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Placental CD4$^+$ T cells isolated from preeclamptic women cause preeclampsia-like symptoms in pregnant nude-athymic rats

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ABSTRACT

Preeclampsia (PE), new onset hypertension during pregnancy, is associated with a proinflammatory profile compared to normal pregnancy (NP). We hypothesize that CD4$^+$ T cells from PE patient placentas cause PE symptoms during pregnancy compared to those from NP women. CD4$^+$ T cells were isolated from placentas of PE and NP women using anti-CD4 magnetic separation, cultured in TexMACS medium at 37 °C in 5% CO_2, and injected intraperitoneally into nude-athymic rats on day 12 of gestation. On day 18, carotid catheters were implanted and on day 19, mean arterial pressure (MAP) was measured and blood and tissues were collected. MAP was 125 ± 2 mmHg in rats with NP CD4$^+$ T cells but increased to 140 ± 4 mmHg in rats with PE CD4$^+$ T cells. Significant differences in circulating cytokines tumor necrosis factor-alpha (TNF-α), interleukin-17 (IL-17) and soluble fms-like tyrosine kinase-1 (sFlt-1) were found with PE vs NP CD4$^+$ T cells (TNF-α- PE = 167.4 pg/mL, NP = 79.4 pg/mL; IL-17-PE = 7.054 pg/mL, NP = 3.185 pg/mL; sFlt-1-PE = 90.7 pg/mL, NP = 58.2 pg/mL. In addition, renal cortical endothelin-1 (ET-1) mRNA expression increased 4.5 fold in rats with PE CD4$^+$ T cells versus those receiving to NP CD4$^+$ T cells. These data indicate an important role for placental PE CD4$^+$ T cells to cause many characteristics of PE during pregnancy.

1. Introduction

Preeclampsia (PE) affects 5–10% of pregnant women each year worldwide. In the United States alone, the disease accounts for 18% of maternal deaths each year and is the number one reason doctors deliver babies prematurely [4]. It is diagnosed by hypertension after week 20 of pregnancy and may be accompanied by proteinuria and edema. Women with PE present with characteristics of chronic inflammation with inflammatory CD4$^+$ T cells and inflammatory cytokines, TNF-α and IL-17 are increased during the disease, while regulatory T cells and anti-inflammatory cytokines are suppressed. The underlying cause of PE is not known, however, evidence supporting the role of the placenta in PE has been presented numerous times [16,18], and it has been shown that delivery of the placenta from mothers with PE reduces the symptoms of the disease [7]. Therefore, the objective of this study was to compare the effect of placental PE patient CD4$^+$ T cells to that of placental NP CD4$^+$ T cells to cause hypertension and other factors that are associated with the pathology of this disease.

Zenclussen et al. [19] demonstrated with adoptive transfer of splenocytes isolated from normal pregnant mice, cultured to promote differentiation of Th-1 like cells that secreted Th-1 specific cytokines provoked symptoms of PE [15]. In addition to these findings, data from our laboratory indicate a role for T cell-mediated pathophysiology during PE [13,14,16,17,19]. CD4$^+$ T cells from the reduced uterine perfusion pressure (RUPP) rat model of PE were adoptively transferred into normal pregnant Sprague-Dawley rats. RUPP CD4$^+$ T cells leads to many of the characteristic pathophysiology seen during PE, such as a significant increase in blood pressure, while there was no significant increase in blood pressure seen in the normal pregnant recipients of normal pregnant CD4$^+$ T cells [16]. Adoptive transfer of RUPP CD4$^+$T cells into normal pregnant rats also led to an increase in circulating inflammatory cytokines, anti-angiogenic factor sFlt-1 and the vasoconstrictor ET-1, which has been previously shown to play a key role in the development of hypertension during PE [13,14,16]. Although the importance of inflammatory T cells in the pathogenesis of PE has been established by adoptive transfer studies using animal models, no studies

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examining the importance of PE patient placental T cells in causing characteristics of PE have ever been performed.

2. Materials and methods

2.1. Human participant selection

Twelve (6 normal pregnant; 6 PE) pregnant women undergoing cesarean-section at the Wiser Hospital for Women and Infants at the University of Mississippi Medical Center (UMMC) in Jackson, MS, were enrolled in the current study. PE was defined according to the 2002 ACOG recommendations of a systolic blood pressure ≤ 140 mmHg and a diastolic pressure ≤ 90 mmHg in previously normotensive women with the presence of proteinuria defined as excretion of ≤0.3 g in a 24hr specimen [1]. All PE patients were treated with labetalol, hydralazine and/or Procardia to decrease their blood pressure and were prophylactically infused with magnesium sulfate upon hospital admission. Women with multiple gestations, chronic hypertension, pre-existing immune disorder (i.e. systemic lupus erythematosus), current tobacco/nicotine/alcohol usage or illegal substance abuse were excluded from the study. Prior to delivery all women signed an UMMC Institutional Review Board (2010–0301) approved consent and their placentas were collected and processed within 30 min of delivery.

2.2. Isolation, expansion and injection of placental $CD4^+$ T cells

On day 1 of isolation, NP and PE placental explants (~4–5 g) were collected from the maternal side of the placenta, rinsed in PBS + 5% penicillin/streptomycin (Pen/Strep) to remove surface blood, followed by tissue digestion in 4% collagenase I, 0.25U DNaseI and 5% Pen/Strep in RPMI1640 buffer for an hour at 37 °C while stirring. The digest was strained with 100 μM filters and centrifuged at 1200 RPM for 10 min. The cell pellet was reconstituted with Hanks Balance Salt Solution (HBSS) and layered carefully over 3 mL of Histopaque solution (Sigma-Aldrich, St. Louis, MO) and centrifuged for 30 min at 1200 RPM with no brake at room temperature. The lymphocyte layer was visualized and removed from the Histopaque via aspiration and placed in a clean tube before addition of 10 mL of RPMI + 5%Fetal calf serum (FCS) + 5% Pen/Strep. Lymphocytes were centrifuged at 3200RPM with brake at room temperature for 5 min tell remove any residual Histopaque. The cell pellet was resuspended in degassed MACS running buffer (Miltenyi Biotec, San Diego, CA), followed by mixing with CD4 microbeads at 4 °C for 30 min. The lymphocyte mixture was centrifuged at 3200 RPM for 10 min and the cell pellet was resuspended in MACS running buffer and filtered with a 70 μm filter. The filtered lymphotcyte mixture was passed through MS columns (Miltenyi Biotec) on a MACS magnetic stand which allowed the CD4-labelled lymphocyte microbeads to adhere to the MS column and all non-labeled cells passed through the column. $CD4^+$ T cells were mechanically released from the columns with the manufacture provided elutant (Miltenyi Biotec) and the resulting $CD4^+$- labeled T cells were then incubated in TexMACs media (Miltenyi Biotec) + 1.022 ng/mL of IL-2 overnight. IL-2 is needed to maintain viability of T cells. Day 2 of isolation, cells were collected and centrifuged at 1600 RPM, counted using a cell counter from Beckman-Coulter, then stored frozen in liquid nitrogen until needed for injection.

2.3. Adoptive transfer of T cells into pregnant rats

Timed-pregnant nude-athymic rats from Harlan (Indianapolis, IN) were used in this study. Nude-athymic rats were chosen because they lack a thymus, meaning they do not produce T cells and will not reject the human T cells injected into them. However, this does render them immunocompromised and thus, animals were continuously housed in a temperature-controlled barrier suite (23 °C) with a 12:12-hour light/dark cycle. All experimental procedures performed in this study were in accordance with the National Institutes of Health guidelines for use and care of animals. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi Medical Center.

The day prior to injecting nude-athymic rats with isolated human placental $CD4^+$ T cells, vials of frozen cells were removed from storage in liquid nitrogen and thawed quickly using ambient temperature water. Cells were washed in HBSS and incubated overnight for further expansion. On the day of injection, $CD4^+$ T cells were removed from incubation, counted, then suspended in sterile saline at a concentration of 1×10^6 cells per 500 μL. In order to be consistent with our other publications performing the adoptive transfer technique [12,13,16,17], on gestational day 12, normal pregnant, nude-athymic rats were anesthetized using isoflurane and quickly injected intraperitoneally with 1×10^6 placental $CD4^+$ T cells isolated from either one NP or one PE patient. A group of normal pregnant (NP) nude athymic rats was used as a blood pressure control in which no $CD4^+$ T cells were injected.

2.4. Measurement of blood pressure in conscious, nude-athymic rats

Under isoflurane anesthesia, on day 18 of gestation, carotid arterial catheters were inserted for blood pressure measurements. The catheters inserted are V3 tubing (SCI) which is tunneled to the back of the neck and exteriorized. On day 19 of gestation, arterial blood pressure was analyzed after placing the rats in individual restraining cages. Arterial pressure was monitored with a pressure transducer (Cobe III Tranducer CDX Sema) and recorded continuously for one hour after a 30 min. stabilization period. The nude-athymic rats were placed under isoflurane anesthesia prior to blood and tissue collection. Kidneys and placentas were harvested and weighed prior to being stored for future analysis. Pups were removed and weights were recorded. Serum and plasma were isolated via centrifugation.

2.5. Determination endothelin expression in the renal cortex

Real time PCR (qRT-PCR) was utilized to determine tissue preproendothelin-1 levels. Kidneys were isolated, weighed and quickly frozen in liquid nitrogen. The tissues were then stored at −80 °C. Total RNA was extracted from the tissues using the RNeasy Protect Mini Kit (Qiagen) and the isolation procedure was performed according to the manufacturer provided instructions. cDNA was synthesized from 1 μg of RNA using the iScript cDNA Synthesis Kit (BioRad). qRT-PCR was performed using iQ SYBR Green Supermix (BioRad) and the CFX96 Touch Real-Time PCR Detection System (BioRad). The following primer sequences provided by Life technologies were used for Preproendothelin (PPET) as previously described: forward 1, ctaggtctaagcgatccttg, and reverse 1, tctttgtctgcttggc. Levels of mRNA were calculated using the mathematical formula $2^{-\Delta\Delta Ct}$ ($2^{\text{avg. Ct gene of interest} - \text{avg Ct beta actin}}$) recommended by Applied Biosystems (Applied Biosystems User Bulletin, No. 2, 1997).

2.6. Measurement of circulating sFlt-1, TNF alpha and IL-17

Circulating levels of the anti-angiogenic factor sFlt-1, TNF alpha and IL-17 were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D Systems, Minneapolis, MN). Appropriate amounts of plasma from each sample were analyzed according to the manufacturer's instructions. The MDD for sFlt-1 assay is 15.2 pg/mL and intra-assay/inter-assay precision is 7.3% CV of 4.8% CV, respectively. The MDD for TNF alpha and IL-17 is 5 pg/mL and intra-assay precision is 3.1% CV and 3.3% CV, respectively, inter-assay precision is 9.3% CV and 4.9% CV, respectively.

2.7. Statistical analysis

All of the data are expressed as mean ± SEM. Comparisons of control with experimental groups were analyzed by the student *t-test*

Table 1
Clinical characteristics of study participants. Data is expressed as mean ± standard error mean.

Variables	Pre-eclampsia	Normal Pregnant	P-value
Maternal Age	25.0 ± 1.9 yrs	25.8 ± 2.1 yrs	0.78
% African American	83%	100%	> 0.99
Gestational age at delivery	34.7 ± 1.6 wks	38.8 ± 0.7 wks	0.04
Fetal Birthweight	1679 ± 312.3 g	3259 ± 243.3 g	0.003
% Multiparous	100%	67%	0.46
Body mass index	30.65 ± 3.5 kg/m^2	37.1 ± 4.7 kg/m^2	0.29
Systolic blood pressure	156.5 ± 5.8 mmHg	125.3 ± 5.6 mmHg	0.003
Diastolic blood pressure	94.5 ± 6.1 mmHg	73.8 ± 6.1 mmHg	0.04

and analysis of variance (ANOVA) with Tukey's post hoc analysis. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Participant's data

The participants' clinical characteristics are presented in Table 1. There were no statistically significant differences in maternal age between women with PE and NP women ($p = 0.78$), maternal race ($p > 0.99$) or in maternal BMI at the time of delivery ($p = 0.29$; Table 1). PE women did delivery significantly earlier compared to NP women ($p = 0.04$) which corresponded to a significantly lower fetal birthweight among infants born to PE women compared to infants born to NP women ($p = 0.003$; Table 1). Women with PE had significantly increased systolic ($p = 0.003$) and diastolic ($p = 0.04$) blood pressures compared to NP women (Table 1).

3.2. MAP was increased in recipients of PE T cells

MAP was measured in pregnant, nude-athymic rat recipients of NP placental CD4$^+$ T cells and PE placental CD4$^+$ T cells. MAP was significantly increased to 140 ± 4 mmHg in recipients of PE T cells (n = 6) vs. normal pregnant (NP) nudes (n = 3) (140 ± 4 vs. 123 ± 5 mmHg, $p < 0.05$) (Fig. 1). MAP was 125 ± 2 mmHg in recipients of NP T cells (n = 6). No change in MAP was noted between NP nude-athymic rats and nude-athymic rats given NP CD4$^+$ T cells (n = 6) (123 ± 5 vs 125 ± 2 mmHg, ns).

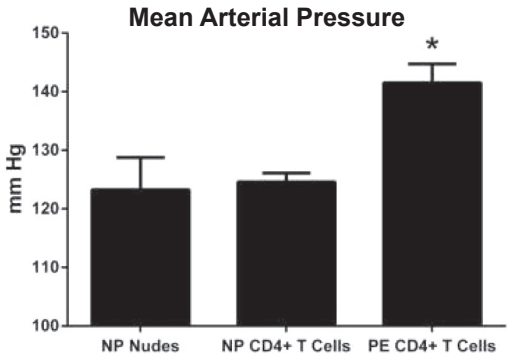

Fig. 1. Blood Pressure is increased in Nude-Athymic Recipients of Preeclamptic CD4$^+$ T cells compared to NP CD4$^+$ T cells. MAP was significantly increased in recipients of PE CD4$^+$ T cells vs. recipients of NP T cells. No change in MAP between NP nude-athymic rats and nude-athymic rats given NP CD4$^+$ T cells. Statistical changes was determined using one-way ANOVA with Turkey's post hoc analysis, $^*p < 0.05$ vs NP.

3.3. TNF-α and IL-17 are increased in recipients of PE T cells

TNF-α production increased to 167.4 ± 30.3 pg/mL in pregnant, nude-athymic recipients of placental PE CD4$^+$ T cells (n = 6) versus 79.4 ± 44.6 pg/mL in nude-athymic recipients of placental NP CD4$^+$ T cells (n = 6) ($p < 0.05$) (Fig. 2A). IL-17 significantly increased to 7.054 ± 1.446 pg/mL in recipients of PE CD4$^+$ T cells versus 3.185 ± 0.6623 pg/mL recipients of NP T Cells ($p < 0.05$) (Fig. 2B).

4. Circulating sFlt-1 and renal cortical PPET-1 increased in recipients of PE T cells

Circulating sFlt-1 production was 71.5 ± 14.7 pg/mL in recipients of NP CD4$^+$ T cells (n = 6) and was significantly increased to 90.7 ± 6.5 pg/mL in recipients of PE CD4$^+$ T cells (n = 6) (Fig. 3A). PPET-1 expression was significantly increased in the renal cortex of PE CD4$^+$ T cells recipients by a greater than 4 fold change compared to nude-athymic rats injected with NP T cells ($p < 0.05$) (Fig. 3B).

5. Discussion

As poor placentation leading to placental ischemia is considered to be one of the primary pathophysiological events leading to the development of PE, it is important to try to identify some of the molecular mechanisms associated with placental ischemia [6,15]. The idea that the imbalance in Tregulatory and Th17 cells can contribute to disease pathology has only recently started to be explored in animal models, however several clinical studies have reported imbalances in these subsets [3,11]. Based on these studies along with those conducted in our lab we set out to determine if CD4$^+$ T cells could lead to the changes blood pressure, inflammation and renal function that accompanies PE.

We demonstrate in this study for the first time that PE CD4$^+$ T cells isolated from the placentas of PE patients increase circulating inflammatory cytokines TNF-α and IL-17 and sFlt-1 when injected into normal pregnant nude athymic rats compared NP recipients of NP CD4$^+$ T cells. We have previously shown TNF-α to cause hypertension associated with oxidative stress, endothelin, and the antiangiogenic factor sFlt-1, while IL-17 stimulates ROS and sFlt-1 in association with hypertension during pregnancy. [4,12,13,16,17]). We have also shown that blocking TNF-α or IL-17 lowers blood pressure and these vasoactive pathways in the RUPP rat model of PE [2,8]. Importantly, when we infuse either TNF-α or sFlt-1 into normal pregnant rats, ET-1 is stimulated and is instrumental in causing the resultant hypertension, which is ameliorated with ETA blockade [4].

The current study is the first study to show that PE placental CD4$^+$ T cells are important to stimulate an elevation in blood pressure during pregnancy by activating ET-1 and sFlt-1 pathways. Moreover, the increase in blood pressure with PE placental T cells injected into normal pregnant, nude-athymic rats, was significantly greater than that observed with NP placental CD4$^+$ T cells. We recognize there were no changes in pup weight, which may be due to the late gestation injection, however injection of CD4$^+$ T cells on gestation day 12 of pregnancy is consistent with our previous adoptive transfer studies. Nevertheless, it is a good model in which to study placental PE CD4$^+$ T cells as mediators of hypertension and the PE phenotype during pregnancy.

A number of underlying mechanisms may be utilized by CD4$^+$ T cells to induce these vasoactive factors via direct or indirect actions. We have previously shown that activated T cells form the RUPP rat model of PE secrete sFlt-1, IL-17 and TNF-α, which could be mechanism where by these cytokines are elevated in the rats receiving PE T cells compared to those receiving NP T cells [13]. In addition to secreting sFlt-1, CD4$^+$ T cells may induce the vasoactive factors sFlt-1 and PPET-1 through indirect mechanisms. The CD4$^+$ T cells from RUPP rats secrete TNFα and directly causes increased vascular and placental levels of

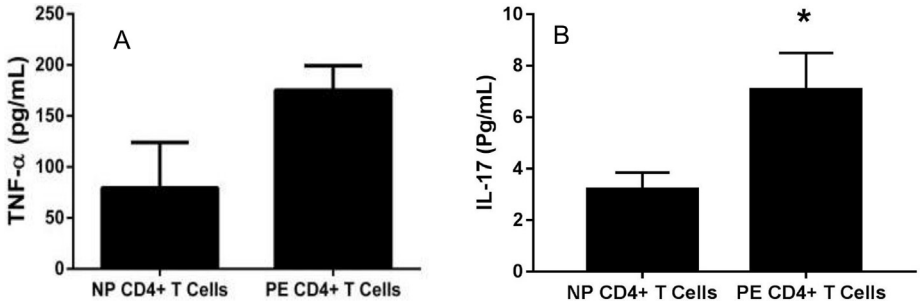

Fig. 2. Circulating inflammatory cytokines TNF-α and IL-17 are increased in response to adoptive transfer of PE CD4$^+$ T cells compared to recipients of normal pregnant CD4$^+$ T cells. A) Plasma TNF-α production had a tendency to increase in pregnant, nude-athymic recipients of placental PE CD4$^+$ T cells vs. nude-athymic recipients of NP CD4$^+$ T cells (not significant). B) IL-17 was increased nude-athymic recipients of placental PE T cells vs nude-athymic recipients of NP T cells. Statistical changes was determined using student *t-test*, $^*p < 0.05$ vs NP.

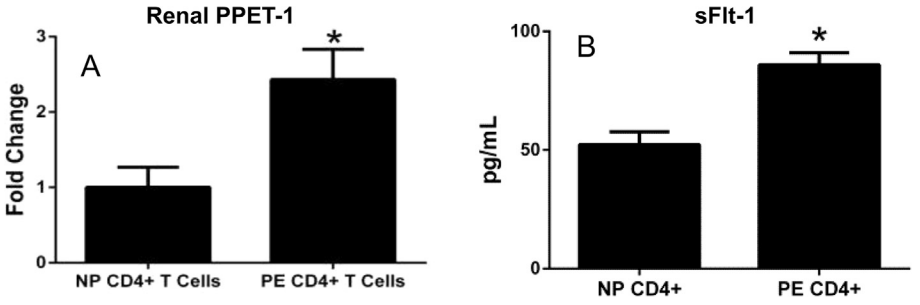

Fig. 3. Renal and circulating vasoconstrictors are increased in Nude-Athymic Recipients of Preeclamptic CD4$^+$ T cells. A) Plasma sFlt-1 increased in nude-athymic recipients of placental PE CD4$^+$ T cells vs. nude-athymic recipients of NP CD4$^+$ T cells. B) PPET-1 expression was significantly increased by 4 fold in the renal cortex of nude-athymic recipients of placental PE CD4$^+$ T cells vs. nude-athymic recipients of NP CD4$^+$ T cells. Statistical changes was determined using student *t-test*, $^*p < 0.05$ vs NP.

TNFα. Increased TNF-α activates endothelial cells, decreases nitric oxide synthase mRNA, and increases the production ET-1 by stimulating expression of PPET-1 mRNA [7–10,18]. The increased population of T cells also stimulate B cells to produce agonistic angiotensin II type 1 receptor autoantibodies (AT1-AA). AT1-AAs induce signaling by the angiotensin II type 1 receptor (AT1-receptor) leading to intracellular cascades and promoter activations in the nucleus to cause upregulation of vasoactive factors including PPET-1 and sFlt1 [5]. The CD4$^+$ T cells can directly or indirectly stimulate pathways to induce the PE phenotype observed in this adoptive transfer model.

This study establishes a role for human preeclamptic CD4$^+$ T cells in mediating PE-like symptoms and adds further evidence to existing studies examining the role of inflammatory T cells and their contribution to the disease. This study not only established a role for the altered CD4$^+$ T cell population in human preeclamptic patients, but it also strongly suggests that the increase in blood pressure and the pathophysiology of preeclampsia is further mediated by the production of sFlt-1 and ET-1. With the increase in IL-17 in the recipient rats, future studies will further examine a role of TH17 cells to mediate some of these effects.

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Conflict of interest/disclosure statement

There are no disclosures or conflicts of interest.

Author's contribution

Ashlyn C. Harmon: Carried out the experiments and drafted original manuscript. Revised and edited the final version.

Tarek Ibrahim: Collected data necessary to the project, thus completing and finalizing the work presented.

Denise C. Cornelius: Performed experiments and analyzed data, revised manuscript and approved final manuscript.

Lorena M. Amaral: Performed experiments and data analysis.

Mark W. Cunningham Jr.: Performed experiments and data analysis.

Kedra Wallace: Edited and revised manuscript.

Babbette LaMarca: Conceived the hypothesis, designed the experiments, and supervised the execution and completion of the project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2018.10.007.

References

[1] ACOG, Diagnosis and management of preeclampsia and eclampsia, Pract. Bull. 33 (2002).
[2] D.C. Cornelius, J.P. Hogg, J. Scott, K. Wallace, F. Herse, J. Moseley, G. Wallukat, R. Dechend, B. LaMarca, Administration of interleukin-17 soluble receptor C suppresses TH17 cells, oxidative stress, and hypertension in response to placental ischemia during pregnancy, Hypertension 62 (2013) 1068–1073.
[3] D. Darmochwal-Kolarz, M. Kludka-Sternik, J. Tabarkiewicz, B. Kolarz, J. Rolinski, B. Leszczynska-Gorzelak, J. Oleszczuk, The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia, J. Rep. Immun. 93 (2) (2012) 75–81.
[4] P. Dhillion, K. Wallace, F. Herse, J. Scott, G. Wallukat, J. Heath, J. Mosely, J.N. Martin Jr., R. Dechend, B. LaMarca, IL-17-mediated oxidative stress is an important stimulator of AT1-AA and hypertension during pregnancy, Am. J. Physiol. Regul. Integr. Comp. Physiol. 303 (2012) R353–358.
[5] F. Herse, B. LaMarca, Angiotensin II type 1 receptor autoantibody (AT1-AA)-mediated pregnancy hypertension, Am. J. Reprod. Immunol. 69 (2013) 413–418.
[6] S.A. Karumanchi, J.P. Granger, Preeclampsia and pregnancy-related hypertensive disorders, Hypertension 67 (2) (2016) 238–242.
[7] B. LaMarca, D. Cornelius, K. Wallace, Elucidating immune mechanisms causing hypertension during pregnancy, Physiology (Bethesda) 28 (2013) 225–233.
[8] B. LaMarca, J. Speed, L. Fournier, S.A. Babcock, H. Berry, K. Cockrell, J.P. Granger, Hypertension in response to chronic reductions in uterine perfusion in pregnant rats: effect of tumor necrosis factor-alpha blockade, Hypertension 52 (2008) 1161–1167.
[9] B. LaMarca, K. Wallace, J. Granger, Role of angiotensin II type I receptor agonistic autoantibodies (AT1-AA) in preeclampsia, Curr. Opin. Pharmacol. 11 (2011) 175–179.
[10] B.B. LaMarca, K. Cockrell, E. Sullivan, W. Bennett, J.P. Granger, Role of endothelin in mediating tumor necrosis factor-induced hypertension in pregnant rats, Hypertension 46 (2005) 82–86.
[11] Z. Jianjun, H. Yali, W. Zhiqun, Z. Mingming, Z. Xia, Imbalance of T-cell transcription factors contributes to the Th1 Type Immunity predominant in pre-eclampsia, Am. J. Rep. Immun. 63 (1) (2010) 38–45.
[12] S. Novotny, K. Wallace, F. Herse, J. Moseley, M. Darby, J. Heath, J. Gill, G. Wallukat, J.N. Martin, R. Dechend, B. LaMarca, CD4 T cells play a critical role in mediating hypertension in response to placental ischemia, J. Hypertens. (2013).
[13] S.R. Novotny, K. Wallace, J. Heath, J. Moseley, P. Dhillon, A. Weimer, G. Wallukat, F. Herse, K. Wenzel, J.N. Martin Jr., R. Dechend, B. Lamarca, Activating

autoantibodies to the angiotensin II type I receptor play an important role in mediating hypertension in response to adoptive transfer of CD4+ T lymphocytes from placental ischemic rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 302 (2012) R1197–1201.

[14] J.M. Roberts, H.S. Gammill, Preeclampsia: recent insights, Hypertension 46 (2005) 1243–1249.

[15] R.I. Sava, K.L. March, C.J. Pepine, Hypertension in pregnancy: taking cues from pathophysiology for clinical practice, Clin. Cardiol. 41 (2) (2018) 220–227.

[16] K. Wallace, S. Novotny, J. Heath, J. Moseley, J. Martin, M. Owens, B. Lamarca, Hypertension in response to CD4+ T cells from reduced uterine perfusion pregnant rats is associated with activation of the endothelin-1 system, Am. J. Physiol. Regul. Integr. Comp. Physiol. 303 (2012) R144–149.

[17] K. Wallace, S. Richards, P. Dhillon, A. Weimer, E.S. Edholm, E. Bengten, M. Wilson, J.N. Martin Jr., B. LaMarca, CD4+ T-helper cells stimulated in response to placental ischemia mediate hypertension during pregnancy, Hypertension 57 (2011) 949–955.

[18] M. Yoshizumi, M.A. Perrella, J.C. Burnett Jr., M.E. Lee, Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life, Circ. Res. 73 (1993) 205–209.

[19] A.C. Zenclussen, S. Fest, R. Joachim, B.F. Klapp, P.C. Arck, Introducing a mouse model for pre-eclampsia: adoptive transfer of activated Th1 cells leads to pre-eclampsia-like symptoms exclusively in pregnant mice, Eur. J. Immunol. 34 (2004) 377–387.