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The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia

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ABSTRACT

The aim of this study was to estimate the prevalence of $CD3^+CD4^+$ T lymphocytes producing IL-17, IL-2, IFN-γ, and IL-4, plus $CD4^+CD25^+FoxP3^+$ T regulatory (Treg) cells, in peripheral blood of patients with preeclampsia and healthy women in the third trimester of normal pregnancy. Another purpose was to assess the immunosuppressive activity of Treg cells from patients with preeclampsia compared with controls. Thirty-four preeclampsia patients and 27 healthy pregnant women were included. The percentages of $CD4^+CD25^+FoxP3^+$ Treg cells and $CD3^+CD4^+$ T lymphocytes with intracellular expressions of cytokines were estimated using monoclonal antibodies and flow cytometry. In vitro functional assays were performed using a Treg Cell Isolation Kit and ^{3}H-thymidine incorporation assays. The percentage of $CD3^+CD4^+$ T lymphocytes producing IL-17A was significantly higher in preeclampsia than in healthy, normotensive pregnant women in the third trimester ($p < 0.001$). The population of $CD4^+CD25^+FoxP3^+$ Treg cells was significantly lower in the study group compared with controls ($p < 0.05$). There was no change in the stimulation index of $CD3^+CD4^+CD25^-$ T lymphocytes from preeclampsia patients without Treg cells and after addition of autologous Treg cells. In normal pregnancy, the stimulation index of $CD3^+CD4^+CD25^-$ T lymphocytes was significantly higher without Treg cells compared with the response after addition of autologous Treg cells ($p < 0.05$). The results suggest up-regulation of the Th17 immune response in preeclampsia. The decreased number and function of Treg cells may be responsible for activating the inflammatory response characteristic of this disorder. In preeclampsia, the predominance of Th17 immunity could act through modulating the Th1/Th2 immune balance.

1. Introduction

Preeclampsia (PE) is a common obstetric syndrome affecting about 5–10% of pregnant women. The symptoms of this syndrome appear during the second and third trimesters of pregnancy. Preeclampsia is a multisystem disorder of human pregnancy and represents the leading cause of both fetal and maternal morbidity and mortality.

The etiology and pathogenesis of this syndrome are not fully understood. Many studies describe alterations in the innate and adaptive immune system that may have an influence on the onset of this disorder. Also, activation of cell-mediated immunity may play a role in the etiology of preeclampsia. In particular, inappropriate activation of the immune system may be associated with the development of this syndrome. Preeclampsia is characterized as a state of the excessive maternal inflammatory response with a predominance of the production of Th1 cytokines, such as

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IL-2, IL-6, IL-8, IFN-γ, TNF-α, as well as IL-12 (Saito et al., 1999; Darmochwal-Kolarz et al., 2002; Saito and Sakai, 2003; Sakai et al., 2004; Tosun et al., 2010; Redman and Sargent, 2007).

Regulatory T lymphocytes $CD4^+CD25^{bright}$ (Treg cells) are known to play an important role in the development and maintenance of tolerance in peripheral tissues (Baecher-Allan et al., 2001; Kuniyasu et al., 2000), as well as playing a role in the induction of transplantation tolerance. They express a high level of CD25 (IL-2Rα), cytotoxic T lymphocyte antigen 4 (CTLA-4), and the transcription factor Foxp3 (Sakaguchi, 2001; Fontenot et al., 2003; Hori et al., 2003; Waldmann et al., 2004).

Recent studies have found that the populations of peripheral blood Treg cells are reduced in preeclampsia (Sasaki et al., 2007; Darmochwal-Kolarz et al., 2007; Santner-Nanan et al., 2009; Toldi et al., 2008; Steinborn et al., 2008). On the other hand, others report that the numbers of peripheral blood Treg cells are similar in preeclampsia and normal pregnancy. These latter studies, however, employed small sample numbers or the authors estimated $CD4^+CD25^+$ T cells and did not evaluate $CD4^+CD25^{high}$ Treg cells (Paeschke et al., 2005; Hu et al., 2008).

Th17 lymphocytes are a recently discovered subset of T $CD4^+$ lymphocytes that produce IL-17. Their discovery has led some to hypothesize a predominance profile of Th1/Th17 over Th2/Treg in chronic inflammatory conditions. Up-regulation of Th17 immunity may contribute to the development and progression of autoimmune diseases, chronic inflammatory diseases or reactions of the graft rejection, and may also induce allergic disorders. Similarly, a deficiency of Th17 cells may lead to recurrent viral, bacterial or fungal infections (Langrish et al., 2005; Schnyder-Candrian et al., 2006; Bettelli et al., 2007; Annunziato et al., 2008; Curtis and Way, 2009; Benghiat et al., 2009; Ivanov et al., 2009).

The aim of the study was to estimate the prevalence of T $CD3^+CD4^+$ lymphocytes producing IL-17, IL-2, IFN-γ, and IL-4, as well as the prevalence of $CD4^+CD25^+FoxP3^+$ T regulatory cells, in peripheral blood of patients with preeclampsia and healthy pregnant women in the third trimester of normal pregnancy. Furthermore, we sought to assess the immunosuppressive activity of T regulatory cells of patients with preeclampsia compared with those of healthy third-trimester pregnant women.

2. Materials and methods

2.1. Patients

The patients participating in this study were admitted to the Department of Obstetrics and Perinatology, Medical University of Lublin, Poland. The study group consists of 34 pregnant women with preeclampsia. Preeclampsia was diagnosed according to accepted criteria by the Committee on Terminology of *American College of Obstetricians and Gynecologists*, and all women with preeclampsia had a blood pressure reading of at least 140/90 mmHg and proteinuria above 0.3 g/24 h. None of patients with preeclampsia had preexisting clinical disorders. The exclusion criteria were chronic hypertension, renal diseases, or autoimmune diseases such as diabetes, lupus or rheumatoid arthritis. Furthermore, none of the pregnancies were or had been complicated by preterm labor or chorioamnionitis. The control group included 27 healthy normotensive pregnant women in the third trimester of normal pregnancy matched for gestational age. All pregnancies from the study and control groups were singleton gestations, and none of the study or control patients had symptoms of labor at the time of enrollment. The women from the control group were followed through the rest of their pregnancy to ensure that they did not subsequently develop preeclampsia. The characteristics of the study and control groups are presented in Table 1. The study was approved by the local Ethics Committee. Informed consent from the patients for peripheral blood sampling was obtained.

2.2. Blood sampling and cell preparation

Venous blood samples were collected from the study patients and controls by venipuncture using sterile, lithium heparin-treated tubes (S-Monovette, SARSTEDT, Aktiengesellschaft & Co., D-51588 Nubrecht, Germany). All samples were obtained before the preeclamptic patients received treatments such as steroids or antihypertensive drugs.

2.3. Isolation of peripheral blood cells and the detection of Th17 and Treg cells

Peripheral blood mononuclear cells (PBMCs) were aseptically separated by a standard density gradient centrifugation (Gradisol L, Aqua Medica, Poland).

Table 1
The clinical characteristics of patients with pre-eclampsia (study group) and healthy normotensive women in the third trimester of an uncomplicated pregnancy (control group).

	Mean ± SD		Significance (p)
	Study group $n = 34$	Control group $n = 27$	
Maternal age	28.68 ± 4.76	27.68 ± 5.31	NS
Gravidity	1.84 ± 1.12	1.93 ± 0.85	NS
Parity	1.63 ± 0.95	1.81 ± 0.81	NS
Time of blood collection (weeks of gestation)	34.05 ± 2.14	34.62 ± 1.63	NS
Systolic pressure (mmHg)	155.35 ± 15.85	110.25 ± 20.15	<0.01
Diastolic pressure (mmHg)	96.47 ± 5.66	73.56 ± 7.18	<0.05
Proteinuria (g/24 h)	1.45 ± 0.65	Absent	–
Uric acid (mg/dl)	5.47 ± 1.38	3.15 ± 1.43	<0.01
Fetal weight (g)	2560 ± 615	3280 ± 365	<0.05