Expression of regulatory T and helper T cells in peripheral blood of patients with pregnancy-induced hypertension

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Summary

Objective: To analyze the expression of regulatory T cells and helper T cells in peripheral blood of patients with pregnancy-induced hypertension (PIH). *Materials and Methods:* Twenty-seven patients hospitalized with PIH were consecutively collected for detection of regulatory T cells (CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+Treg) and helper T (CD+3, CD+4, CD+8, CD+4/CD+8) cells in peripheral blood. Meanwhile, 20 normal hospitalized pregnant women served as the control group. *Results:* In the comparison of regulatory T cells, the level of serum CD4+ CD25+ Treg and CD4+ CD25+ Foxp3+Treg in PIH group was significantly lower than control group (all $p < 0.05$). In the comparison of help T cells, the expression level of serum CD+4/CD+8 in PIH group was obviously higher than control group, while the expression level of CD+8 was significantly lower than control group (all $p < 0.05$). *Conclusions:* There were obvious abnormal expressions of regulatory T cell and helper T cells in peripheral blood of patients with PIH.

Key words: Pregnancy-induced hypertension; Regulatory T cells/peripheral blood; Helper T cells/peripheral blood.

Introduction

Pregnancy-induced hypertension (PIH) is a disease occurring in late pregnancy. In severe cases, there may be fetal growth retardation, maternal placental abruption, premature birth, and postpartum hemorrhage, which is one of the main causes leading to the death of pregnant women and perinatals in China. Immune factors have a close relation to the onset of PIH. In recent years, according to the study involving maternal immune process in gestation, regulatory T cells were gradually recognized as the regulator of Th1 and Th2 cells [1-4]. CD_4^+ CD_{25}^+ Treg is a unique subtype of CD_4^+T [5], of which the main function is to inhibit the autoreactive T cells from immune response, the activation of conventional T cells, and to promote the secretion of inhibitory cytokine, as well as to preserve the homeostasis of the body and induce tolerance to grafts. CD_4^+ CD_{25}^+ Foxp3$^+$Treg are the Foxp3 transcription-factor of X chromosome which is necessary for the development, growth, and function of CD_4^+ CD_{25}^+ Treg. Expression of CD_4^+ CD_{25}^+ Treg and CD_4^+ CD_{25}^+ Foxp3$^+$Treg in the maternal peripheral blood during various stages of pregnancy plays an inhibitory effect on maternal immunological rejection to a semi-allogeneic fetus during the dominant control of fetal-maternal immune. Under normal conditions, the absolute number of CD_4^+ CD_{25}^+Treg and CD_4^+ CD_{25}^+ Foxp3$^+$Treg in peripheral blood during pregnancy increases and dynamically changes. The preservation of normal pregnancy depends on the stability of the immune balance, which once has been broken, pathological pregnancy will occur. According to recent findings [6, 7], the onset of PIH was closely-related to the imbalance of maternal immune, although there are few researches or reports addressing regulatory T cells in peripheral blood of PIH patients. In this research, the expression of regulatory T cells and helper T cells in the peripheral blood was studied to determine the possible immune mechanism in PIH.

Materials and Methods

Twenty-seven patients with a systolic blood pressure ≥ 140 mm Hg and a diastolic blood pressure ≥ 90 mm Hg or urine protein from - to ++++ after 20 weeks gestation in the present obstetrics department from January 2009 to December 2009 were consecutively selected for PIH. This study was conducted in accordance with the declaration of Helsinki and approved from the Ethics Committee of the Fourth Affiliated Hospital of China Medical University. Written informed consent was obtained from all participants. Exclusion criteria included: patients recently suffering from acute and/or chronic infectious diseases, patients with autoimmune diseases, patients with reproductive tract infections which was confirmed by TORCH, chlamydia and mycoplasma examination, and patients suffering from liver, kidney, and systemic blood diseases. Twenty healthy pregnant women hospitalized simultaneously were selected as the control group. Descriptive statistics about patients and control groups were summarized in Table 1 with similar mean age and mean gestational age (all $p > 0.05$).

Fasting cubital venous blood was obtained, centrifuged at 2,500 r/min for ten min to separate the serum and stored at -70°C. Type FC-500-MPL of flow cytometry was utilized to detect regulatory Tin peripheral blood (CD_4^+ CD_{25}^+Treg and CD_4^+ CD_{25}^+ Foxp3$^+$Treg). Indirect immunofluorescence was used to determine the level of regulatory T in peripheral blood (CD^+_3, CD^+_4, CD^+_8, and CD^+_4/CD^+_8).

SPSS 10.0 was adopted for data analysis. Data were expressed as mean ± SD. *T*-test was used for comparison between groups. A $p < 0.05$ was considered statistically significant.

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Table 1. — *General information of the two groups.*

Group	Age in years	Mean age in years	Gestational age in weeks	Mean gestational age in weeks
PIH group (n = 27)	25 - 39	29.12 ± 5.39	36 - 40	37.91 ± 3.66
Control group (n = 20)	24 - 37	28.64 ± 4.72	35 - 39	38.23 ± 3.25

Table 2. — *The comparison of the expression levels of peripheral blood CD^+3, CD^+4, CD^+8, and CD^+4/CD^+8 between the two groups.*

Group	CD^+3	CD^+4	CD^+8	CD^+4/CD^+8
PIH group (n = 27)	66.28 ± 9.34	35.62 ± 4.53	21.80 ± 3.25[a]	1.78 ± 0.25[b]
Control group (n = 20)	67.79 ± 10.55	36.17 ± 4.88	27.72 ± 3.64	1.39 ± 0.17

[a]$p < 0.05$, [b]$p < 0.01$ (compared with control group).

Table 3. — *The comparison of the expression levels of peripheral blood $CD_4^+ CD_{25}^+$ Treg, and $CD_4^+ CD_{25}^+$ Foxp3$^+$Treg between the two groups.*

Group	$CD_4^+CD_{25}^+$ Treg	$CD_4^+CD_{25}^+$ Foxp3$^+$Treg
PIH group (n = 27)	9.06 ± 2.56[b]	2.27 ± 0.85[b]
Control group (n = 20)	14.82 ± 3.35	3.98 ± 1.26

[a]$p < 0.05$, [b]$p < 0.01$ (compared with control group).

Results

Expression levels of peripheral blood

The comparison of the expression levels of peripheral blood CD^+3, CD^+4, CD^+8, and CD^+4/CD^+8 between two groups: the results suggested that the expression level of peripheral blood CD^+4/CD^+8 in PIH group was higher than control group, while the expression level of CD^+8 was lower than control group (all $p < 0.05$) (Table 2).

Expression levels of peripheral blood

The comparison of the expression levels of peripheral blood $CD_4^+CD_{25}^+$ Treg, and $CD_4^+CD_{25}^+$ Foxp3$^+$Treg between two groups: the expression levels of peripheral blood $CD_4^+CD_{25}^+$ Treg and $CD_4^+CD_{25}^+$ Foxp3+Treg were significantly lower than control group (all $p < 0.01$) (Table 3).

Discussion

Immune factors have a close relation to the onset of PIH. According to the recent findings [8-10], regulatory T cells played an important role in the balance between the regulation of human peripheral immune tolerance and response to the immunological stress caused by infection. It is well known that pregnancy induces enhancement of immunosuppression to ensure the stable growth of the fetus. Numerous Foxp3 related factors played an important role as immunosuppression factor [11-13]. According to other studies, $CD_4^+CD_{25}^+$ Treg played an important role in pregnancy maintenance [14, 15]. It was also confirmed that its expression was enhanced during normal pregnancy implying the immunosuppressive effect of T cells for the preservation of pregnancy [16-18]. Therefore, it was proposed that the onset of PIH was closely related to the disruption of maternal immune balance during pregnancy. The results in this study showed that the expression level of serum $CD_4^+CD_{25}^+$ Treg, $CD_4^+CD_{25}^+$ Foxp3$^+$Treg and CD^+8 was significantly decreased while the expression level of CD^+4/CD^+8 significantly increased in PIH group in line with other reports [6, 9]. Treg cells suppress the response of immune system to its own and foreign antigens mainly through the "active" way, but the amount and functional changes of $CD_4^+CD_{25}^+$ Treg in patients with PIH still remain unknown. Previous studies [19, 20] reported that the number of $CD_4^+CD_{25}^+$ Treg cells in peripheral blood of PIH patients was significantly decreased when compared with normal pregnancy or normal non-pregnant women, suggesting that the decreased expression of Foxp3 in PIH women was probably related to the reduction of $CD_4^+CD_{25}^+$ Treg cells' number. After further analysis, it was found that T lymphocytes cells of these patients that were activated, followed the lack of regulatory cells, especially reducing Treg cells leading to maternal immune rejection towards the fetus. It is believed that the significant decrease of Treg cells in PIH patients affecting the immunomodulatory in the third trimester, prompted a shift in the Thl/Th2 balance from Th2 to Thl and disrupted maternal-fetal immune tolerance, resulting in decreased immunosuppressive protection from embryonic antigen and embryonic susceptibility to immune attack. Therefore, a series of pathophysiological changes occurred including the onset and progression of PIH.

In conclusion, Treg cells, which are important immunoregulatory cells, have the effect of inducing maternal immune tolerance and preserving internal environment stability. There are significantly lower expressions and absolute amounts of $CD_4^+CD_{25}^+$ Treg in peripheral blood in pregnant women, which might be one of the causes of PIH. It is believed that producing more $CD_4^+CD_{25}^+$ Treg cells via different ways and the balance between regulatory T cells and effector T cells may become a new option for the treatment of PIH.

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