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The prevalence of regulatory T and dendritic cells is altered in peripheral blood of women with pre-eclampsia

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ABSTRACT

Objectives: Regulatory T cells (Tregs) and dendritic cell (DC) subsets play an essential role in the development of pregnancy immune tolerance. We aimed to investigate the proportion of $CD4^+CD25^+FOXP3^+$ Tregs and DC subsets in peripheral blood of patients with pre-eclampsia compared to normal pregnant women.

Study design: Peripheral blood samples were collected from 25 women with pre-eclampsia and 30 women with normal, healthy pregnancies. The ratio of $CD4^+CD25^+$ T cells in all $CD4^+$ T cells, expression of FOXP3, and the ratio of myeloid DC (mDC) versus plasmacytoid DC (pDC) were assessed by flow cytometry. Levels of IL-10 and TGF-β in culture supernatants of purified $CD4^+CD25^+$ T cells were analyzed by enzyme-linked immunosorbent assay.

Results: The mean ratio of $CD4^+CD25^+$ T cells in $CD4^+$ T lymphocytes in peripheral blood was lower in pre-eclampsia women than in normal pregnancies. The expression of Tregs marker FOXP3 in the pre-eclampsia group was significantly lower than the control group. The levels of IL-10 and TGF-β in supernatants of $CD4^+CD25^+$ T cells were significantly decreased in the pre-eclampsia group compared with the control group. The ratio of mDC/pDC was significantly higher in the pre-eclampsia group when compared with the control group.

Conclusions: A decreased proportion and secretion of related inhibitory cytokines of $CD4^+CD25^+FOXP3^+$ Tregs was found in the peripheral blood of pre-eclampsia, while the ratio of mDC/pDC increased. It is speculated that Tregs and DCs may play a role in the decreased immunosuppressive function of pre-eclampsia patients.

1. Introduction

Pre-eclampsia is a major cause of fetal and maternal morbidity and mortality worldwide [1]. The main clinical symptoms of this complication include hypertension and proteinuria. Potential etiological factors for this disorder include poor placentation, immune response imbalance, excessive maternal inflammation and endothelial dysfunction [2].

As a semi-homograft, the maintenance and development of an embryo depend on the establishment of maternal immune tolerance. Regulatory T cells (Tregs) and dendritic cells (DCs) have an essential role in regulating maternal immune balance during pregnancy by inducing both immune suppression and antigen-specific immune response [3,4]. In a hypoxic environment, the release of damage-associated molecular patterns (DAMPs) can activate innate cells (the macrophages, dendritic cells, natural killer cells) [5,6]. The activation of these immune cells initiate an immune response, lead to pregnancy immune imbalance, and pregnancy pathology [2]. Pre-eclampsia (PE) is closely associated with the imbalance of the immune system.

Tregs are a subset of $CD4^+$ T cells characterized by a suppressive effect on the immune response and identified by flow cytometry due to the expression of transcription factor (FOXP3), which is required for their development. Tregs exert their immune modulation through a variety of different mechanisms, including the induction of lymphocyte apoptosis, inhibition of $CD4^+$ and $CD8^+$ T cell function, and down-regulation of the expression of CD80/CD86 on DCs [7]. Most studies have previously reported that Tregs is decreased in both peripheral blood and decidua in women with pre-eclampsia, compared with that of normal pregnancies [8,9]. However, some researchers have demonstrated different results [10]. Studies on the function of Tregs in pre-eclampsia are rarely reported.

DCs are specialized antigen-presenting cells capable of initiating

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and regulating immune responses. Studies have found DCs are related to immune tolerance and play an important role in pathological pregnancy [11]. Two distinct lineages of DCs have been described in humans, depending on their source and function. Myeloid dendritic cells (mDC) express markers primarily as $CD11c^{hi}$, $HLA\text{-}DR^{hi}$, $BDCA3^+$ or $BDCA1^+$ $CD1a^-$. Plasmacytoid dendritic cells (pDC) have been described in human peripheral blood and lymphoid tissue and characterized with $HLA\text{-}DR^+$, $CD123^{hi}$, $BDCA2^+$, $BDCA4^+$, $CD1a^-$, and $CD11c^-$ [12–15]. Both mature DC subsets highly express MHC-II antigens and co-stimulatory molecules, CD80, and CD86. Given mature mDC powerful antigen-presenting function, could activate initial T lymphocytes and induce an adaptive immune response. In contrast, pDC is relatively weak in phagocytosis and antigen processing [16]. pDC could induce immune tolerance via the production of inhibiting factor IL-10 in $CD4^+$T cells [12]. In studies, the immune tolerance state of the body can be assessed by monitoring the total number as well as the proportion of DC subsets.

Researches on the changes of immune tolerance in pre-eclampsia would be valuable for further illustrating the pathogenesis of the disease and searching for new treatment ideas. In this study, we aim to explore the changes of immune tolerance by investigating the prevalence of peripheral Tregs and the ratio of DC subsets in pre-eclampsia. Furthermore, $CD4^+$ $CD25^+$ T cells were separated for detecting the expression of FOXP3 and inhibitory cytokines.

2. Materials and methods

2.1. Patients and blood sampling preparation

The patients participating in this patient control study were recruited in the Department of Obstetrics and Gynaecology of Beijing Chao-Yang Hospital, Capital Medical University (Beijing, China), between August 1, 2016, and April 30, 2017. Twenty-five preeclamptic patients and 30 healthy pregnant women as a control group were included in the study. Pre-eclampsia is defined according to the 2013 guidelines set out by the American College of Obstetricians and Gynecologists (ACOG). ACOG's definition characterizes pre-eclampsia as having a blood pressure of at least 140/90 mmHg on two occasions at least 4 h apart after 20 weeks of gestation in a female with previously normal blood pressure and proteinuria equal to or greater than 300 mg per 24-hour urine collection, or a dipstick reading of 1+, or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following: platelet count less than 100,000/microliter, elevated serum creatinine and liver transaminases, pulmonary edema or cerebral or visual symptoms [17]. Exclusion criteria for all participants included chronic hypertension, renal disease, diabetes mellitus, hematological diseases, acute or chronic infection, and smokers. Patients who were in active labor or experienced preterm rupture of membranes were also excluded from the study. The gestational age at sampling varied from 27 to 40 weeks. Preterm healthy pregnancies were recruited from outpatient women, while healthy full-term women were recruited from inpatient women who intend to deliver. The local Ethics Committee approved the study design. Informed consent was obtained from patients for peripheral blood sampling. Patients with pre-eclampsia and full-term healthy pregnancies underwent an induction to give birth vaginally or were delivered via Caesarean section, according to their conditions, within 48 h after the peripheral blood sampling. The healthy preterm pregnancies had blood sampling at pregnancy ages matched with the study group and delivered all at term.

2.2. Isolation of peripheral blood mononuclear cells

Blood (18–20 ml) was taken by venipuncture from each participant and collected in sterile heparinized tubes. Following dilution in a ratio of 1:1 with phosphate-buffered saline (PBS), the blood was slowly added to the upper layer of human lymphocyte separation solution (Ficoll – Paque; Haoyang biological products co. LTD, Tianjin, China) along the tube wall in a ratio of 2:1. Peripheral blood mononuclear cells (PBMCs) were separated by a standard density gradient centrifugation (20 min, 800*g*, 22 °C). The suspended cells in the middle layer were collected, washed in 5 ml PBS and centrifugated (400*g*, 5 min). The cell suspension was washed twice in PBS and was then suspended in PBS. The number of peripheral mononuclear cells was counted under the microscope, and the cells concentration was calculated. The diluted cell suspension was stained with 0.4% trypan blue solution (Sigma, USA) 10 μl, and the number of live cells was counted. Cell viability was required to be more than 95% by trypan blue exclusion assay.

Table 1
Characteristics of normal pregnant women and pre-eclampsia patients.[a]

Characteristic	Normal pregnant women (n = 30)	Pre-eclampsia patients (n = 25)	*P* value[b]
Age (y)	30.0 ± 2.9	32.0 ± 4.6	0.152
BMI	27.0 ± 2.1	28.2 ± 2.9	0.140
Primiparas (%)	26(86.7)	19(76.0)	0.009
Proteinuria dipstick reading	NONE	(+) 5(20.0) ≥(++) 20(80.0)	–
Corticosteroids use[c]	NONE	6(24.0)	–
Duration of pregnancy (wk)			
At sampling	37 (35–38)	37 (35–38)	0.791
At delivery	39 (38–39)	37 (35–38)	< 0.001
Neonatal delivery weight (g)	3523 ± 338	2715 ± 1015	< 0.001
Blood pressure (mmHg)			
Systolic	121 ± 8	159 ± 13	< 0.001
Diastolic	70 ± 10	94 ± 13	< 0.001

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

[a] Values are given as mean ± SD or median (interquartile ranges), unless indicated otherwise.

[b] The Student's *t*-test was used to assess all characteristics, other than pregnancy duration at sampling and at delivery (non-parametric Mann–Whitney *U* test); $P < 0.05$ was considered statistically significant.

[c] In less than 34 week pregnancies, corticosteroids were prescribed to promote fetal lung maturation in pre-eclampsia patients.

2.3. Isolation of $CD4^+CD25^+$ T cells, purity verification, and cell culture

Several 2×10^6 peripheral blood mononuclear cells were used for analyzing the proportion of $CD4^+CD25^+$T cells and DC subsets utilizing a FACScalibur flow cytometer (BD Biosciences, USA).

Most cells were prepared for $CD4^+CD25^+$T cells isolation. The cells were isolated using anti-CD4/CD25 MicroBeads and a MiniMACS separator (Miltenyi Biotec GmbH, Germany) according to the manufacturer's instructions. The purity of isolated $CD4^+CD25^+$ T cells was verified by flow cytometric analysis with anti-CD4 and anti-CD25 staining. $CD4^+CD25^+$ T cells were resuspended in the desired volume of RPMI 1640 FCS (10%) medium (5×10^6 cells/ml), then cultured at 37 °C in 5% CO_2 in humidified air overnight for recovery.

2.4. Phenotyping of Tregs and DCs

Peripheral blood mononuclear cells (5×10^5) were stained for 20 min with FITC-conjugated CD4 and APC-conjugated CD25 antibodies (BD Biosciences PharMingen, San Diego, USA). FITC and APC Mouse IgG1, κ (BD Biosciences PharMingen, San Diego, USA) were used as matched control isotype antibodies. The population of lymphocytes was gated from PBMCs according to forward and side scatter characteristics. Regions were placed to identify first $CD4^+$ T cells and then $CD4^+CD25^+$ population. $CD4^+CD25^+$ T cells were counted.

Peripheral blood mononuclear cells (5×10^5) were stained with FITC-conjugated Lineage cocktail (CD3/CD14/CD16/CD19/CD20/

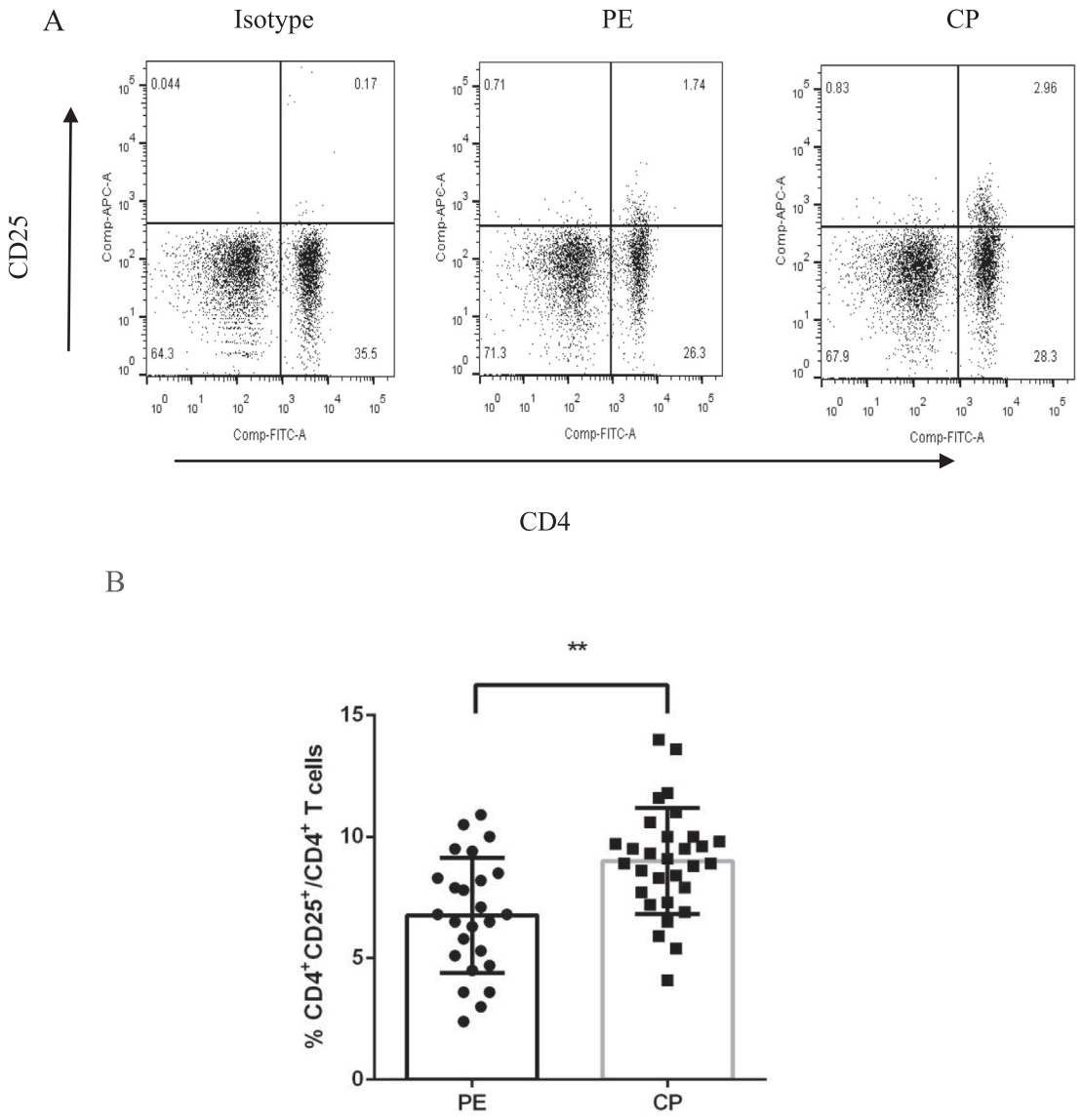

Fig. 1. Decreased presence of $CD4^+CD25^+$ T cells in the peripheral blood of patients with pre-eclampsia. Peripheral blood from patients with pre-eclampsia (PE) and control pregnant woman (CP) were used to isolate peripheral blood mononuclear cells (PBMCs) and then stained with anti-CD4 (FITC-conjugated) and anti-CD25 (APC-conjugated) antibody. The IgG isotype served as controls. Lymphocytes were gated from PBMCs according to forward scatter (FSC) and side scatter (SSC) characteristics. $CD4^+CD25^+$ T cells were gated from $CD4^+$ T cells and the proportion of $CD4^+CD25^+$ T cells in $CD4^+$ T cells were analyzed. (A) The representative flow cytometric dot plots of $CD4^+CD25^+$ T cells in $CD4^+$ T cells from patients with pre-eclampsia and control pregnant woman. (B) Comparisons of percentages of $CD4^+CD25^+$ T cells in $CD4^+$ T cells from above two groups. Data is presented as mean ± SD. **: Statistically significant difference when compared with controls, $P < 0.01$.

CD56) (Exbio, USA), APC-conjugated CD11c, PE- Cy7-conjugated CD123 and PerCP-Cy5.5-conjugated HLA-DR Abs (BD Biosciences PharMingen, USA). Lineage cocktail staining referred to as Lin^+ cells were excluded from PBMCs. Then, the Lin^- mononuclear cells fraction was analyzed with flow cytometry (FCM) for $HLA\text{-}DR^+CD123^+$ and $HLA\text{-}DR^+CD11c^+$ antigens. Lin^- $HLA\text{-}DR^+CD11c^+CD123^-$ cells were counted as circulating myeloid DCs (mDC). Lin^- $HLA\text{-}DR^+$ $CD123^+$ $CD11c^-$ cells were counted as circulating plasmacytoid DCs (pDC). The identification of circulating DCs by flow cytometry in peripheral blood is presented in Fig. 4. Samples were washed, fixed with 1% paraformaldehyde, and analyzed by flow cytometry.

The isolated $CD4^+CD25^+$ T cells after culture overnight were collected for detection of intranuclear FOXP3. $CD4^+CD25^+$ T cells were reacted with 1 ml freshly prepared fixation/permeabilization working solution (BD Biosciences PharMingen, USA) for 40 min at 4 °C. After washing the cells with 1× permeabilization buffer, cells were stained with PE-conjugated FOXP3 antibody (BD Biosciences PharMingen, USA) for 50 min at 4 °C in the dark. After washing twice, cells were analyzed by FCM. Isotype-matched PE-conjugated mouse IgG1 antibody was used as a control (BD Biosciences PharMingen, USA).

All samples were analyzed on a BD FACS Aria flow cytometer (BD Biosciences, USA).

2.5. Detection of cytokines (L-10 and TGF-β) with ELISA method

After culturing the isolated $CD4^+CD25^+$ T cells overnight, the supernatants were collected from each well and stored at −80 °C until detection assays were performed. The concentration of human IL-10 and TGF-β in the incubation medium was analyzed with enzyme-linked immunosorbent assay (ELISA) kits (Expandbio, Beijing, China), strictly following the manufacturer's protocols. All samples were run through the protocol twice.

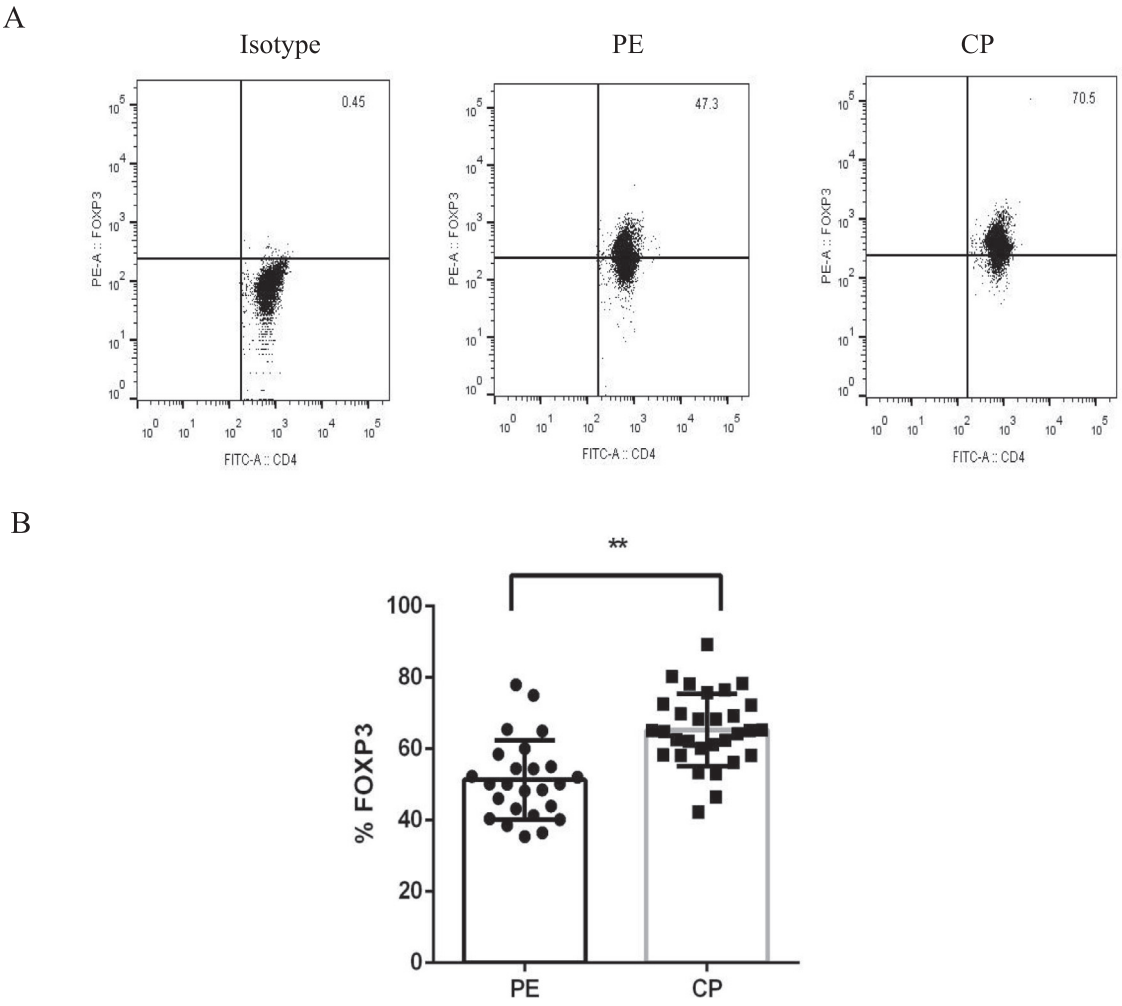

Fig. 2. Decreased expression of FOXP3 in $CD4^+CD25^+$ T cells among patients with pre-eclampsia. $CD4^+CD25^+$ T cells were isolated using anti-CD4/CD25 MicroBeads from peripheral blood of pre-eclampsia patients (PE) and control pregnancy women (CP) and were stained with PE-conjugated FOXP3 antibody. The IgG isotype served as controls. The expression of FOXP3 in the purified $CD4^+CD25^+$ T cells were analyzed. (A) The representative flow cytometric dot plots of FOXP3 in the purified $CD4^+CD25^+$ T cells from pre-eclampsia group and normal pregnancy women. (B) Comparisons of percentages of $FOXP3^+$ cells in the purified $CD4^+CD25^+$ T cells from above group. Data is presented as mean ± SD. Statistically significant difference when compared with controls, **: $P < 0.01$.

2.6. Statistical analysis

The data was analyzed using SPSS version 17.0 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test was used to verify the normal distribution of data. Continuous variables with normal distribution were presented as mean ± SD; these values were compared using a Students *t*-test. Variables with non-normal distribution were presented as the median (interquartile range); differences between such variables were calculated using the non-parametric Mann–Whitney *U* test. Statistical significance was defined as a two-sided $P < 0.05$.

3. Results

The present study included 25 patients in the pre-eclampsia group and 30 normal pregnant women in the control group. We recruited both late-onset pre-eclampsia (n = 19) and early onset pre-eclampsia (n = 6). The characteristics of the participants are summarized in Table 1. The gestational age at sampling varied from 27 to 40 weeks. Duration of pregnancy age at delivery and neonatal delivery weight were both lower in the pre-eclampsia group than in the control group ($P < 0.001$).

3.1. The ratio of $CD4^+CD25^+$ T cells in $CD4^+$ T lymphocytes

The ratio of $CD4^+CD25^+$ T cells in $CD4^+$ T lymphocytes in the peripheral blood was lower in pre-eclampsia women than in normal pregnancy respectively [(6.76 ± 3.37)% versus (9.01 ± 2.18)%, $P < 0.001$.] (Fig. 1A, B).

3.2. Expression of FOXP3 in pre-eclampsia

The viability of $CD4^+CD25^+$T cells was (96.28 ± 4.87) % as determined directly after the purification process by means of trypan blue exclusion. The purity of $CD4^+CD25^+$T cells was (94.15 ± 5.96)%, as assessed by flow cytometry.

The positive expression ratio of Tregs FOXP3 in the pre-eclampsia group (51.31 ± 11.10) % was significantly lower than in the normal gestation group (65.27 ± 10.27) % ($P < 0.001$), after which, $CD4^+CD25^+$ T cells were incubated for 24 h. (Fig. 2A, B, C)

3.3. Expression of IL-10 and TGF-β in the supernatants

IL-10 levels in supernatants of $CD4^+CD25^+$ T cells in pre-eclampsia group [(338.34 ± 40.01) ng/L] were lower compared with the control group [(382.06 ± 33.68) ng/L] ($P < 0.001$) (Fig. 3A). TGF-β levels in

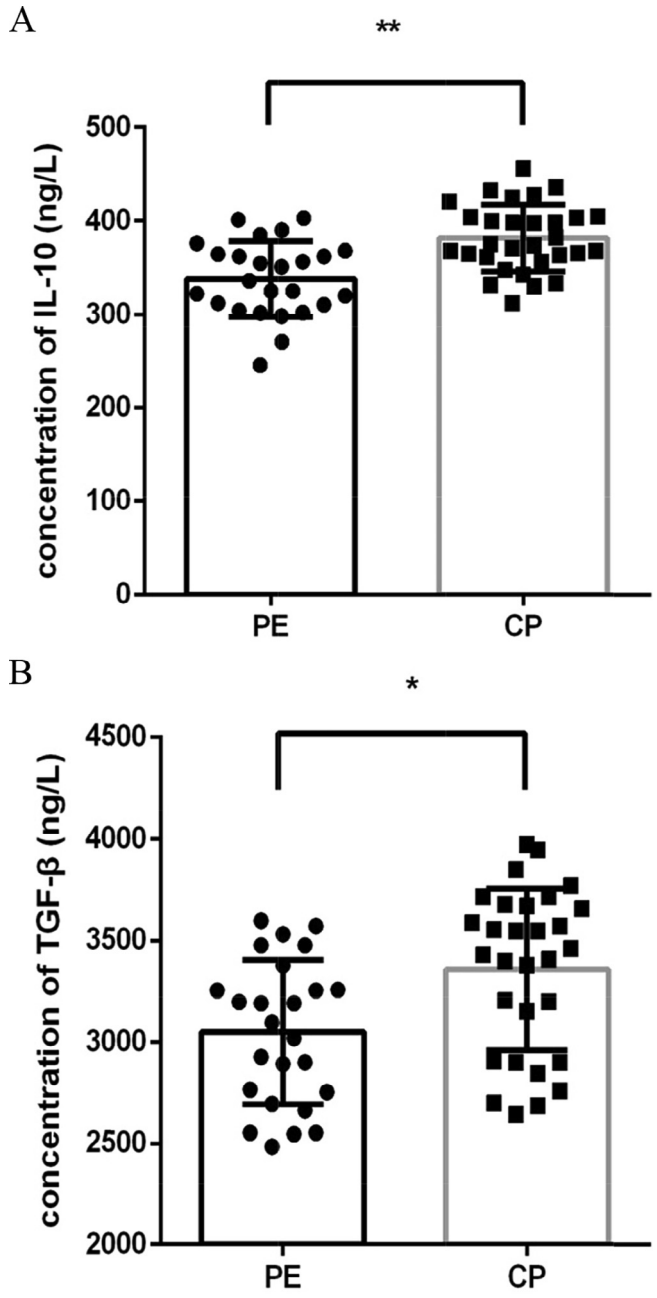

Fig. 3. Concentrations of IL-10 and TGF-β decreased in $CD4^+CD25^+$T cells in pre-eclampsia. After incubation for 24 h, the concentration of IL-10 (Fig A) and TGF-β (Fig B) in supernatants of $CD4^+CD25^+$ T cells from pre-eclampsia patients (PE) and control pregnant women (CP) were analyzed with ELISA kits. Results were shown as the mean ± SD. Statistically significant difference when compared with controls, *: $P < 0.05$, **: $P < 0.01$.

supernatants of $CD4^+CD25^+$ T cells in the pre-eclampsia group were also lower compared to the control group [(3049.41 ± 355.67) ng/L versus (3358.10 ± 399.98) ng/L] ($P = 0.017$). (Fig. 3B)

3.4. The ratio of DC subsets in PBMCs

The ratio of myeloid and lymphoid dendritic cells (mDC/pDC) in pre-eclampsia and control groups are presented in Fig. 4. It was observed that the ratio was significantly higher in the pre-eclampsia group (1.86 ± 0.76) when compared with the control group (0.78 ± 0.41). The difference was statistically significant ($P < 0.001$).

4. Discussion

The findings of the present study were threefold. First, the ratio of $CD4^+CD25^+$ T cells out of the total number of $CD4^+$ T lymphocytes in the peripheral blood was lower in pre-eclampsia women. Second, the expression of FOXP3, IL-10 and TGF-β were significantly lower in purified $CD4^+CD25^+$ T cells among women with pre-eclampsia, than in the control. Third, the ratio of myeloid DCs to plasmacytoid DCs (mDC/pDC) in the peripheral blood was significantly higher in pre-eclampsia when compared with normal pregnancy.

Regulatory T cells are a subset of mature T cells with immunomodulatory function. Tregs play an essential role in inducing priority of Th2 cells development in Thl/Th2 balance and maintaining normal pregnancy. Several studies have found that the decrease of Tregs is associated with obstetric complications such as pre-eclampsia, fetal growth restriction, and premature birth [8,18–20]. In recent years, several studies of Tregs on hypertensive disorders complicating pregnancy were performed [21,22]. Toldi et al. [21] found the proportion of $CD4^+CD25^+$ $FOXP3^+$ T lymphocytes in peripheral blood to be higher in the normal gestation group than in either pre-eclampsia or non-gestation groups. In an investigation made in two different countries (Japan and Poland), Sasaki et al. [8] found the proportion of $CD4^+CD25^{bright}$ Tregs in the peripheral blood of pre-eclampsia was significantly lower than that of normal pregnancy group. At the same time, the results of flow cytometry analysis of Tregs in placental tissue were consistent with those from peripheral blood [8].

It has been confirmed that some soluble factors and membrane binding factors play a vital role in the development, survival, and activity of Tregs [23,24]. FOXP3 is traditionally considered a characteristic marker of Tregs which is expressed explicitly in $CD4^+CD25^+$ T cells [25]. TGF-β and IL-10 serve as the main soluble inhibitory factors in mediating the function of immune suppression. In this study, the ratio of $CD4^+CD25^+$ T cells out of the total of $CD4^+$ T cells in the peripheral blood of pre-eclampsia patients was measured. Meanwhile, $CD4^+CD25^+$ T cells were purified for culture in vitro using magnetic bead separation, the expression of FOXP3, TGF-β, and IL-10 was then assessed. The results indicated the ratio of $CD4^+CD25^+$ $FOXP3^+$ Tregs out of the total $CD4^+$ T cells in the peripheral blood of preeclamptic women was significantly lower than the values found in the normal pregnancy group. The result was consistent with previous studies. We found the levels of TGF-β and IL-10 in a culture supernatant of $CD4^+CD25^+$ T cells in the preeclampsia group were lower than in the normal gestation group. This has been rarely reported. As some of the essential cytokines for immunomodulatory function, the decrease of TGF-β and IL-10 levels may lead to the decline of Tregs Immunosuppressive ability. The results of this study suggest that the ratio of total $CD4^+$ T cells of Tregs and the relative inhibitory cytokines decreased in pre-eclampsia in contrast with the finding from the normal pregnancy group. This lower ratio may lead to an imbalance of immune regulation in pre-eclampsia.

Current theories suggest pre-eclampsia is related to shallow implantation of the placenta, which leads, via a chain of events, to vascular endothelial injury and organ dysfunction [26]. Several studies demonstrate an imbalance of local immune regulation may cause insufficient invasion of trophoblast cells, which may lead to shallow implantation of the placenta [27]. In addition to regulatory T cells, dendritic cells also play a vital role in immunomodulation. The research of dendritic cells during pregnancy mainly focuses on the maternal-fetal interface. However, the little amount of DCs in decidua makes it challenging to perform functional studies.

Investigations in DCs mainly focused on flow cytometry and immunohistochemistry analysis. Due to the small amount and lack of independent specific markers in DCs, it is difficult to separate cells and perform further studies. At present, both of Lineage (CD3/CD14/CD16/CD19/CD20/CD56) negative and HLA-DR positive cells are regarded as DCs in many published papers [28]. Several studies have found an

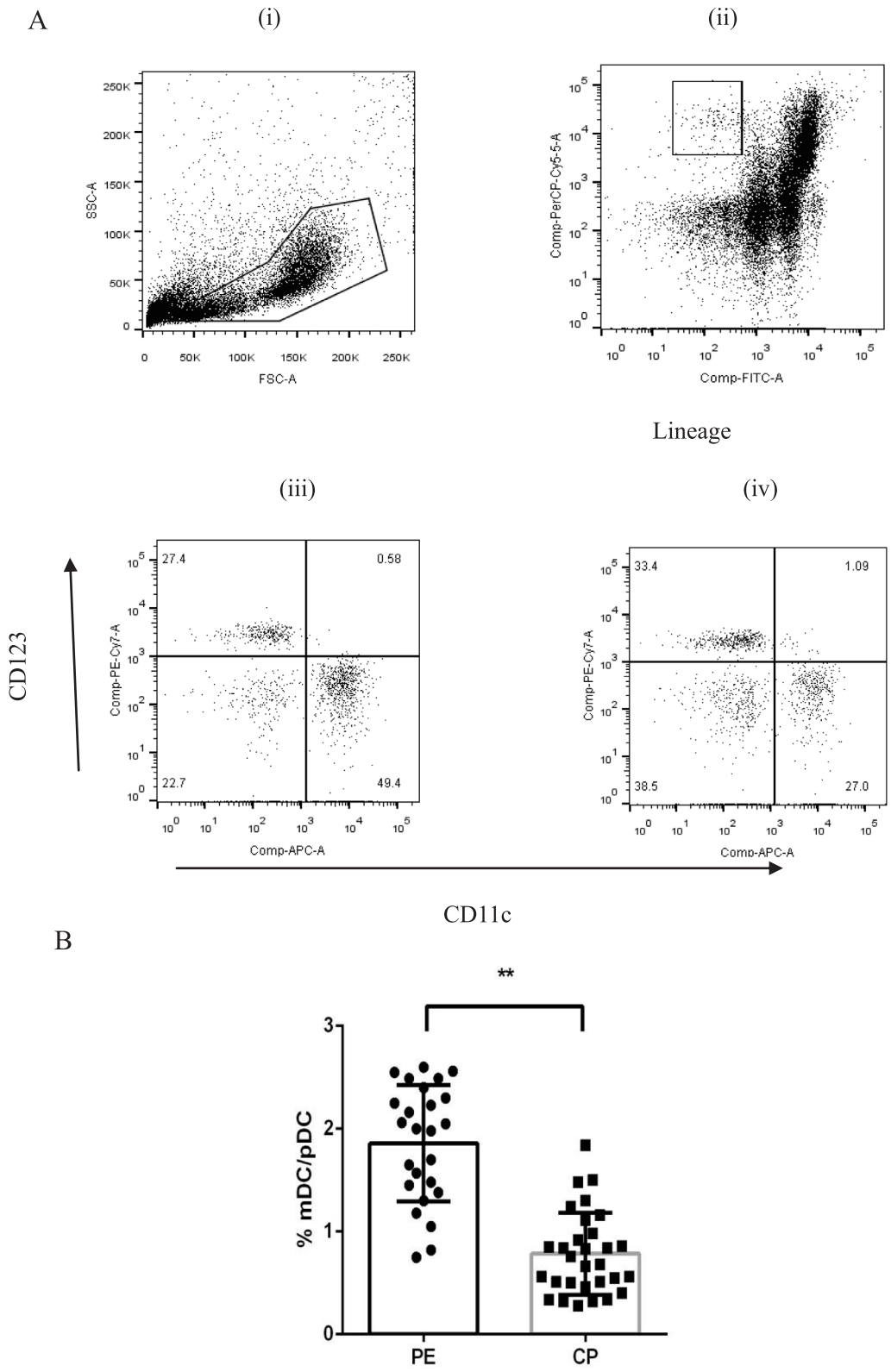

Fig. 4. The identification of circulating DCs by flow cytometry in PBMCs of patients with pre-eclampsia. Peripheral blood from patients with pre-eclampsia (PE) and control pregnant women (CP) were used to isolate PBMCs and then stained with anti-Lin (FITC-conjugated), anti-HLA-DR (PerpCP-Cy5.5-conjugated), anti-CD11c (APC-conjugated) and anti-CD123 (PE-Cy7-conjugated) antibody. (A) According to the order (i)-(ii)-(iii) or (i)-(ii)-(iv) in the figure, the target gate was selected to obtain the ratio of mDC/pDC in PBMCs. (i) PBMCs were gated according to forward scatter (FSC) and side scatter (SSC) characteristics. (ii) $Lin^-HLA\text{-}DR^+$ cells were gated from PBMCs, these events were then analyzed for ratio of $CD11c^+CD123^-$ (mDC) and $CD123^+CD11c^-$ (pDC) cells. The identification of DC subsets in patients with pre-eclampsia (iii) and control pregnancy group (iv). (B) Comparisons of ratio of mDC/pDC cells from above two groups. Results were shown as the mean ± SD. **: $P < 0.01$.

increase in CD83$^+$ and DC-sign$^+$ APCs in the deciduae of pre-eclamptic women. DC-sign$^+$ APCs are probably a differentiated subset of HLA-DR$^+$CD11c$^+$ traditional mDCs [29,30]. Darmochwal-Kolarz et al. [31] evaluated the populations of peripheral blood myeloid and lymphoid dendritic cells in 15 normal pregnant women and 25 patients with pre-eclampsia. It was found that the prevalence of pDCs (BDCA-2$^+$ and CD123$^+$) in pre-eclampsia women decreased, and the ratio of mDCs (CD19$^-$, CD1c$^+$) to pDCs was significantly higher than the control group. In this study, mononuclear cells in peripheral blood were stained with lineage (CD3/CD14/CD16/CD19/CD20/CD56) and HLA-DR antibodies and estimated by the flow cytometry method. The results indicated that the ratio of lineage$^-$ HLA-DR$^+$ CD11c$^+$ CD123$^-$ cells (mDC) and lineage$^-$ HLA-DR $^+$CD123$^+$CD11c$^-$ cells (pDC) in the peripheral blood of pre-eclampsia was significantly higher than that in normal pregnancy. Our result was consistent with those of Darmochwal-Kolarz et al. [31], although the label markers in DC subsets were different. Previous studies suggested that DCs may participate in immune regulation by secreting inhibiting factors and interacting with Tregs [32]. The increase in the ratio of mDC/pDC suggests that the ability of DCs to induce immune tolerance is reduced in pre-eclampsia. The specific mechanism of involvement of DCs in the pathogenesis of pre-eclampsia is still unknown. Further research is necessary to explore the role of DCs in pre-eclampsia.

Tregs and DCs could interact [33] with each other and maintain the balance of immune regulation of the body. DCs could not only induce T cell immunity by antigen uptake and presentation but also mediate immune tolerance by acting on Tregs [34]. In vitro studies, it was shown that Tregs could inhibit the antigen presentation of DCs derived from macrophages and monocytes. Tregs could decrease the expression of DCs costimulatory molecules and inhibit the maturation of mDC mediated by toll-like receptors (TLR) [35]. DCs and Tregs play immunomodulatory effects by different functional subsets which were characterized by different membrane molecules and various secreted cytokines. The conditions of Tregs or DC subsets in pre-eclampsia have been observed separately in various studies. In this study, both Tregs and DC subsets were analyzed. It was found that the prevalence of Tregs and the level of inhibiting cytokines were decreased, while the mDC/pDC ratio was increased in maternal peripheral blood of pre-eclampsia when compared with the normal pregnancy findings. Therefore, it is speculated that Tregs and DC subsets may be closely related to each other in the complex immune network of the body and contribute to the maintenance of immune regulation and homeostasis during pregnancy. Abnormalities in the ratio or function of the Tregs and DC may lead to an imbalance in immune regulation and contribute to the pathogenesis of pre-eclampsia. Exploring the interaction between DCs and Tregs may lead to a better understanding of pre-eclampsia.

In our previous study, we observed that DAMPs, such as HMGB1 and calprotectin, were increased in the serum of women with pre-eclampsia [36]. In vitro experiments have found that HMGB1 can inhibit the function of Tregs in mice [37]. Therefore, it is speculated that excessive inflammatory response may lead to immune disorder in pre-eclampsia. This will provide the evidence required to further research into pre-eclampsia and to enhance treatment modalities.

A limitation of the present study was the study design, which prevented the analysis of Tregs and DCs for the duration of pregnancy. Future studies should, therefore, ascertain the prevalence of Tregs and DCs throughout pregnancy in a large population determine their role in pre-eclampsia. The assertation of the prevalence of both Tregs and DCs will contribute to the prediction and treatment of pre-eclampsia.

5. Conclusions

The findings of the present study suggest Tregs and DC subsets may play a role in the pathogenesis of pre-eclampsia. Additional research is still necessary to elucidate the roles of both Tregs and DCs in the pathogenesis of poor placental implantation. New ways need to be found to improve immune regulation and correct the immune homeostasis of the maternal body. The findings of such studies will contribute to the generation of novel treatments for pre-eclampsia.

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Conflicts of interest

The authors have no conflicts of interest.

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