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Potential role and mechanism for high mobility group box1 in childhood chronic immune thrombocytopenia

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Abstract. – OBJECTIVE: High mobility group box1(HMGB1) can be used as PAMP or alarmins to stimulate the innate immune system; however, previous research on immune thrombocytopenic purpura (ITP) mainly focused on its adaptive immunity. The study aimed to determine whether HMGB1 is associated with chronic ITP (cITP) during childhood and investigate its role in innate immunity in childhood cITP.

PATIENTS AND METHODS: We recruited 80 patients to measure the expression of HMGB1, IL-17, and IL-10; 55 patients were recruited to measure the expression of TLR2 and TLR4 in monocyte and CD1c+dendritic cells, and 30 volunteers were included as controls. We focused on the expression of the NLRP3 inflammasome during childhood cITP. Furthermore, the impact of HMGB1 on the NLRP3 inflammasome was explored.

RESULTS: The expressions of HMGB1 and IL-17 increased in children with cITP, while that of IL-10 decreased; HMGB1 was correlated with the expression of IL-17 and IL-10. The expression of TLR2 in CD14++CD16+, CD14+CD16++ monocytes increased significantly in comparison with the controls; the contrary was observed regarding TLR4. The expression of NLRP3, IL-1β, and IL-18 was significantly higher in CD14 and CD1c, respectively. As the concentration of HMGB1 increased, the expression of NLRP3, IL-1β, and IL-18 increased in different degrees.

CONCLUSIONS: HMGB1 could be used as an early warning alarm for childhood cITP and is involved in developing cITP. HMGB1 could affect the incidence and development of chronic childhood ITP via the NLRP3, TLR2/TLR4 pathways.

Key Words:
HMGB1,NLRP3, TLR2/TLR4,Childhood cITP.

Introduction

Immune thrombocytopenic purpura (ITP) is a common syndrome characterized by persistent clinical bleeding in children. The incidence of ITP is 2.2-5.3 in 10 million children. ITP is classified as newly diagnosed, persistent, and chronic ITP (cITP). Most children with ITP have newly diagnosed ITP, but 10%-20% develop chronic ITP. In 1951, William Harrington infused plasma from a patient with ITP into himself and rapidly suffered the same symptoms as the patient. The cause of ITP was then considered autoimmune[1,2]. However, the mechanisms underlying the development of ITP remain unclear.

High mobility group box 1 (HMGB) 1 is a member of the HMGB family. It is a DNA-binding protein that is widely distributed in nearly all eukaryotic nuclei. It has three domains: box A, box B, and an acidic C-terminus, which stabilizes the nucleosome structure, regulates gene transformation, and maintains chromatin integrity. In addition to its intracellular functions, HMGB1 has roles in the extracellular space, where it can move freely and affect autophagy and inflammatory disorders[3]. HMGB1 contributes to the pathogenesis of autoimmune diseases. HMGB1 levels are higher in the synovial fluid, serum, and synovial tissue of patients with rheumatoid arthritis (RA). In addition to its roles in RA, HMGB1 may promote the progression of primary Sjögren's syndrome, systemic lupus erythematosus (SLE), and other autoimmune diseases[4-6].

Studies have shown that the expression of HMGB1 is associated with the risk of ITP. In this study, we assessed the levels of HMGB1 in the sera of children with cITP and the expression of

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Toll-like receptor (TLR) 2, TLR4, NLRP3, IL-1β, and IL-18 in patient peripheral blood mononuclear cells (PBMCs) and dendritic cells to explore the relationship between HMGB1 and cITP pathogenesis.

Materials and Methods

Patients and Control Subjects

All of the children with cITP were diagnosed at the First Affiliated Hospital of Zhengzhou University from July 2015 to November 2017. Eighty (40 males, 39 females, 7.48 ± 2.44 years) patients were subjected to an enzyme-linked immunosorbent assay (ELISA) and 55 patients to flow cytometry analysis; 30 (18 male, 12 female, 6.75±1.12 years) age- and sex-matched control subjects were also included. cITP diagnosis was based on The American Society of Hematology 2011 evidence-based practice guideline[7]. Patients were excluded if they presented immunological diseases, circulatory diseases, malignancies, or had received transfusions. Patients were evaluated for symptoms of acute or chronic infection two weeks before hospitalization. The present investigation was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All the subjects were informed and signed an informed consent statement. The characteristics of the patients are displayed in Table I.

Enzyme-Linked Immunosorbent Assay

We collected plasma from patients and controls using ethylenediamine tetraacetic acid (EDTA). We then tested the concentrations of HMGB1, IL-17, and IL-10 with ELISA kits (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

Flow Cytometry and Cell Sorting

We purchased the following antibodies from eBioscience (San Diego, CA, USA): anti-CD14-PE, anti-CD16-PE, anti-CD1c-PE, anti-TLR2-FITC, and anti-TLR4-FITC. Red blood cells were eliminated from the blood samples with lysing buffer (BD Biosciences, San José, CA, USA). We fixed the samples in 3.8% formaldehyde and incubated them in the dark at 4°C for 30 min with the monoclonal antibodies. Subsequently, we analyzed the samples with flow cytometry. We acquired data for 20,000-50,000 gated cells per sample on a FACSArray™ instrument (BD Biosciences, San José, CA, USA). We evaluated the data using FCS Express 4 Flow Cytometry (De Novo Software, Los Angeles, CA, USA). We used the same cell preparation for cell sorting. Monocytes and dendritic cells were sorted on a FACSAria™ Cell Sorter (BD Biosciences, San José, CA, USA).

Quantitative Real-Time Polymerase Chain Reaction

We used quantitative real-time polymerase chain reaction (qPCR) to test the expression of NLRP3, IL-1β, and IL-18 by monocytes and dendritic cells. We purified RNA from sorted monocytes and dendritic cells using a GenElute™ Single Cell RNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA). We performed qPCR according to the protocols described for the PrimeScript™ RT Reagent Kit with gDNA Eraser and SYBR®

Table I. Clinical data and laboratory examinations.

	cITP	Controls
Female/Male	39/41	12/18
Age (years)	7.48 ± 2.44	6.75 ± 1.12
Age at diagnosis (years)	3.09 ± 1.14	n.a.
Pre-treatment platelets ($\times 10^9$/L)	28.93 ± 18.13	261.94 ± 53.1*
Post-treatment platelets ($\times 10^9$/L)	130.53 ± 62.93	261.94 ± 53.1*
MPV	8.74 ± 1.69	7.95 ± 0.89
Lymphocyte count ($\times 10^9$/L)	3.03 ± 1.27	4.74 ± 2.30
CRP (> 5 mg/L)	4.86 ± 1.07	4.26 ± 1.19
PCT (> 0.2 ng/mL)	0.20 ± 0.16	0.18 ± 0.09
Th cells (%)	39.83 ± 4.75	21.61 ± 2.36*
TS (%)	22.37 ± 4.72	44.00 ± 7.18*
B cells (%)	17.83 ± 5.34	15.53 ± 4.67
NK cells (%)	9.00 ± 3.20	6.87 ± 3.09
Th:Ts ratio	1.22 ± 0.55	0.56 ± 0.24*

MPV, mean platelet volume; CRP, C-reactive protein; PCT, procalcitonin.

Premix Ex Taq™ II reagent (TaKaRa, Dalian, China) using the StepOne™ Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). We used the following primers: NLRP3 forward, 5′-GCTGGCATCTGGGGAAACCT-3′; NLRP3 reverse, 5′-CTTAGGCTTCGGTCCACACA-3′; IL-1β forward, 5′-CTGAGCTCGCCAGTGAAATG-3′; IL-1β reverse, 5′-TGTCCATGGCCACAACAACT-3′; IL-18 forward, 5′-AAGATGGCTGCTGAACCAGT-3′; IL-18 reverse, 5′-GAGGCCGATTTCCTTGGTCA-3′; GAPDH forward, 5′-GCACCGTCAAGGCTGAGAAC-3′; and GAPDH reverse, 5′-TGGTGAAGACGCCAGTGGA-3′. We used the ΔΔCt method to determine the relative expression of the target genes by normalizing our data to that of GAPDH as an internal control. All experiments were repeated three times.

Cell Culture

Due to the nature of children, it was not possible to draw large volumes of blood; therefore, we collected peripheral blood (60 mL) in tubes containing heparin from six healthy adult donors. We isolated PBMCs from blood using Ficoll-Paque Premium (GE Healthcare, Chicago, IL, USA). PBMCs were cultured in complete medium, comprising Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) with L-glutamine (0.5 mM), 10% fetal calf serum (FCS; Gibco, Grand Island, NY, USA), penicillin (100 IU/mL), streptomycin (100 μg/mL), and M-CSF (20 ng/mL). Complete medium was changed three times, once every other day. Recombinant HMGB1(rHMGB1) protein (0, 50, 100, or 200 ng/mL) was added to the medium on the sixth day. After 24 h, culture supernatant was collected to measure the concentrations of IL-1β and IL-18 by ELISA (IBL, Hamburg, Germany). On the last day (day eight), we collected cells to extract whole proteins.

Western Blotting

We separated whole proteins using 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, we electrotransferred the proteins to polyvinylidene difluoride (PVDF) membranes (with a constant current of 300 mA for 2.5 h). The membranes were blotted with anti-NLRP3 (catalog number ab91525, Abcam, Cambridge, MA, USA) and anti-GAPDH, followed by incubation period with secondary antibodies. The membranes were then washed with Tris-Buffered Saline with Tween-20® buffer three times for 10 min after antibody incubation. The bands for the target proteins were exposed to an automated imaging system (Amersham Imager 600, GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Statistical Analysis

The data were analyzed using a one-way analysis of variance (ANOVA) and displayed as the mean ± standard deviation. We performed a linear regression analysis to assess correlations. Differences were considered statistically significant when $p<0.05$.

Results

Patient Characteristics

We collected clinical and laboratory results from children in the cITP and control groups (Table I). The pre-treatment (28.93 ± 18.13×10^9/L) and post-treatment (130.53 ± 62.93×10^9/L) platelet counts of cITP group were significantly lower than those of the controls (261.94 ± 53.1×10^9/L); however, post-treatment platelet counts of cITP group did reach the normal range. The T helper (Th) cell, B cell, and NK cell counts were higher in patients with cITP than in controls. The percentage of Th cells (39.83% ± 4.75%) in the blood of patients with cITP was significantly higher than that in the controls (21.61% ± 2.36%), whereas the percentage of regulatory T cells (Treg) (22.37% ± 4.72%) was significantly lower than in the controls (44.00% ± 7.18%). The ratio of Th:Ts (1.22 ± 0.55) in patients with cITP was significantly lower than in the controls (0.56 ± 0.24). There were no differences in sex, age, mean platelet volume, number of lymphocytes, C-reactive protein concentration, or concentration of procalcitonin between the groups (Table I).

Expression of HMGB1 in the Serum of Patients With cITP and Controls

To investigate if HMGB1 was involved in the pathogenesis of cITP, we first examined the levels of HMGB1, IL-17, and IL-10 by ELISA in plasma from patients with cITP and controls. The expression of HMGB1 in patients with cITP (35.44 ± 0.93 ng/mL) was almost seven times higher than what was observed in controls (5.37 ± 0.44 ng/mL; $p<0.001$; Figure 1). In addition, the levels of IL-17 in the plasma of patients with cITP (20.43 ± 0.50 pg/mL) were significantly higher than what

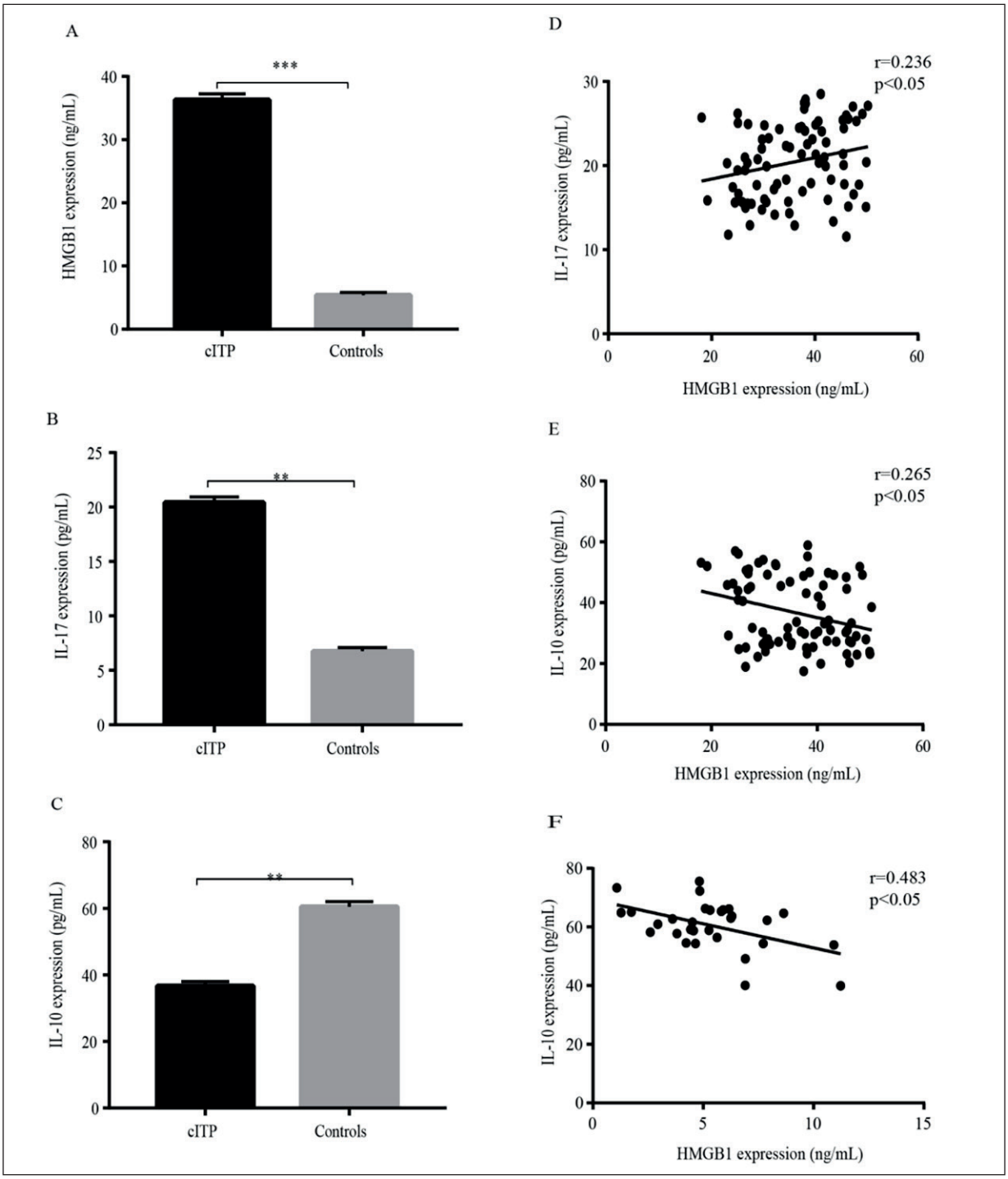

Figure 1. Expression of HMGB1(**A**), IL-17 (**B**), and IL-10 (**C**) in the plasma of patients with cITP and healthy controls. **D**, Expression of HMGB1 positively correlated with the expression of IL-17 in the plasma of patients with cITP. **E**, Expression HMGB1 is negatively correlated with the expression of IL-10 in the plasma of patients with cITP. **F,** Expression of HMGB1 is negatively correlated with the expression of IL-10 in the plasma of controls.

was observed in the controls (6.76 ± 0.34 pg/mL; $p<0.05$; Figure 2). The expression of IL-10 in the plasma of cITP patients (35.73 ± 1.29 pg/mL) was significantly lower than what was observed in controls (60.52 ± 1.48 pg/mL; $p<0.05$) (Figure 1A-C).

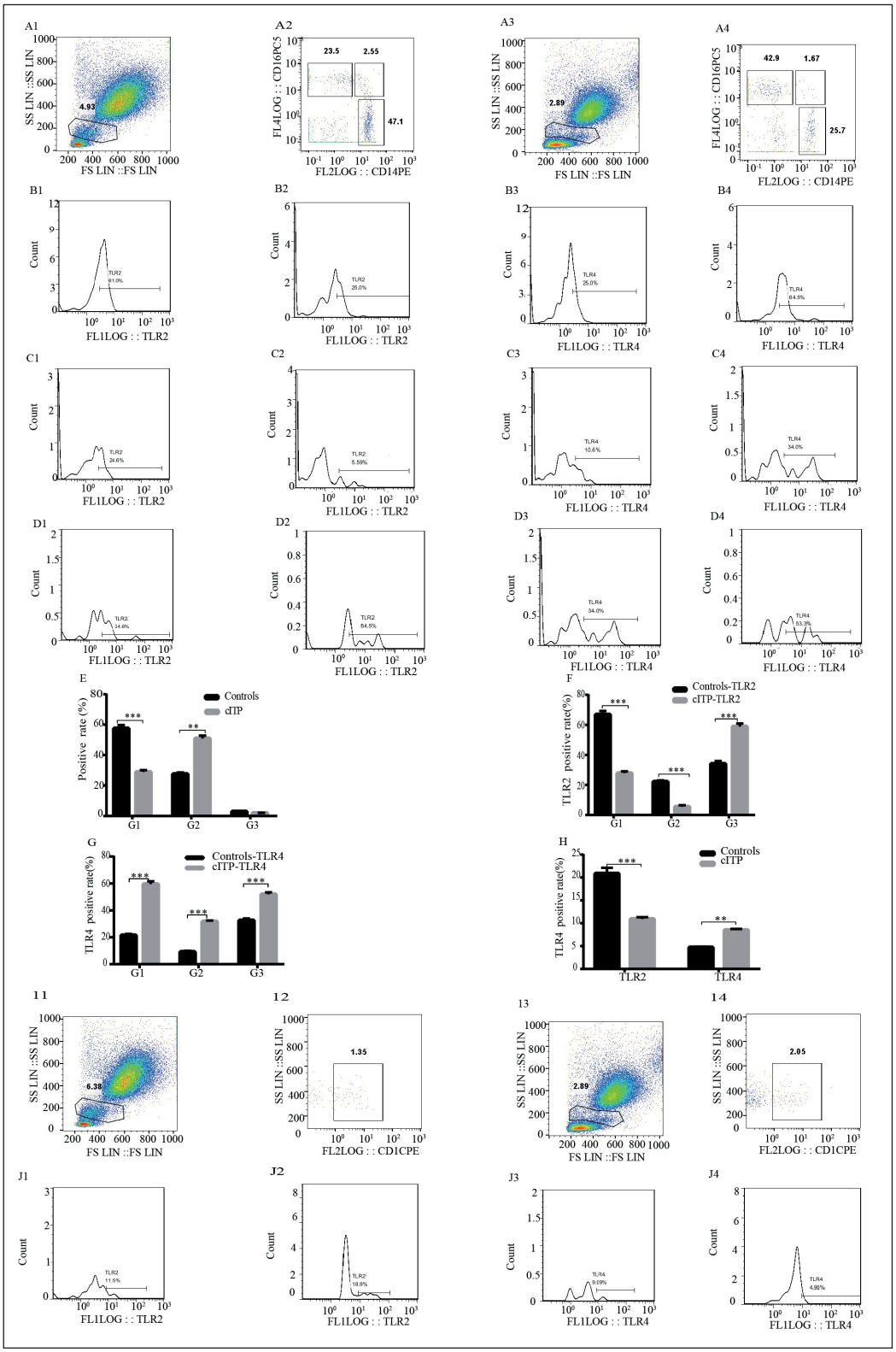

Figure 2. Expression of CD14++CD16−, CD14+CD16++, and CD14++CD16+ monocytes in controls and patients with cITP (**A1-A4, E**); the expression of TLR2 and TLR4 in CD14++CD16−(G1) monocytes (**B1-B4**), CD14+CD16++(G2) monocytes (**C1-C4**), and CD14++CD16+(G3) monocytes (**D1-D4**) of controls and patients with cITP; expression of TLR2 (**F**) and TLR4 (**G**) in monocyte subgroups in patients with cITP and controls; **I1-I4**, the expression of CD1c+ in dendritic cells in patients with cITP and controls; expression of TLR2 and TLR4 in CD1c+ dendritic cells of patients with cITP and controls (**J, H**).

Correlations Between the Expression of HMGB1, IL-17, and IL-10 in Patients with cITP and Controls

We analyzed the correlations between the expression of HMGB1 and IL-17 or IL-10 in the two groups. We found that the expression of HMGB1 was positively correlated with the expression of IL-17 ($r = 0.236$; $p<0.05$) and negatively correlated with the expression of IL-10 ($r = -0.265$; $p<0.05$) in the plasma of patients with cITP. In the plasma obtained from the control subjects, HMGB1 was negatively correlated with IL-10 ($r = -0.483$; $p<0.05$); furthermore, there was no statistical correlation between HMGB1 and IL-17 in the controls (data not shown) (Figure 1D-E).

Surface Expression of TLR2 and TLR4 on $CD14^{++}CD16^{D}$, $CD14^{+}CD16^{++}$, $CD14^{++}CD16^{+}$ Monocytes and CD1c+ Dendritic Cells

We analyzed the expression of TLR2 and TLR4 in $CD14^{++}CD16^{-}$ (G1), $CD14^{+}CD16^{++}$ (G2), and $CD14^{++}CD16^{+}$ (G3) monocytes from patients with cITP and controls (Figure 2A). The expression of $CD14^{++}CD16^{-}$ monocytes in patients with cITP (28.9% ± 1.26%) was significantly lower than that the controls (57.59 ± 3.26% $p<005$). The proportion of $CD14^{+}CD16^{++}$ monocytes among the patients with cITP (50.98% ± 1.84%) was higher than that among controls (27.55% ± 1.07% $p<0.05$). There were no differences between the two groups (2.78 ± 0.1%, 1.98±0.08%) regarding $CD14^{++}CD16^{+}$ (Figure 2A1-2A4).

The expression of TLR2 in $CD14^{++}CD16^{-}$and $CD14^{+}CD16^{++}$monocytes was significantly lower among patients with cITP (27.93% ± 1.12%; 5.5% ± 1.03%) as compared to the controls (66.87% ± 2.37%; 22.14% ± 0.99%). The expression of TLR2 in $CD14^{++}CD16^{+}$ monocytes (58.93% ± 1.94%) was significantly higher among patients with cITP as compared to the controls (34.10% ± 1.83%; $p<0.05$). However, the expression of TLR4 in monocytes among the subgroups(G1,G2,G3) was significantly higher amongst patients with cITP (G1: 59.52% ± 2.04%, G2: 31.79% ± 0.72%, G3: 51.92±1.45%) than amongst the controls (G1: 21.43% ± 1.01%, G2: 9.13% ± 0.84%, G3: 32.6% ± 1.37%; $p<0.05$) (Figure 2B1-G). There were no significant differences regarding the proportion of $CD1c^{+}$ dendritic cells between the two groups. However, we observed a lower proportion of TLR2-expressing $CD1c^{+}$ dendritic cells (cITP: 10.92% ± 0.41%, control: 20.86% ± 1.27%; $p<0.05$) and a higher proportion of TLR4-expressing $CD1c^{+}$ dendritic cells (cITP: 8.51% ± 0.23% and controls: 4.72% ± 0.15%; $p<0.05$) amongst the patients with cITP than amongst the controls (Figure 2H-J4).

Expression of NLRP3, IL-1β, and IL-18 in $CD14^{+}$ Monocytes and $CD1c^{+}$ Dendritic Cells

After sorting $CD14^{+}$ monocytes and $CD1c^{+}$ dendritic cells from patients with cITP and controls, we assessed the mRNA expression of NLRP3, IL-1β, and IL-18. Compared to controls, the group with cITP expressed significantly higher levels of NLRP3 (5.86 ± 0.44), IL-1β (2.67 ± 0.47), and IL-18 (2.77 ± 0.29) in $CD14^{+}$ monocytes (for all comparisons, $p<0.05$). The expression of NLRP3 (4.34 ± 0.17), IL-1β (3.20 ± 0.17), and IL-18 (2.37 ± 0.17) was significantly higher in cITP group in CD1c+ dendritic cells than in the controls (for all comparisons, $p<0.05$) (Figure 3 A1-C).

Expression of NLRP3, IL-1β, and IL-18 in Human Peripheral Blood Macrophages

Macrophages induced by M-CSF and positive cells were 80.1%. As the concentration of rHMGB1 increased, the expression of NLPR3 also gradually increased. The expression of NLPR3 was statistically significant among the groups regarding the administration of 0 ng/mL, 100 ng/mL, and 200 ng/mL ($p<0.05$). In comparison with the 50 ng/mL and 100 ng/mL groups, the relative protein expression of NLPR3 increased significantly ($p<0.05$). We found no significant differences amongst the other groups.

The expression of IL-1β in the 50 ng/mL, 100 ng/mL, 200 ng/mL groups increased significantly in comparison to 0 ng/mL group ($p<0.05$). 50 ng/mL group was significantly different from 100 ng/mL and 200 ng/mL groups respectively ($p<0.05$).

Similarly, as the concentration of rHMGB1 increased, the expression of IL-18 also increased. Unlike the 0 ng/mL group, the difference between 100 ng/ml and 200 ng/mL groups was significant ($p<0.05$). The expression of IL-18 in the 200 ng/mL group was significantly higher than that in 50 ng/mL group ($p<0.05$). There were no significant differences among other groups (Figure 3D-H).

Discussion

Pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) are recognized by innate immune cells through pattern recognition recep-

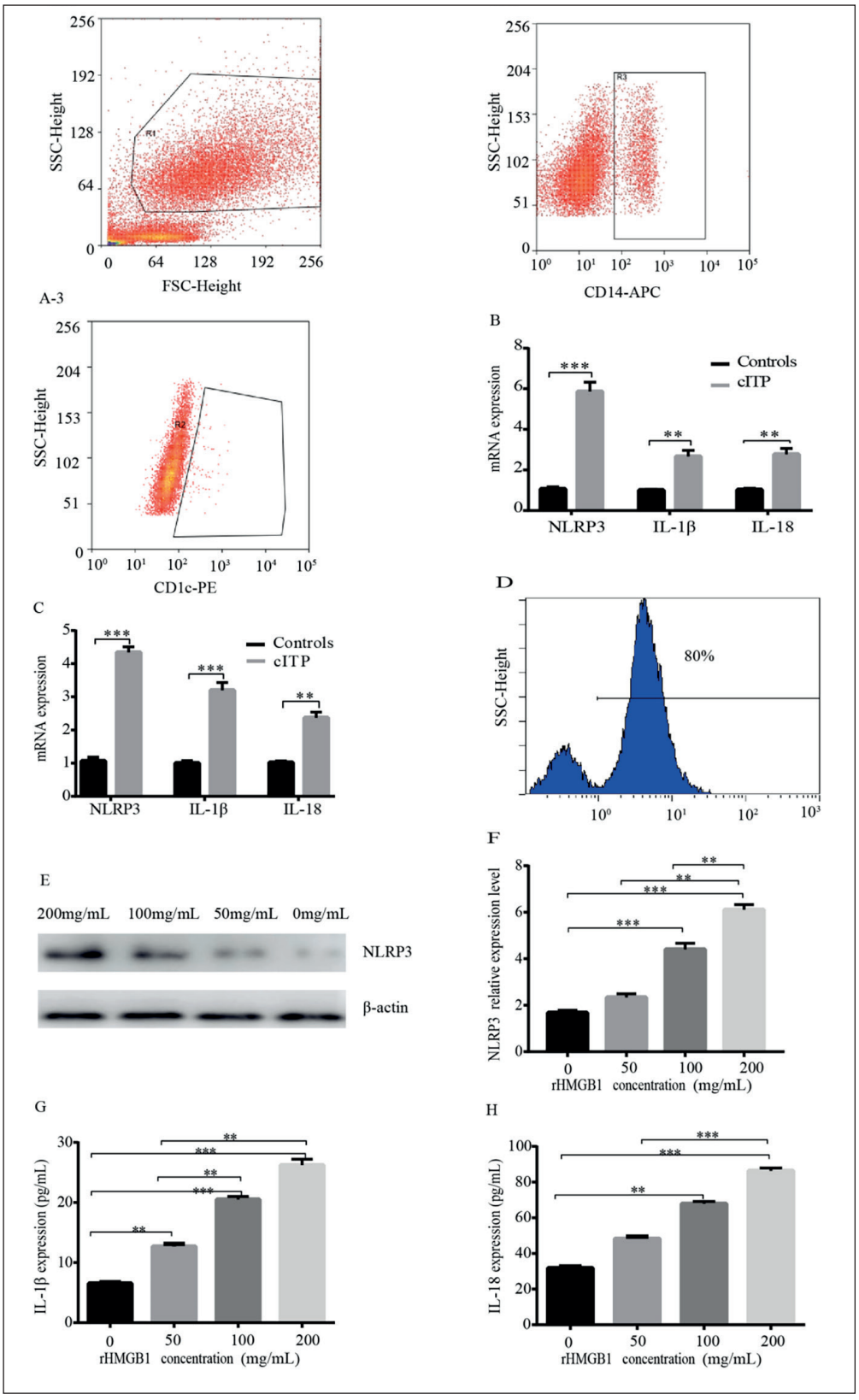

Figure 3. Sorting CD14+ monocytes and CD1c+ dendritic cells (**A1-3**), Expression of NLRP3, IL-1β, and IL-18 in CD14+ monocytes (**B**) and CD1c+ dendritic cells (**C**) of patients with cITP and controls. **D**, Induced macrophages and flow identification. **E-H**, Expression of NLRP3, IL-1β, and IL-18 stimulated by different rHMGB1 concentrations.

tors, through which they activate innate immune responses. Adaptive immune responses can be activated either directly or indirectly. HMGB1, an important PAMP and DAMP, is also known as an alarmin[8]. At least three receptors mediate the pro-inflammatory and immune activator reactions to HMGB1: RAGE, TLR2, and TLR4. The TLR family has been widely studied and it is considered a bridge that links innate and adaptive immunity. NLRP3 is a member of the NLR family. NLRP3 plays an important role in innate immunity. In the past, it was thought that NLRP3 could identify "non-self" antigens after a cell had been invaded by a pathogen, leading it to form a complex with ASC and caspase-1 to promote the maturation and release of IL-1β and IL-18, thus driving the development of acute inflammatory reactions[9]. However, Wang et al[10] have demonstrated that NLRP3 can identify "danger signals" from non-pathogenic agents, leading to target cell damage, chronic or acute inflammatory responses, and autoimmune reactions, such as those in diabetic nephropathy.

cITP is a chronic autoimmune disease. The levels of NLRP3 and ASC mRNA in peripheral blood monocytes and of IL-18 in the plasma of patients with acute ITP (without infections or diseases) become elevated over normal levels but decrease after treatment. The pathogenesis of acute ITP is suspected to be related to NLRP3-mediated inflammation[11]. However, no previous reports investigated the link between cITP in children and NLRP3.

Human monocytes are classified into three subgroups based on the expression of CD14 and CD16. The classic $CD14^{++}CD16^{-}$ subgroup, which can be quickly recruited to the inflammation site, is involved in phagocytosis, inflammatory cell regulation, and antigen presentation[12]. The second subgroup, non-classical $CD14^{-}CD16^{++}$ monocytes, can secrete TNF-α and IL-1β upon TLR stimulation. $CD14^{+}CD16^{+}$ subgroup is an intermediate between the other subgroups. All monocytes are believed to play inflammatory roles[13]. In patients with RA, fewer $CD14^{++}CD16^{-}$ cells were detected than in healthy controls and patients with SLE, whereas there were no statistical differences between the numbers in healthy controls and patients with SLE[14]. During earlier studies involving ITP, monocytes were divided into $CD14^{hi}CD16^{-}$ and $CD16^{+}$ groups. The number of $CD16^{+}$ cells was elevated in ITP and correlated with platelet counts $<50\times10^{9}/L$[15].

The expression of TLRs differs by disease. The expression of TLR2 in all three groups of monocytes in RA patients was higher than what was observed in controls[16]. Iwahashi et al[17] detected that in RA the expressions of TLR2 and TLR4 in $CD16^{+}$ monocytes were higher than in controls. We observed fewer $CD14^{++}CD16^{-}$ than $CD14^{-}CD16^{++}$ cells in patients with cITP; CD16 is related to inflammation and cytokine production. In addition, the proportion of $CD14^{++}CD16^{-}$ cells of controls was lower than what has been reported in PBMCs of adults[18]. To determine whether this discrepancy relates to the differences between adults and children requires further investigation. We also found that high expression of TLR4 may be associated with cITP.

There are at least two types of dendritic cells among PMBCs: monocyte-derived dendritic cells (mDCs) and plasmacytoid dendritic cells[19]. mDCs can be further divided into $CD1c^{+}$ and $CD141^{+}$ subsets. Each mDC subset has a characteristic TLR expression pattern that contributes to its functional specificity. The relative expression of TLR2 and TLR4 in $CD1c^{+}$ mDCs is higher than that in $CD141^{+}$ mDCs[20]. Activation of TLRs promotes the maturation of dendritic cells and the activation of macrophages, thus driving adaptive immune response[21]. The relative expression of TLR4 in mDCs from ulcerative colitis and patients with Crohn's disease is higher than what is observed in controls, but the relative expression of TLR2 in Crohn's disease mDCs does not differ from that in healthy mDCs[22]. We found no differences in the proportions of $CD1c^{+}$ mDCs in patients with cITP and controls; however, the expression of TLR2 was lower while the expression of TLR4 was higher in cells from patients with cITP.

The levels of PBMC microRNA-33 and NLRP3 inflammasome activity are higher in RA patients than in controls[23]. The expression of NLRP3 and NLRC4 in the PBMCs of RA patients is significantly higher than that in controls; in addition, NLRP3 gene polymorphisms are associated with the progression and severity of RA[24]. U1-small nuclear glycoprotein stimulates the activation of NLRP3 inflammasome in $CD14^{+}$ monocytes and induces IL-1β; knocking out NLRP3 inflammatory inhibits the expression of IL-1β[25].

The expression of NLRP3 in autoimmune diseases differs. An NLRP3 gene polymorphism is associated with type 1 diabetes and abdominal diseases in children in Brazil[26], suggesting that NLRP3 mutations may lead to autoimmune diseases. Yang et al[27] have shown that the expression

of NLRP3, NLRP1, caspase-1, and IL-lβ mRNA in the PBMCs of patients with SLE was lower than that found in controls; furthermore, the activity of SLE negatively correlates with their expression[27]. The level of NLRP3, caspase-1, IL-18, and IL-1β in the skin of patients with Sjogren's Syndrome (SS)[28] was higher than that in the skin of controls; furthermore, NLRP3 correlates positively with the expression of ET-1, which promotes fibrosis. These effects may be related to the recognition and binding of TLRs by NLRP3 and the activation of NLRP3 inflammatory inflammasome, which induces the maturation and release of IL-1β, IL-18, and various inflammatory reactions[29]. Due to the particularity of children, we were unable to meet the amount of blood required for the experiment of cell culture. Thus, we collected adult blood for further investigations. We found that increasing the expression of HMGB1 could stimulate the expression of NLRP3, IL-18, and IL-1β, especially at high levels of rHMGB1, where we observed significant differences between experimental and control groups. HMGB1 is an important early warning factor in innate immunity. NLRP3 is activated by various receptors (such as TLRs) and undergoes a series of changes, caspase-1 cleaves precursors pro-IL-1β and pro-IL-18 resulting in active IL-1β and IL-18, which participate in the regulation of various immune and inflammatory reactions[30]. In addition to directly mediating inflammation, they can also induce inflammatory cascades and promote the release of downstream inflammatory factors. In human monocyte cell lines, NLRP3 mRNA is overexpressed in unstimulated macrophages from female patients with SLE[31]. HMGB1 stimulates monocytes and macrophages *via* RAGE, TLR2, and TLR4, leading to the induction of inflammatory cytokines, including TNF-α and IL-1β[32,33]. Human lymphocytes and monocyte-derived macrophages can also activate NLRP3 inflammatory bodies[34]. In our study, different concentrations of recombinant HMGB1 and ATP were used to stimulate human primary macrophages. We found that HMGB1 activated NLRP3 inflammatory bodies, leading to the release of IL-1β and IL-18.

Briefly, in our study the expression of HMGB1 increased in the cITP of children. The expression rate of TLR2 in CD14++CD16+and CD14+CD16++ monocytes increased significantly compared to the controls. Also, we observed that the expression of NLRP3, IL-1β, and IL-18 was significantly higher in CD14 and CD1c, respectively.

Conclusions

We have done animal researches and found that blocking HMGB1 in cITP animal models can significantly improve symptoms and change the expression of related factors. For future studies, our studies must include more samples to further explore the innate immune response.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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