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Indirubin regulates MPL and TNF expression in peripheral blood mononuclear cells from patients with primary immune thrombocytopenia

Kai Shao[a,b,1], Tengkai Wang[c,1], Tiantian Li[a], Aijun Zhang[d], Meijuan Cai[e], Guanghui Zhao[e], Qingsong Fu[a], Qian Wang[e], Xinguang Liu[e], and Ming Hou[f]

[a]*Department of Clinical Laboratory, Qilu Hospital of Shandong University (Qingdao), Qingdao, China;* [b]*Qingdao Key Lab of Mitochondrial Medicine, Qilu Hospital of Shandong University (Qingdao), Qingdao, China;* [c]*Department of Internal Medicine, Qilu Hospital of Shandong University, Jinan, China;* [d]*Department of Pediatrics, Qilu Hospital of Shandong University, Jinan, China;* [e]*Department of Clinical Laboratory, Qilu Hospital of Shandong University, Jinan, China;* [f]*Department of Hematology, Qilu Hospital of Shandong University, Jinan, China*

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Indirubin, a traditional Chinese medicine, is currently used to treat certain autoimmune diseases such as primary immune thrombocytopenia (ITP) in clinics. However, the effects of indirubin on expression of related genes in peripheral blood mononuclear cells (PBMCs) from ITP patients have not been investigated. In the present study, PBMCs were isolated from 19 adult patients with well-characterized active ITP and 20 healthy controls (HCs) and then treated with increasing concentrations of indirubin. The mRNA expression levels of thrombopoietin receptor (MPL), GATA binding protein 3 (GATA3), DNA methyltransferase 3B (DNMT3B), interleukin-6 (IL6), tumor necrosis factor (TNF), and interferon gamma (IFN-γ) were determined by quantitative real-time polymerase chain reaction (PCR). We found that indirubin had no cytotoxic effect on PBMC viability. Significantly lower MPL ($p < 0.05$) and GATA3 ($p < 0.05$) expression together with markedly higher IL6 ($p < 0.05$), TNF ($p < 0.0001$), and IFN-γ ($p < 0.001$) mRNA levels were observed in ITP patients compared with HCs. Notably, indirubin significantly enhanced MPL expression and inhibited TNF expression in PBMCs from ITP patients ($p < 0.05$). In summary, indirubin may play a direct role in thrombopoiesis by activating cellular MPL and normalizing TNF expression to suppress inflammation in ITP. This study may thus improve our understanding of indirubin and provide important information for optimizing therapeutic strategies for ITP patients.

Indirubin, a traditional Chinese medicine (TCM) formulation with anticancer and anti-inflammatory activities, is used in clinics for the treatment of chronic myelocytic leukemia and certain autoimmune diseases such as primary immune thrombocytopenia (ITP) [1]. Indirubin and its multiple synthetic derivatives are potent cyclin-dependent kinases (CDKs) and glycogen synthetase kinase 3 (GSK-3) inhibitors [2]. Anti-inflammatory and immune modulatory functions of indirubin have also been identified in several models [3,4]. In a lipopolysaccharide-induced mastitis mouse model, indirubin reduced inflammation by inhibiting the production of interleukin-1β (IL1β), interleukin-6 (IL6), and tumor necrosis factor (TNF) [5].

Primary immune thrombocytopenia is an acquired autoimmune disease characterized by immune-mediated peripheral platelet destruction and suboptimal platelet production in bone marrow [6,7]. The pathogenesis of ITP includes the production of antiplatelet autoantibody and the targeting of T-cell responses. Antiplatelet autoantibody production is under the control of T helper (Th) cells, and elevated antiplatelet T-cell reactivity in ITP has been observed [1]. Thrombopoietin is the principal hematopoietic cytokine that stimulates thrombopoiesis by activating cells through the thrombopoietin receptor (MPL) [8]. Direct stimulation of MPL will increase platelet production by the bone marrow [9]. Inflammation can trigger ITP, and ITP can in turn induce inflammation through platelet dysfunction. Inflammatory cytokine-mediated immunity plays an important role in the pathogenesis of ITP [10].

ITP is a common disease of the blood system that often recurs because of the lack of an ideal treatment modality.

Offprint requests to: Qian Wang, Department of Clinical Laboratory, Qilu Hospital of Shandong University, 107 Wenhuaxi Road, Jinan 250012, China; E-mail: sd.wangqian@163.com

[1]KS and TW contributed equally to this work.

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Modern medicine uses glucocorticoids, gamma immunoglobulin, immunosuppressants, and others as the main treatment, even splenectomy, but there are still many patients whose condition is not obviously relieved. TCM treatment of ITP has obvious advantages in improving both clinical symptoms and long-term efficacy. However, there are few reports about the effective clinical intervention and mechanism of ITP with TCM. Our present study reveals that indirubin positively enhances MPL expression and inhibits TNF expression in PBMCs from ITP patients. Our findings offer a better understanding of the potential mechanism of indirubin in the treatment of ITP and expand new research ideas in the field of TCM treatment of ITP under the guidance of TCM theory.

Methods

Patients

We analyzed blood samples and clinical data from adult patients with well-characterized active ITP and from HCs. A total of 19 active ITP patients and 20 gender- and age-matched HCs were enrolled in this study. The diagnosis of ITP was based on recently reported criteria [11]. Patients with platelet counts below 100×10^9/L were defined as having active ITP. All included cases were assessed by an expert committee and clinical data were recorded in a database using standardized language and adapted software.

Standard protocol approval, registration, and patient consent

All subjects gave written informed consent to participate in this study, which was approved by the ethics committee of Qilu Hospital of Shandong University (Qingdao), China.

Preparation of PBMCs

Blood samples were collected from active ITP patients and HCs with EDTA anticoagulation. PBMCs were isolated from blood samples by gradient centrifugation (400*g*, 20 min) on Ficoll–Paque (HaoYang Biological Manufacture, Tianjin, China) [9].

Cell viability assay

PBMCs (1×10^6/mL) isolated from HCs were seeded in 24-well plates with RPMI 1640 medium (containing 10% fetal bovine serum and 2 mmol/L glutamine/penicillin/streptomycin) and stimulated with 400 ng/mL phorbol 12-myristate 13-acetate (Multi Sciences, Hangzhou, China). The effect of indirubin (1, 5, and 10 μmol/L) on PBMC viability was investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay after treatment with indirubin (Meilun Biological Technology, Dalian, China) for 24 hours. The absorbance was read at 540 nm with a microplate reader (Molecular Devices, San Jose, CA).

Cell culture

After collection from active ITP patients and HCs with EDTA anticoagulation, PBMCs (1×10^6/ml) were immediately cultured in 24-well plates as previously described for cell viability assays. Increasing concentrations of indirubin (dissolved in DMSO) were added, and cells were cultured for 24 hours at 37°C in 5% CO_2 for further investigation. Groups were divided as follows: HC group, ITP control group, 1 μmol/L indirubin-treated ITP group, and 10 μmol/L indirubin-treated ITP group.

Extraction of total RNA and reverse transcription of mRNA

After incubation, the PBMCs were collected for extraction of total RNA. Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA). The amount of RNA was determined using a spectrophotometer (Titertek-Berthold Colibri, Germany). The reverse transcription reactions were carried out using the All-in-One First-strand cDNA Synthesis Kit (GeneCopoeia, Rockville, MD) following the manufacturer's procedures.

Real-time PCR

Messenger RNA expression levels of MPL, GATA3, DNMT3B, IL6, TNF, and IFN-γ were quantified by universal quantitative real-time PCR using All-in-One qPCR Mix (GeneCopoeia) on a StepOnePlus Real-Time PCR system (ABI, Waltham, MA). β-Actin was quantified as an internal reference gene to normalize differences. All primer sequences are listed in Table 1. The amplification was performed with a two-step PCR protocol (95°C for 10 min, followed by 45 cycles of 95°C for 10 sec, 60°C for 20 sec, and 72°C for 15 sec). Each sample was run in triplicate. The relative quantification of gene expression was obtained by comparison with the relative expression of the β-actin internal reference using the $2^{-\Delta\Delta Ct}$ method [7].

TNF-α enzyme-linked immunosorbent assay

Tumor necrosis factor α concentration in cell culture supernatant was measured with the ELISA kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Statistics

Unless otherwise indicated, data are expressed as the mean ± SEM. The difference between two independent groups was evaluated using an unpaired Student *t* test or Mann–Whitney *U* test. Correlation was performed with Spearman's rank correlation coefficient. Statistical significance was assumed at

Table 1. Primers for real-time polymerase chain reaction

Gene	Nucleotide sequence of primers (5′–3′)	bp
β-Actin	F: GGCACCCAGCACAATGAAG	131
	R: CCTCATACTCCTGCTTGCTG	
MPL	F: CCCACTTTGGAACCCGATACG	111
	R: GAGTCCGAGTCTGGTTTAGGA	
GATA3	F: GCCCCTCATTAAGCCCAAG	80
	R: TTGTGGTGGTCTGACAGTTCG	
DNMT3B	F: CCCAGCTCTTACCTTACCATCG	199
	R: GGTCCCCTATTCCAAACTCCT	
Interleukin-6	F: CCTGAACCTTCCAAAGATGGC	75
	R: TTCACCAGGCAAGTCTCCTCA	
Tumor necrosis factor	F: CCTCTCTCTAATCAGCCCTCTG	220
	R: GAGGACCTGGGAGTAGATGAG	
Interferon γ	F: TCGGTAACTGACTTGAATGTCCA	93
	R: TCGCTTCCCTGTTTTAGCTGC	

F=Forward primer; *R*=reverse primer.

$p < 0.05$. All analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY).

Results

Clinical characteristics of ITP patients

The main clinical characteristics of ITP patients in this study, including disease duration, platelet count and major therapy, are listed in Table 2.

Effect of indirubin on PBMC viability

The cytotoxic effect of indirubin was investigated in PBMCs from HCs by MTT assay. We observed no significant change after treatment with increasing concentrations of indirubin (1, 5, and 10 μmol/L) for 24 hours (Fig. 1).

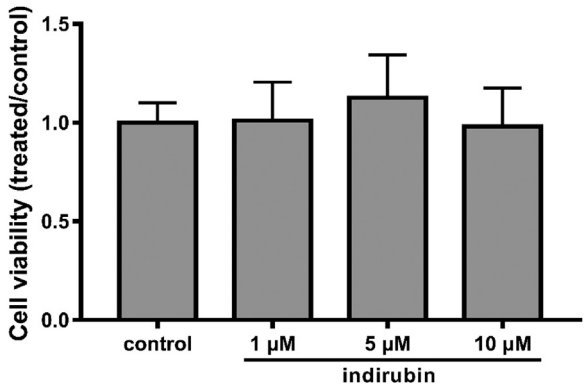

Figure 1. Effect of indirubin on the viability of PBMCs from HCs. Error bars represent SEM.

Indirubin enhanced MPL expression in PBMCs from ITP patients

Direct stimulation of MPL can increase platelet production by the bone marrow [8]. The relative mRNA expression level of MPL in PBMCs from active ITP patients was significantly lower compared with that from HCs ($p < 0.05$, Fig. 2A). The indirubin treatment enhanced MPL expression in a dose-dependent manner. There was a significant difference between the 10 μmol/L indirubin-treated ITP group and the ITP control group ($p < 0.05$, Fig. 2A), suggesting that indirubin could restore MPL expression in PBMCs from ITP patients. Analysis of the relationship between MPL expression and clinical data revealed a significantly positive correlation ($r = 0.54$, $p < 0.05$) between MPL expression and platelet count in ITP patients (Fig. 2B). However, MPL expression did not correlate with gender, age, disease duration, or treatment.

Indirubin inhibited TNF expression in PBMCs from ITP patients

TNF participates as a candidate susceptibility factor for ITP [10]. As was illustrated in Figure 3A, TNF expression in PBMCs from ITP patients was remarkably higher than that in HCs ($p < 0.0001$). After treatment with indirubin, TNF expression was significantly lower than in the ITP control group ($p < 0.05$) but was still higher than in the HC group ($p < 0.05$). There was no significant difference between the 1 and

Table 2. Clinical features of ITP patients

Patient no.	Sex	Age	Disease duration (m)	Platelet count ($\times 10^9$/L)	Major therapy
1	M	40	25	36	None
2	F	59	50	5	IVIG
3	M	59	9	16	GC, IVIG
4	F	54	121	16	GC, DAC
5	F	61	1	59	GC
6	M	67	108	22	GC
7	F	61	1	7	GC
8	M	71	52	8	GC
9	F	55	75	56	None
10	F	67	110	26	GC
11	M	71	49	19	GC
12	F	54	122	48	GC
13	F	32	80	12	GC
14	F	65	245	15	None
15	M	38	1	13	GC
16	F	54	124	14	GC
17	F	48	127	4	None
18	F	29	12	97	DAC
19	F	62	3	3	GC
Median		59	52	16	
Range		29–71	1–245	3–97	

DAC=Decitabine; *GC*=glucocorticoid; *IVIG*=intravenous immunoglobulin.

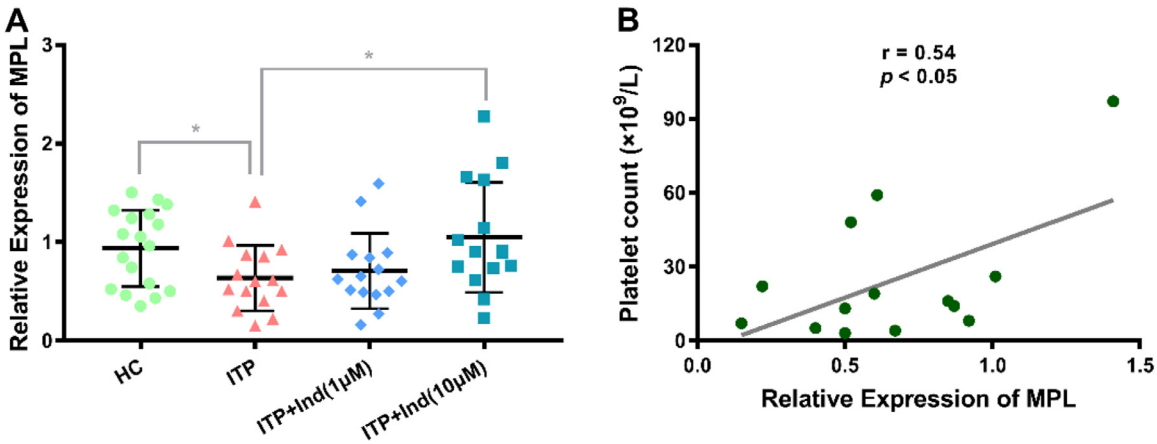

Figure 2. MPL expression in PBMCs from ITP patients and HCs. **(A)** Effect of indirubin on MPL mRNA expression in PBMCs from ITP patients and HCs. **(B)** Association between MPL expression and platelet count in ITP patients. *$p < 0.05$. Error bars represent SEM.

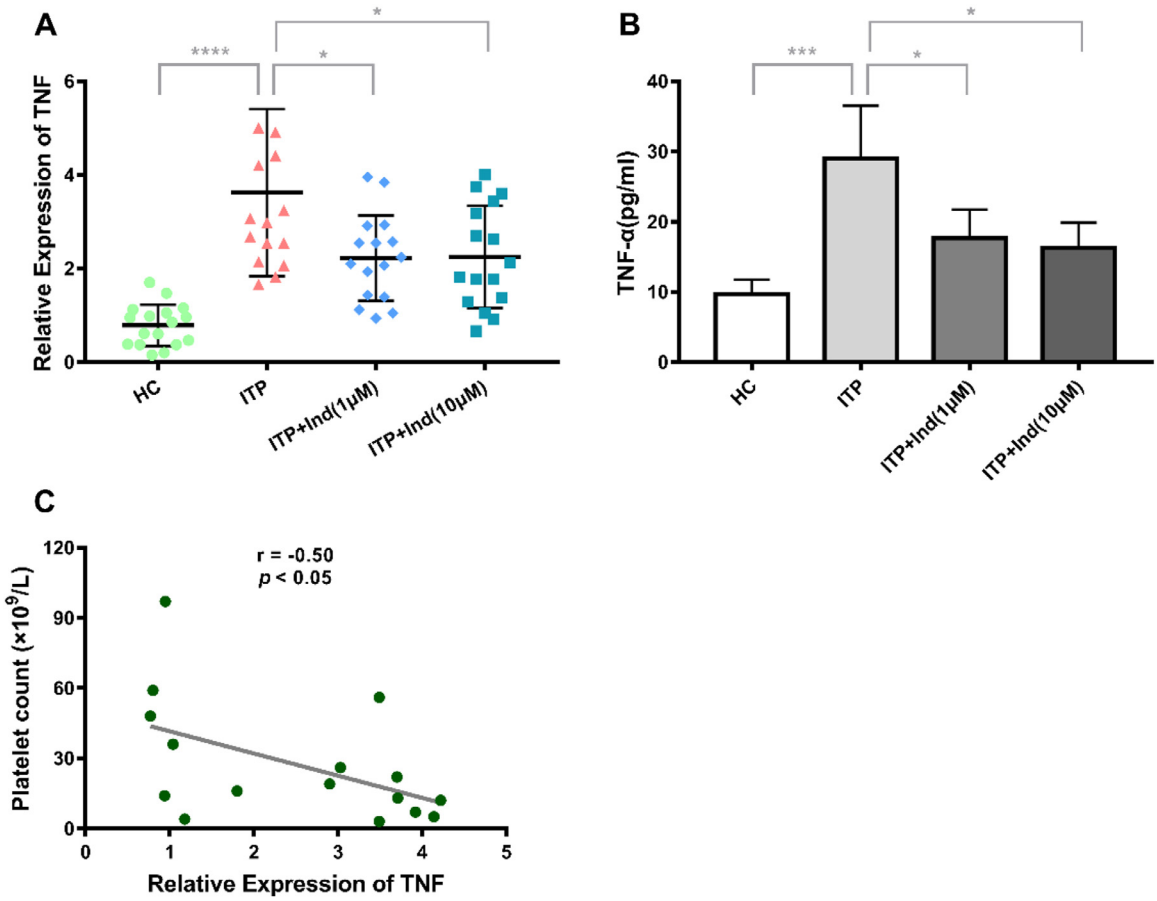

Figure 3. TNF expression in PBMCs from ITP patients and HCs. **(A)** Effect of indirubin on TNF mRNA expression in PBMCs from ITP patients and HCs. **(B)** Effect of indirubin on TNF-α concentration in the cell culture supernatants of PBMCs from ITP patients and HCs. **(C)** Association between TNF expression and platelet count in ITP patients. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$. Error bars represent SEM.

10 μmol/L indirubin-treated ITP groups. To further evaluate the effect of indirubin on TNF modulation, TNF-α concentrations in the cell culture supernatants were also determined by ELISA (Fig. 3B). The results were consistent with the levels of expression of TNF mRNA. Analysis of the relationship between TNF expression and platelet count in ITP patients (Fig. 3C) showed a negative correlation ($r = -0.50$, $p < 0.05$). Moreover, TNF expression did not correlate with other clinical data.

Effects of indirubin on expression of GATA3, DNMT3B, IL6, and IFN-γ in PBMCs from ITP patients

To further evaluate the effect of indirubin on associated transcription factors and inflammatory cytokines, mRNA expression levels of GATA3, DNMT3B, IL6, and IFN-γ in PBMCs from ITP patients and HCs were detected by real-time PCR. As illustrated in Figure 4, significantly lower GATA3 levels together with markedly higher IL6 and IFN-γ mRNA levels were observed in PBMCs from ITP patients compared with HCs (GATA3, $p < 0.05$; IL6, $p < 0.05$; IFN-γ, $p < 0.001$). There was no difference between DNMT3B in ITP patients' PBMCs compared with HCs ($p > 0.05$). After indirubin treatment, there were no significant differences in mRNA expression levels of GATA3, DNMT3B, IL6, or IFN-γ in PBMCs from ITP patients ($p > 0.05$).

Discussion

ITP is an acquired immune-mediated bleeding disorder caused by the production of antiplatelet autoantibodies that bind to platelet membrane glycoproteins or by targeted T-cell responses mediating the destruction of platelets [12–16]. More recently, it has been observed that antiplatelet autoantibody production is under the control of Th cells in ITP [1]. The dominant clinical manifestation is an increased risk of bleeding associated with a reduced platelet count. Although many studies have deepened our understanding of ITP, the etiology is still not clear. It is widely accepted that both genetic and environmental factors play important roles in the pathogenesis of ITP [17]. In clinics, most adult patients show chronic disease progression and require long-term glucocorticoid treatment, administration of immunosuppressive reagents, or even splenectomy. However, many side effects are inevitable [18]. ITP is closely related to inflammation and platelets have inflammatory functions in both innate and adaptive immunity [6,19]. ITP can induce inflammation through platelet dysfunction. Furthermore, platelets mediate inflammation and regulate immune-mediated inflammatory cytokines such as TNF and IFN-γ. Even

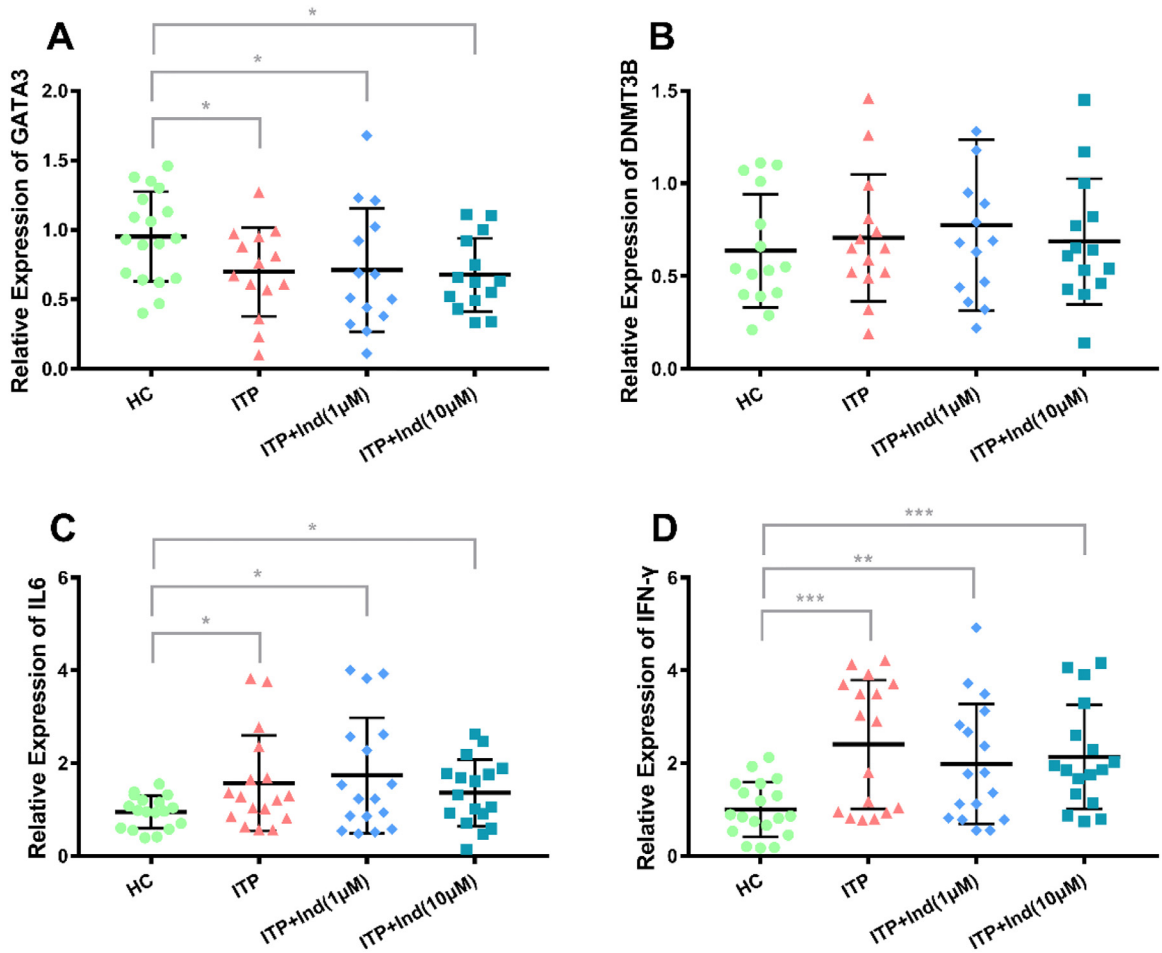

Figure 4. Effect of indirubin on **(A)** GATA3, **(B)** DNMT3B, **(C)** IL6, and **(D)**IFN-γ mRNA expression in PBMCs from ITP patients and HCs. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Error bars represent SEM.

after being phagocytosed by the mononuclear phagocyte system, platelets can continue to regulate inflammation [20].

Indirubin is the active ingredient of Danggui Longhui Wan, a mixture of plants that is used in traditional Chinese medicine [2]. For its anticancer and anti-inflammatory activities, indirubin is extensively used for the treatment of chronic myelocytic leukemia, certain autoimmune diseases, and other chronic diseases such as Alzheimer's disease and diabetes [21–24]. Indirubin and its multiple synthetic derivatives are potent CDKs and GSK inhibitors and may also suppress NF-κB activation. It has been found that indirubin and its derivatives have antiproliferative activity and can induce apoptosis. Zhang et al. [25] provided evidence for the antitumor angiogenesis activity of indirubin and suggested that indirubin was a potential drug candidate for angiogenesis-related diseases. Nam et al. [26] identified indirubin derivatives as a promising structural class for development of new therapeutics for wild-type or T315I-mutant Bcr-Abl-positive chronic myelogenous leukemia patients. Moreover, anti-inflammatory and immune modulatory functions of indirubin have been identified in some models [5]. Kim et al. [3] found that indirubin treatment suppresses skin inflammation and reduces TNF, IL4, IL6, and IFN-γ production in mice. Qi et al. [4] reported that indirubin alleviates lipopolysaccharide-induced oxidative stress and inflammation by reducing malondialdehyde abundance and IL1β and TNF-α expression in mice. However, the effects of indirubin on associated transcription factors and cytokine production in PBMCs from ITP patients have not been investigated. TNF participates as a candidate susceptibility factor in ITP and initiates a pro-inflammatory response to facilitate proliferation of cytotoxic T cells [6]. Moreover, TNF has been reported as a pro-inflammatory cytokine of innate immunity which participates in ITP processes [10]. Thrombopoietin is the principal hematopoietic cytokine that stimulates thrombopoiesis by activating MPL [8]. Lack of MPL expression may cause decreased platelet production in ITP patients. The U.S. Food and Drug Administration (FDA) has approved two types of MPL agonists, eltrombopag and romiplostim, for the treatment of ITP [27–29]. Currently, indirubin is used to treat ITP in clinics [1]. However, the effect of indirubin on MPL expression in PBMCs from ITP patients has not yet been investigated. Thus, in the present study, we investigated mRNA expression of MPL, GATA3, DNMT3B, IL6, TNF, and IFN-γ in PBMCs isolated from 19 well-characterized active ITP patients and 20 HCs. Our study found significantly lower MPL and GATA3 expression together with markedly higher IL6, TNF, and IFN-γ mRNA levels in PBMCs from ITP patients compared with HCs.

Our results confirmed markedly higher TNF mRNA levels in PBMCs from ITP patients compared with HCs. Responses to different therapeutic strategies, such as high-dose dexamethasone, splenectomy, and rituximab, are often associated with the correction of cytokine abnormalities in ITP [30–33]. An anti-inflammatory function of indirubin has been identified in some models. However, the effect of indirubin on TNF expression in PBMCs from ITP patients is still unclear although indirubin has been used in ITP treatment for several years in China. Notably, we found that indirubin treatment inhibits TNF expression in PBMCs from ITP patients, indicating that indirubin may correct TNF expression to suppress inflammation in ITP patients. More in-depth studies are necessary to clarify its exact molecular mechanisms of action.

In the present study, we found that indirubin treatment enhanced MPL expression in a dose-dependent manner, suggesting that indirubin could stimulate MPL expression to increase platelet production in ITP patients. Our data indicated that indirubin may have a direct effect on thrombopoiesis by activating cells through MPL. However, considering the enormous heterogeneity and complexity of ITP pathogenesis, the precise role of MPL in indirubin treatment of ITP awaits more investigation.

Conclusions

Our study found significantly lower MPL and GATA3 expression together with markedly higher TNF, IL6, and IFN-γ mRNA levels in PBMCs from ITP patients compared with HCs. Moreover, indirubin enhanced MPL mRNA expression and inhibited TNF mRNA expression in PBMCs from ITP patients. These data indicate that indirubin may play a direct role in thrombopoiesis by activating cellular MPL and in suppression of inflammation in ITP patients by normalizing TNF expression. This study may thus improve our understanding of the mechanism of indirubin and provide important information for optimizing therapeutic strategies for ITP.

Acknowledgments

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Conflict of interest disclosure

The authors declare no conflicts of interest.

References

1. Zhang A, Ning B, Sun N, Wei J, Ju X. Indirubin increases CD4+CD25+Foxp3+ regulatory T cells to prevent immune thrombocytopenia in mice. PloS One. 2015;10:e0142634.
2. Hoessel R, Leclerc S, Endicott JA, et al. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. Nat Cell Biol. 1999;1:60–67.
3. Kim MH, Choi YY, Yang G, Cho IH, Nam D, Yang WM. Indirubin, a purple 3,2-bisindole, inhibited allergic contact dermatitis via regulating T helper (Th)-mediated immune system in DNCB-induced model. J Ethnopharmacol. 2013;145:214–219.
4. Qi T, Li H, Li S. Indirubin improves antioxidant and anti-inflammatory functions in lipopolysaccharide-challenged mice. Oncotarget. 2017;8:36658–36663.
5. Lai JL, Liu YH, Liu C, et al. Indirubin inhibits LPS-induced inflammation via TLR4 abrogation mediated by the NF-kB and MAPK signaling pathways. Inflammation. 2017;40:1–12.
6. Li J, Ma S, Shao L, et al. Inflammation-related gene polymorphisms associated with primary immune thrombocytopenia. Front Immunol. 2017;8:744.
7. Li H, Hao Y, Zhang D, et al. Aberrant expression of long noncoding RNA TMEVPG1 in patients with primary immune thrombocytopenia. Autoimmunity. 2016;49:496–502.
8. Gudbrandsdottir S, Ghanima W, Nielsen CH, Feng X, Hasselbalch HC, Bussel J. Effect of thrombopoietin-receptor agonists on circulating cytokine and chemokine levels in patients with primary immune thrombocytopenia (ITP). Platelets. 2017;28:478–483.
9. Qu MM, Liu XN, Liu XG, et al. Cytokine changes in response to TPO receptor agonist treatment in primary immune thrombocytopenia. Cytokine. 2017;92:110–117.
10. Wang N, Li GN, Wang XB, Liang T, Hu L. TNF-alpha promoter single nucleotide polymorphisms and haplotypes associate with susceptibility of immune thrombocytopenia in Chinese adults. Human Immunol. 2014;75:980–985.
11. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113:2386–2393.
12. George JN. Management of immune thrombocytopenia—Something old, something new. N Engl J Med. 2010;363:1959–1961.
13. Jansen AJ, Swart RM, te Boekhorst PA. Thrombopoietin-receptor agonists for immune thrombocytopenia. N Engl J Med. 2011;365:2240–2241.
14. Li J, van der Wal DE, Zhu G, et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. Nat Commun. 2015;6:7737.
15. Mahevas M, Patin P, Huetz F, et al. B cell depletion in immune thrombocytopenia reveals splenic long-lived plasma cells. J Clin Invest. 2013;123:432–442.
16. Provan D, Newland AC. Guidelines for immune thrombocytopenia. N Engl J Med. 2011;364:580–581. author reply 581.
17. Cines DB, Bussel JB, Liebman HA, Luning Prak ET. The ITP syndrome: pathogenic and clinical diversity. Blood. 2009;113:6511–6521.
18. Boruchov DM, Gururangan S, Driscoll MC, Bussel JB. Multiagent induction and maintenance therapy for patients with refractory immune thrombocytopenic purpura (ITP). Blood. 2007;110:3526–3531.
19. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. Nat Rev Immunol. 2011;11:264–274.
20. Lang D, Dohle F, Terstesse M, et al. Down-regulation of monocyte apoptosis by phagocytosis of platelets: Involvement of a caspase-9, caspase-3, and heat shock protein 70-dependent pathway. J Immunol. 2002;168:6152–6158.
21. Blazevic T, Heiss EH, Atanasov AG, Breuss JM, Dirsch VM, Uhrin P. Indirubin and indirubin derivatives for counteracting proliferative diseases. Evidence-Based Complementary Altern Med. 2015;2015:654098.
22. Huang M, Wang L, Zeng S, et al. Indirubin inhibits the migration, invasion, and activation of fibroblast-like synoviocytes from rheumatoid arthritis patients. Inflammation Res. 2017;66:433–440.
23. Kosuge Y, Saito H, Haraguchi T, et al. Indirubin derivatives protect against endoplasmic reticulum stress-induced cytotoxicity and down-regulate CHOP levels in HT22 cells. Bioorg Med Chem Lett. 2017;27:5122–5125.
24. Kumagai T, Aratsu Y, Sugawara R, Sasaki T, Miyairi S, Nagata K. Indirubin, a component of Ban-Lan-Gen, activates CYP3A4 gene transcription through the human pregnane X receptor. Drug Metab Pharmacokinet. 2016;31:139–145.
25. Zhang X, Song Y, Wu Y, et al. Indirubin inhibits tumor growth by antitumor angiogenesis via blocking VEGFR2-mediated JAK/STAT3 signaling in endothelial cell. Int J Cancer. 2011;129:2502–2511.
26. Nam S, Scuto A, Yang F, et al. Indirubin derivatives induce apoptosis of chronic myelogenous leukemia cells involving inhibition of Stat5 signaling. Mol Oncol. 2012;6:276–283.
27. Bussel JB, Provan D, Shamsi T, et al. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. Lancet. 2009;373:641–648.
28. Kuter DJ, Bussel JB, Lyons RM, et al. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. Lancet. 2008;371:395–403.
29. Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood. 2011;117:4190–4207.
30. Guo C, Chu X, Shi Y, et al. Correction of Th1-dominant cytokine profiles by high-dose dexamethasone in patients with chronic idiopathic thrombocytopenic purpura. J Clin Immunol. 2007;27:557–562.
31. Ling Y, Cao X, Yu Z, Ruan C. Circulating dendritic cells subsets and CD4+Foxp3+ regulatory T cells in adult patients with chronic ITP before and after treatment with high-dose dexamethasome. Eur J Haematol. 2007;79:310–316.
32. Peng J, Liu C, Liu D, et al. Effects of B7-blocking agent and/or CsA on induction of platelet-specific T-cell anergy in chronic autoimmune thrombocytopenic purpura. Blood. 2003;101:2721–2726.
33. Rocha AM, Souza C, Melo FF, et al. Cytokine profile of patients with chronic immune thrombocytopenia affects platelet count recovery after *Helicobacter pylori* eradication. Br J Haematol. 2015;168:421–428.