

Expression and Significance of Regulatory B Cells in Patients with Immune Thrombocytopenia

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Abstract

Objective: To detect the expression and significance of regulatory B cells in patients with immune thrombocytopenia. **Methods:** 73 ITP patients were divided into glucocorticoids treatment group (n = 42) and recombinant human thrombopoietin (rhTPO) treatment group (n = 31). According to the therapeutic effect, it was divided into effective group and ineffective group. The expression of CD19+ CD24hiCD38hi Breg in peripheral blood was detected by flow cytometry before and after treatment. The expression levels of transforming growth factor (TGF)- β 1, interleukin (IL-10) and interferon (IFN)- γ were detected by ELISA before and after treatment. 30 volunteers were selected as the control group. **Results:** The expression of CD19+ CD24hiCD38hi Breg and cytokines IL-10 and TGF- β 1 in 73 ITP patients before treatment was lower than that in the control group, while the expression of IFN- γ was higher than that in the control group (p < 0.05). The expression levels of CD19+ CD24hiCD38hi Breg, IL-10 and TGF- β 1 in the effective group were significantly higher than before treatment, while the expression of IFN- γ was significantly lower than before treatment (p < 0.05). The expression of CD19+ CD24hiCD38hi Breg, IFN- γ , IL-10 and TGF- β 1 in the invalid group had no significant change compared with before treatment. **Conclusion:** Abnormal expression of CD19+ CD24hiCD38hi Breg and related cytokines is involved in the pathogenesis of ITP.

Keywords

Immune Thrombocytopenia, CD19+ CD24hiCD38hi Breg, Cytokines

1. Introduction

Immune thrombocytopenia (ITP) is an autoimmune disease that is characterized

by low platelet counts due to increased platelet destruction and insufficient platelet production. Previous studies have shown that the main pathogenesis of ITP is PLT destruction mediated by PLT-specific autoantibodies, and the common types are anti-platelet membrane glycoprotein (GP) IIb/IIIa and anti-GPIIb/IIIa antibodies [1] [2]. Regulatory B cells (Bregs) identified in mouse and in human have been shown to downregulate inflammation associated with numerous pathological processes, including autoimmune diseases, transplant rejection, anti-tumor responses, and infections [3] [4] [5]. Studies on regulatory B cells' involvement in the pathogenesis of ITP are rare. In this study, flow cytometry was used to detect the expression levels of regulatory B cells and related immune cytokines in peripheral blood of ITP before and after treatment, so as to explore their possible role in the pathogenesis of ITP.

2. Materials and Methods

2.1. Patients

A total of 73 ITP patients and 30 age- and gender-matched healthy donors were enrolled in this study. The diagnosis of ITP was based on the recently reported criteria [6]. There were 27 males and 46 females, aged from 16 to 82 years, with a median age of 47 years, 25 patients over 40 years old, and 41 patients with PLT count $\leq 10 \times 10^9/L$, all of which met the ITP diagnostic criteria. The clinical data of the patients are shown in **Table 1**. In the control group, there were 30 patients, including 12 males and 18 females, aged from 22 to 68 years, with a median age of 38 years. Our research was approved by the hospital based ethics committee, and written informed consent was obtained from all participants.

Table 1. The clinical data of the patients.

	Total No. (n = 73)	The rhTPO group (n = 31)	The GCs group (n = 42)	χ^2	P
Age				0.095	0.758
Median age y (-)	47 (16 - 86)	47 (21 - 78)	47 (16 - 82)		
≤ 40 y [n (%)]	48 (65.8%)	21 (67.7%)	27 (64.3%)		
>40 y [n (%)]	25 (34.2%)	10 (32.3%)	15 (35.7%)		
Gender				0.069	0.793
Female [n (%)]	46 (63.0%)	19 (61.3%)	27 (64.3%)		
Male [n (%)]	27 (37.0%)	12 (38.7%)	15 (35.7%)		
PLT count				0.575	0.448
$\leq 10 \times 10^9/L$ [n (%)]	41 (56.2%)	19 (61.3%)	22 (52.4%)		
$>10 \times 10^9/L$ [n (%)]	32 (43.8%)	12 (38.7%)	20 (47.6%)		

GCs: Glucocorticoid treatment group; rhTPO: Recombinant human thrombopoietin treatment group.

2.2. Evaluation Criteria of Curative Effect

Refer to relevant diagnostic criteria [6]. 1) Completely response: After treatment, $PLT \geq 100 \times 10^9/L$ and no bleeding symptoms. 2) Effective: $PLT > 30 \times 10^9/L$ after treatment and increased by at least 2 times compared with basic PLT count and no bleeding symptoms. 3) Ineffective: $PLT \leq 30 \times 10^9/L$ after treatment or PLT count increased less than 2 times of the base value or bleeding symptoms.

2.3. Treatment

The GCs group patients: Dexamethasone 40 mg/d was given intravenously for 4 days. The rhTPO group patients: rhTPO 15,000 units were injected subcutaneously once a day for 14 days.

2.4. FAS

To detect the percentage of Breg cells, heparinized PB (100 ul) was incubated with antibodies FITC-CD38, PE-CD24, PECy7-CD19 and the appropriate isotype controls (Biolegend, San Diego, CA, USA) for 20 min at room temperature. Then, 2 ml of FACS lysing solution (BD Biosciences, San Jose, CA, USA) was added to the samples and they were incubated for 10 min at room temperature, washed twice in phosphate-buffered saline (PBS). Analysis of all samples was conducted using a Beckman Navios.

2.5. Enzyme-Linked Immunosorbent Assay (ELISA) for Cytokines

The concentrations of c-interferon (IFN- γ), transforming growth factor-b (TGF- β 1) and IL-10 in the cell culture supernatants were measured using an ELISA kit (ABclonal Technology, Wuhan, P. R. China) according to the manufacturer's instructions.

2.6. Statistical Analysis

Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Unless indicated, data were expressed as the mean standard error of the mean (SEM). The percentage of Breg cells in PB among ITP patients, ITP patients and controls were analysed by one-way analysis of variance. $p < 005$ was considered significant.

3. Results

3.1. Decreased Percentage of CD19+ CD24hiCD38hi Breg in Patients with ITP

To detect the abnormal frequency of Breg cells in ITP, we analysed the percentage of Breg cells in PB samples obtained from patients with ITP, patients in ITP and controls using FACS. A low proportion of CD19+ CD24hiCD38hi Breg in the CD19+ population was observed in patients with ITP compared with healthy controls (Figure 1).

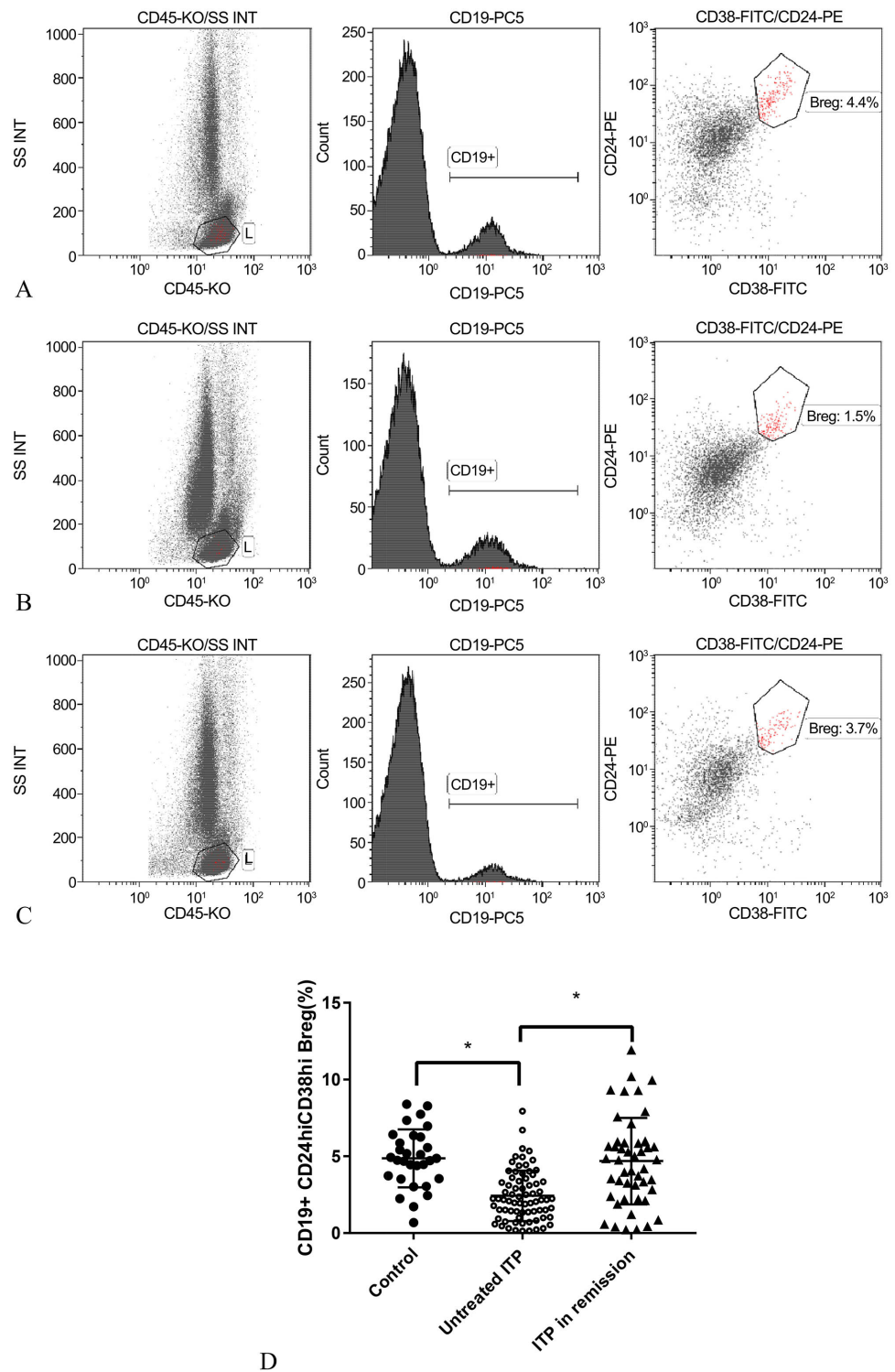


Figure 1. Decreased frequencies of CD19+ CD24hiCD38hi Breg cells in peripheral blood from patients with immune thrombocytopenia. (A) Dot plot of flow cytometric analysis of CD19+ CD24hiCD38hi Breg cells in peripheral blood from control. (B) Dot plot of flow cytometric analysis of CD19+ CD24hiCD38hi Breg cells in peripheral blood from untreated patients. (C) Dot plot of flow cytometric analysis of CD19+ CD24hiCD38hi Breg cells in peripheral blood from ITP patients in remission. (D) Frequencies of CD19+ CD24hiCD38hi Breg cells in the CD19+ T population in patients with ITP, in remission and controls.

3.2. Expression of IFN- γ , TGF- β 1 and IL10 in Patients with ITP

The expression of cytokines IL-10 and TGF- β 1 was lower than that of the control group, and the expression of IFN- γ was higher than that of the normal control group, with statistically significant differences between the two groups. Furthermore, the expression of IL-10, TGF- β 1 in patients who are responses to treatment were increased compared with before treatment. These differences between before and after treatment were uniform statistical significance. While, the expression of INF- γ of response to treatment was lower than that of no response ITP, these differences were not statistical significance (**Figure 2**).

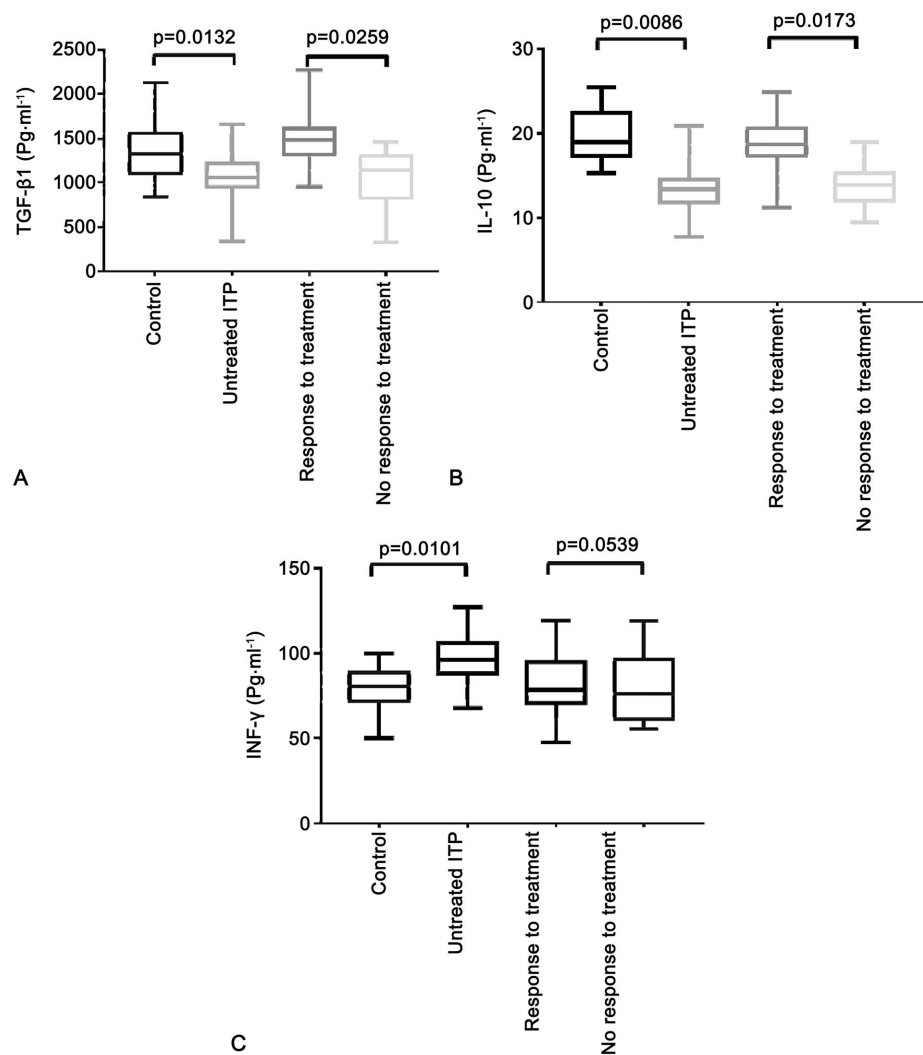


Figure 2. Expression of IFN- γ , TGF- β 1 and IL10 in patients with ITP. (A) The expression of cytokines TGF- β 1 was lower than that of the control group. The expression of TGF- β 1 in patients who are responses to treatment were increased compared with before treatment. (B) The expression of cytokines IL-10 was lower than that of the control group. The expression of IL-10 in patients who are responses to treatment were increased compared with before treatment. (C) The expression of IFN- γ of patients was higher than that of the normal control group, the expression of INF- γ of response to treatment was lower than that of no response ITP, these differences were not statistical significance.

4. Discussion

The pathogenesis of ITP has not been fully clarified, and existing studies have shown that platelet autoantibodies secreted by B lymphocytes are the main cause of ITP. Platelets bound to autoantibodies are eventually cleared by the body's mononuclear phagocyte system, resulting in peripheral thrombocytopenia⁷. To elucidate the regulation mechanism of platelet autoantibody production is of great significance to clarify the pathogenesis and treatment of ITP. In this paper, the expression of Breg cells in ITP patients was detected by FCM to explore its possible mechanism in ITP.

Breg cells function mainly by secreting IL-10, TGF- β . IL-10 is a potent anti-inflammatory cytokine that plays a central role in inhibiting the host immune response to pathogens, thus preventing injury and maintaining the dynamic balance of the body's internal environment. IL-10 can directly or indirectly inhibit the activation of CD4+T cells and the differentiation of Th0 cells into Th1 and Th2 cells, thereby reducing the secretion of IFN- γ by Th1 cells. At the same time, it can also induce the apoptosis of effector T cells and regulate the Th1/Th2 balance, thus inhibiting the occurrence of autoimmunity [7] [8] [9] [10]. The results of this study showed that compared with the control group, peripheral blood CD19+ CD24hiCD38hi B cells and IL-10 expression were significantly decreased in ITP patients. In ITP patients, Breg and related cytokine IL-10 decreased significantly, which weakened the inhibitory ability of Breg and cytokine IL-10 on T cells, resulting in increased destruction and decreased formation of platelets. TGF- β is a cytokine in the body that regulates the body's inflammatory response. It regulates the proliferation, differentiation and activation of lymphocytes, macrophages and other immune-related cells through autocrine and paracrine forms [11]. TGF- β can induce secretion of Treg cells, and Treg cells play an immunosuppressive role by secreting TGF- β and IL-10. The results of this study showed that the levels of TGF- β in peripheral blood of ITP patients decreased compared with normal controls.

5. Conclusion

In summary, Breg can inhibit the activity of reactive T cells and inhibit the production of antibodies by secreting cytokines IL-10 and TGF- β , and can promote the differentiation and proliferation of Treg to mediate immune tolerance, and its dysfunction and reduction may lead to the occurrence of autoimmune diseases. Breg immune deficiency exists in ITP patients. Exploring the targeted treatment of Breg to inhibit the occurrence and development of ITP is expected to provide new ideas for the treatment of ITP.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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