and clinical data indicated a significant association between plasma IL-10 levels and platelet counts in ITP patients ($r = 0.3409, p = 0.0109$) (Figure 4C). Negative correlation was found between serum IL-22 levels and platelet counts in ITP ($r = -0.4521, p = 0.005$) (Figure 4D). For the remission ITP group, serum IL-10 and PBMC IL-10 ($r = 0.4684, p = 0.0003$) (Figure 5A), serum IL-22 and PBMC IL-22 ($r = 0.4935, p = 0.0001$) (Figure 5B), and IL-10 levels and platelet counts ($r = 0.3850, p = 0.0037$) (Figure 5C) were positively correlated, while IL-22 levels and platelet counts were negatively correlated ($r = -0.3495, p = 0.0089$) (Figure 5D). For the control group, serum IL-10 and PBMC IL-10 ($r = 0.4616, p = 0.0004$) (Figure 6A), serum IL-22 and PBMC IL-22 ($r = 0.2858, p = 0.0344$) (Figure 6B), and IL-10 levels and platelet counts ($r = 0.3294, p = 0.0141$) (Figure 6C) were positively correlated, and IL-22 levels and platelet counts were negatively correlated ($r = -0.3593, p = 0.0071$) (Figure 6D).

3.5 | ROC analysis

The sensitivity and specificity of serum IL-10 and IL-22 concentrations for active ITP patients were estimated using Receiver operating characteristic-curve cut-off values (ROC curve). ROC curves for IL-10 were shown in Figure 7A. The areas under the ROC curves (AUCs) were 0.9286, implying that IL-10 can distinguish the active ITP patients from the healthy individuals efficiently. ROC curves for IL-22 were shown in Figure 7B and the AUCs were 0.8311, showing that IL-22 can also distinguish the active ITP patients from the healthy individuals.

4 | DISCUSSION

Pro and anti-immune cells/cytokines influence and restrain each other to maintain a delicate balance in the human immune system. The disruption of immunological homeostasis in autoimmune illnesses like ITP is largely due to functional and numerical deficiencies in regulatory immune cells. ITP is hypothesized to be the outcome of immunoregulation problems caused by processes in B cells, T cells, and associated cytokines. In ITP patients, platelet breakdown and an increased risk of bleeding are connected to immunological dysfunction in T cells. In ITP patients, the imbalances of Th17/Treg and Th1/Th2 cells have been identified. In addition, in ITP patients, the inflammatory state generated by the imbalances between these T cells contributes to vascular endothelial damage and platelet loss. TNF-α, IL-23, IL-22, IL-10, IL-8, IL-6, IL-1, and other cytokines influence the activity of these T cells. As a result, the expression of cytokine may affect the platelet destruction severity and endothelial damage in the vascular system. Proinflammatory and anti-inflammatory cytokines are divided into two categories based on their function. Under physiological conditions, the expression of pro- and anti-inflammatory mediators is typically balanced; otherwise, immunological diseases develop.
In addition, immune cells create cytokines, and related cytokines regulate immune cell development and function. According to studies, proinflammatory factors are overexpressed and anti-inflammatory ones are under-expressed, which leads to the establishment and progression of ITP. ITP has been related to immunoregulation difficulties caused by Th1/Th2 and Th17/Treg cell biases, as well as aberrant Breg secretion. Another kind of immunoregulatory cell is the breg cell, which generates the cytokines IL-10 and IL-22.

The immunoregulatory cytokine IL-10 is generated primarily by lymphocytes and monocytes. It prevents T cells and monocytes from producing pro-inflammatory cytokines like TNFα. The severity of chronic ITP in Japanese patients is influenced by IL-10 gene polymorphism. Treg cells from IL-10−/− mice have been demonstrated to lose Th17 cells suppression ability. In mice, Treg cells' ability to suppress Th17 cells is dependent on IL-10. Tregs' IL-10 secretion was discovered to be decreased in patients with ITP. In newly diagnosed ITP patients, insufficient IL-10 production impairs Treg inhibitory power against Teffs. In our research, we discovered that active ITP patients had reduced serum IL-10 levels than healthy controls and ITP patients in remission. Similarly, the mRNA expression of IL-10 was found to be significantly decreased in the active ITP group than in the ITP remission and control groups. Moreover, in ITP patients, the serum IL-10 concentrations correlated positively with platelet counts, although there was no significant difference. ROC analysis showed that IL-10 can distinguish active ITP patients from healthy individuals.

According to research, Th17 and Th22 lymphocyte subsets, as well as natural killer (NK) cells, can release IL-22. ITP patients with excessive IL-22 expression, low IL-10 expression, a low platelet...
count, and a lengthy course of disease were at increased bleeding risk. 
Previously, high levels of IL-22 and the Th22 T cell subset were reported in individuals with ITP, implying a function for this population of T cells in the pathogenesis of ITP. In our current study, the active ITP group had significantly greater serum IL-22 levels than the control and ITP remission groups. Also, the mRNA expression of IL-22 was found to be significantly elevated in the active ITP group than in the ITP remission and control groups. The serum IL-22 levels were negatively correlated with the platelet counts; however, no significant difference was found. Furthermore, AUC showed that IL-22 can be used to differentiate active ITP patients from healthy individuals. We hypothesized that the cytokines IL-10 and IL-22 played a larger role in the pathogenesis of ITP. As a result, cytokines should be considered when diagnosing or treating ITP patients. However, the mechanism underlying the imbalance of these cytokines in ITP patients is unknown.

5 | CONCLUSION

To summarize, the current study shows that active ITP patients have much lower IL-10 levels compared to control and ITP remission participants. On the other hand, the active ITP patients exhibited higher levels of IL-22 than those of control and ITP remission groups. Furthermore, IL-10 and IL-22 can differentiate the active ITP patients from the healthy individuals. Additionally, the platelet counts were considerably reduced in active ITP patients compared to control and ITP remission groups. The imbalance of IL-10 and IL-22 levels in patients with ITP at the start stage may be a potential indication of the disease's clinical development. More research is needed to understand the mechanism and function of IL-10 and IL-22, which may be viable targets for ITP immune modulation.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Yan Liu https://orcid.org/0000-0003-3905-1780

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