

# Preeclampsia: From Inflammation to Immunoregulation

Denise C Cornelius

Departments of Emergency Medicine and Pharmacology and Toxicology, The University of Mississippi Medical Center, Jackson, MS, USA.

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**ABSTRACT:** Preeclampsia (PE) affects 5% to 7% of pregnant women each year worldwide, accounts for up to 18% of maternal deaths in the United States each year, and is the number 1 cause of premature births. Preeclampsia is associated with hypertension after the 20th week of gestation with or without proteinuria, in conjunction with fetal growth restriction, maternal endothelial dysfunction, and chronic immune activation. The mechanisms leading to the development of PE are unclear. However, it is thought that shallow trophoblast invasion and insufficient remodeling of uterine spiral arteries result in placental ischemia. Consequently, an immune imbalance characterized by increases in proinflammatory CD4<sup>+</sup> T cells and cytokines along with decreases in regulatory T cells and anti-inflammatory cytokines occurs. This imbalance leads to chronic inflammation and ensuing oxidative stress, proinflammatory cytokines, and autoantibodies. Studies performed in our laboratories, using the *Reduced Uterine Perfusion Pressure* (RUPP) rat model of placental ischemia, have demonstrated a role for this immune imbalance to mediate PE pathophysiology and identified potential mechanisms of immunoregulation that may be of benefit in the treatment of PE. Therefore, the purpose of this commentary is to review studies demonstrating the positive effects of immunoregulatory factors in the RUPP rat model of PE. Restoration of the immune balance in PE may be a potential strategy for the development of therapeutic interventions that could improve maternal and fetal outcomes associated with this maternal syndrome.

**KEYWORDS:** Preeclampsia, inflammation, T-helper 17 Cells, immune regulation, regulatory T cells

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**CORRESPONDING AUTHOR:** Denise C Cornelius, Departments of Emergency Medicine and Pharmacology and Toxicology, The University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA. Email: dcornelius@umc.edu

## Introduction

Preeclampsia (PE) affects 5% to 7% of pregnant women each year worldwide, accounts for up to 18% of maternal deaths in the United States each year, and is the number 1 cause of premature births.<sup>1,2</sup> Hallmark characteristics of PE are new-onset hypertension, proteinuria, edema, chronic immune activation, and maternal endothelial dysfunction. According to the National High Blood Pressure Education Program, a blood pressure of at least 140/90 mm Hg during pregnancy in women who were not previously hypertensive constitutes a preeclamptic pregnancy.<sup>3</sup> Preeclampsia often ends with premature delivery of the fetus and therefore is also a major cause of fetal and perinatal morbidity and mortality, in addition to maternal death.

Preeclampsia is diagnosed after 20 weeks of gestation with a worsening of the disease state throughout the remainder of the pregnancy up until delivery. However, initiating events that lead to the development of the disease are hypothesized to begin during implantation. In normal pregnancy, immune cells in the decidua, including macrophages, uterine natural killer (NK) cells, dendritic cells (DCs), and regulatory T cells (T<sub>regs</sub>), facilitate migration and invasion of trophoblasts into the uterine wall during establishment of the placenta. These immune cells establish tolerance toward the fetal-derived trophoblasts and facilitate trophoblast-mediated uterine spiral artery remodeling. This remodeling creates high capacitance vasculature with low resistance, leading to increased blood flow to the placenta and fetus. Dendritic cells promote an anti-inflammatory, CD4<sup>+</sup> T-helper 2 (T<sub>H2</sub>) dominant state in the uterus to further support maternal immunotolerance of the fetus and

fetal antigens. CD8<sup>+</sup> T cells with suppressive function are thought to possibly have a role in mediating fetal tolerance by hampering B-cell antibody production.<sup>4,5</sup> Other studies have shown hormone downregulation of B-cell differentiation and deletion of placental reactive B cells during normal pregnancy.<sup>6</sup> Furthermore, effector cells such as inflammatory DCs and cytotoxic NK cells are decreased in the circulation during normal pregnancy. These pregnancy-specific changes are regulated by the release of cytokines and angiogenic factors.<sup>1</sup>

As in normal pregnancy, alterations in the immune system also occur in PE. However, rather than immune changes that promote tolerance and inhibit reactivity to the fetus and placenta, immune cells such as CD4<sup>+</sup> T-helper 1 (T<sub>H1</sub>) cells, cytotoxic NK cells, and autoreactive B cells secrete factors that instigate an increase in innate immune activation and inflammation in the maternal circulation and uteroplacental unit. These immune changes result in shallow trophoblast invasion of the uterine wall and insufficient spiral artery remodeling early in pregnancy. Placental ischemia ensues due to the reduced blood flow, and low oxygen and nutrient delivery lead to intrauterine growth restriction (IUGR) of the fetus. The placental ischemia augments oxidative stress and stimulates the release of hypoxia-induced anti-angiogenic factors including soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), which are implicated in the development of hypertension.<sup>7</sup> This shift in the immune response during PE suggests a role for inflammation in the development and progression of the disease.



The *Reduced Uterine Perfusion Pressure* (RUPP) rat model of placental ischemia is an ideal animal model in which to study PE. This model exploits the reduction in uterine blood flow through placement of restrictive clips on the abdominal aorta and ovarian arteries and mimics many of the characteristics of PE observed in humans. These features include hypertension, proteinuria, impaired renal function, increased vascular reactivity, IUGR, and chronic immune activation. Reduced Uterine Perfusion Pressure models have been performed in a number of animals, including rats, dogs, rabbits, sheep, and primates. The RUPP rat model is the most well-characterized and utilized model.<sup>8</sup> Importantly, the RUPP rat has been used in numerous studies to investigate the altered immune status in PE and to elucidate immune mechanisms that contribute to PE pathophysiology.

### PE: An Inflammatory State

Preeclampsia is associated with chronic immune activation that results in elevated levels of inflammatory cytokines released by proinflammatory helper T-cell subsets.<sup>9</sup> The proinflammatory cytokines tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin (IL) 6, and IL-17 are typically secreted by activated T<sub>H</sub>1 and T<sub>H</sub>17 cells to instigate a cytotoxic and inflammatory immune response to foreign pathogens or injury. During PE, these cytokines are significantly increased in the maternal circulation and the placenta, resulting in chronic systemic and local placental inflammation, which contributes to the pathophysiologic complications that manifest during PE.<sup>10,11</sup>

The increased TNF- $\alpha$  and IL-6 in the vasculature contribute to increased endothelial expression of adhesion molecules and permeability, resulting in endothelial dysfunction during PE.<sup>12–14</sup> Expression of adhesion molecules in the vasculature promotes leukocyte rolling and extravasation, leading to increased endothelial permeability. Studies have demonstrated that TNF- $\alpha$  signaling results in endothelial cell activation, decreased nitric oxide synthase (NOS) messenger RNA (mRNA), and increased production of preproendothelin 1 (PPE-1) mRNA.<sup>15,16</sup> Decreased nitric oxide (NO) bioavailability results in a loss of vasodilatory ability of endothelial cells and leads to endothelial dysfunction and the development of hypertension and IUGR in PE.<sup>17</sup> Preproendothelin 1 is the precursor to the potent vasoconstrictor endothelin 1 (ET-1), which has been shown to be increased in the circulation of preeclamptic women.<sup>18</sup> Furthermore, PPE-1 mRNA expression is increased in the endothelial cells of women with PE compared with women with normal pregnancies.<sup>2</sup> Interleukin 6 has also been shown to mediate the mRNA expression of PPE-1 and play a role in stimulating endothelial permeability.<sup>19</sup>

Previous studies demonstrated that a 2-fold increase in plasma levels of TNF- $\alpha$  resulted in significant increases in mean arterial pressure (MAP); PPE-1 expression in placenta, aorta, and kidney; and renal vascular resistance in pregnant

rats.<sup>16,20</sup> Furthermore, increased TNF- $\alpha$  caused reductions in neuronal and inducible NOS expression, renal plasma flow, and glomerular filtration rate (GFR).<sup>20,21</sup> Reduced Uterine Perfusion Pressure rats also show a 2- to 3-fold increase in serum TNF- $\alpha$  in response to placental ischemia.<sup>21</sup> Inhibition of TNF- $\alpha$  in RUPP rats, with etanercept, significantly decreased MAP, serum, placental, and renal levels of TNF- $\alpha$  and blunted renal and placental PPE-1 mRNA expression.<sup>22</sup> Furthermore, etanercept treatment improved pup weight, cardiac hypertrophy, and angiogenesis in RUPP rats.<sup>22,23</sup> Similarly, serum IL-6 doubles in response to placental ischemia in pregnant rats.<sup>10</sup> To test the role of IL-6 in contributing to PE-like characteristics in pregnancy, IL-6 levels were increased 2- to 3-fold in pregnant rats and were found to be associated with significantly increased MAP, decreased renal plasma flow and GFR, and increased plasma renin activity.<sup>10</sup> To our knowledge, no studies to directly inhibit IL-6 have been performed in the RUPP rat model.

Interleukin 17 activates and promotes proliferation of T<sub>H</sub>17 cells in a feedback loop and is a key cytokine essential for proliferation, recruitment, activation, and migration of neutrophils.<sup>24–26</sup> Interleukin 17 signaling to neutrophils stimulates cytokine-mediated cell-to-cell communication and neutrophil release of antimicrobial substances, such as reactive oxygen species (ROS). Interleukin 17 has been linked to oxidative stress and can induce expression of cytokines in nonimmune cells, including endothelial cells.<sup>26–28</sup> The circulating population of T<sub>H</sub>17 cells is significantly increased in PE compared with normal pregnancy.<sup>29,30</sup> Moreover, mRNA expression of the T<sub>H</sub>17-specific transcription factor, RAR-related orphan receptor  $\gamma$  (ROR- $\gamma$ ), is increased in peripheral blood mononuclear cells and the decidua of preeclamptic women.<sup>31</sup>

We have previously published studies that demonstrated a pathophysiologic role of T<sub>H</sub>17 cells and IL-17 in PE. Dhillon et al<sup>32</sup> observed that administration of IL-17 into normal pregnant rats resulted in significant increases in MAP, placental ROS, and circulating T<sub>H</sub>17 cells and production of an agonistic autoantibody to the angiotensin II type I receptor (AT1-AA). CD19<sup>+</sup>/CD5<sup>+</sup> B cells produce AT1-AAs and are significantly increased in the circulation of preeclamptic women. Furthermore, these AT1-AA-producing cells are present in the placenta of preeclamptic patients, but not in women with normal pregnancies.<sup>33</sup> Blockade of the AT1 receptor with losartan attenuated the rise in blood pressure and decreased placental ROS in response to chronic IL-17 infusion. Depletion of B cells with rituximab also blunted the blood pressure response and decreased circulating T<sub>H</sub>17 cells in response to IL-17 infusion. Administration of the superoxide dismutase mimetic, Tempol, also inhibited the increase in blood pressure and markedly reduced placental ROS and circulating AT1-AAs in these same animals. These data suggest that IL-17 plays a role in the pathophysiology associated with PE, including hypertension, via placental oxidative stress and activation of

the AT1 receptor by AT1-AAAs produced by IL-17-stimulated B cells.<sup>32</sup>

To support a proposed role for IL-17 in oxidative stress-mediated pathophysiology in pregnancy, we performed studies to examine the effect of IL-17 blockade on various pathways activated in the RUPP rat model of PE. Infusion of soluble IL-17 receptor C (IL-17RC) into RUPP rats resulted in significant decreases in the population of circulating  $T_H17$  cells, oxidative stress, AT1-AA production, and MAP and improved fetal growth. To examine the mechanism of IUGR inhibition, placental weight and uterine artery resistance index were measured. Infusion of IL-17RC caused an increase in pup weight and placental weight and a decrease in the uterine artery resistance index.<sup>34</sup> Currently, there are several IL-17 blockers, including the anti-IL-17A monoclonal antibodies secukinumab and ixekizumab and the anti-IL-17 receptor subunit A monoclonal antibody brodalumab, that are under investigation for clinical treatment of rheumatoid arthritis and psoriasis.<sup>35,36</sup> These biologics may be therapeutic options for the treatment of PE; however, information on their safety during pregnancy is limited and requires further investigation.<sup>37</sup>

### PE: Impaired Immunoregulation

In addition to an increase in proinflammatory T cells and inflammatory cytokines, it has been suggested that PE is characterized by a decrease in  $T_{regs}$  and IL-10, the anti-inflammatory cytokine produced by  $T_{regs}$ . A number of clinical studies have investigated the status of  $T_{regs}$  in the circulation of normal pregnant versus preeclamptic women. The majority of studies have consistently observed a decrease in  $T_{regs}$  (CD4<sup>+</sup>/FoxP3<sup>+</sup> or CD4<sup>+</sup>/CD25<sup>+</sup>/FoxP3<sup>+</sup>) in preeclamptic women compared with normal pregnant women.<sup>38</sup> However, there are a few studies that report no significant change in  $T_{regs}$  between women with normal pregnancies and those with PE.<sup>39–42</sup> The inconsistencies of the data presented in these studies could be affected by the use of less specific markers to identify  $T_{regs}$  (CD4/CD25 only or CD4/CTLA4).<sup>39,40</sup> Some of these studies that reported no changes in total  $T_{regs}$  in preeclamptic women did, however, report alterations in  $T_{reg}$  subsets.<sup>41,42</sup> Larger studies with more standardized protocols across institutions will be necessary to conclusively determine  $T_{reg}$  status in PE.

However, given the high consistency of studies reporting decreased  $T_{regs}$  or at least reduced regulatory function in PE, a role for decreased  $T_{regs}$  in contributing to PE pathophysiology remains worthy of consideration. Regulatory T cells have been shown to be decreased in the periphery and decidua of women with PE, and the decrease in  $T_{regs}$  is directly proportional to PE severity.<sup>43</sup> Several studies report a significant decrease in  $T_{regs}$  in patients with severe, early-onset PE compared with patients with mild, late-onset PE.<sup>39,44,45</sup> Studies have also shown that decreases in decidual  $T_{regs}$  result in increased apoptosis in trophoblast cells and shallow invasion of trophoblasts into the deciduas.<sup>46,47</sup> This suggests the direct involvement of  $T_{regs}$  in

spiral artery remodeling and the initial phenomenon resulting in the development of PE.

Interleukin 10 is a  $T_{reg}$ -associated anti-inflammatory cytokine responsible for stimulating the differentiation of  $T_{regs}$  from naïve T cells.<sup>48–50</sup> Interleukin 10 is important during pregnancy because of its ability to inhibit secretion of  $T_H1$  inflammatory cytokines and thus provide an important counterbalance for controlled inflammation at the fetal-maternal interface.<sup>51</sup> Pro-inflammatory cytokines such as interferon  $\gamma$  (IFN- $\gamma$ ), IL-2, and TNF- $\alpha$  are downregulated by IL-10.<sup>52,53</sup> Interleukin 10 levels are persistently higher during normal pregnancy and usually do not fall until labor begins.<sup>54</sup> In PE, lower levels of IL-10 have been observed in circulation and in the placenta of patients.<sup>55–57</sup> We have previously shown that placental explants from preeclamptic patients released lower amounts of IL-10 in *in vitro* culture under normal oxygen and hypoxic conditions when compared with placental explants from normal pregnant patients.<sup>58</sup> Furthermore, patients with early-onset PE were also found to have lower circulating IL-10 compared with women with late-onset PE.<sup>45,59,60</sup> Interleukin 10 decreases inflammatory cytokines that are linked to oxidative stress while promoting vascular healing, a process that is necessary for spiral artery remodeling and placental perfusion. Interleukin 10 also restores endothelin-dependent relaxation and increases endothelial NOS expression in ET-1-treated aortic rings.<sup>61</sup> Importantly, in the pregnant DOCA/saline rat model of PE, IL-10 treatment yielded decreased circulating ET-1 and IFN- $\gamma$  levels, restored relaxation responses in aortic rings, decreased urinary protein output, and improved litter size.<sup>62</sup>

Studies recently published by our laboratory show that adoptive transfer of  $T_{regs}$  from normal pregnant rats into RUPP rats lowers blood pressure, blunts fetal growth restriction, and reduces inflammatory cytokines. This was accompanied by a corresponding increase in circulating anti-inflammatory cytokines and significantly lower placental ET-1 expression and placental and renal ROS. Normal pregnant  $T_{regs}$  also attenuated the production of agonistic AT1-AAAs in RUPP rats.<sup>63</sup> Supplementation of normal pregnant  $T_{regs}$  occurred prior (gestational day 12) to induction of placental ischemia (gestational day 14). These effects suggest the importance of immune regulation during normal pregnancy to inhibit AT1-AA production and maintain appropriate levels of inflammation, oxidative stress, and ET-1, all of which are responsible for increasing blood pressure. Lower levels of these factors may ultimately result in a more normal fetal weight and safer blood pressures in response to placental ischemia. We have also performed preliminary adoptive transfer studies of  $T_{regs}$  from RUPP rats into normal pregnant rats and did not observe any PE-like features in normal pregnant recipients of RUPP-induced  $T_{regs}$ . This suggests that the function of  $T_{regs}$  may not be altered in response to placental ischemia.

The results of the aforementioned studies suggest that the proinflammatory cytokine profile seen in RUPP rats can be



somewhat improved by increasing the  $T_{reg}$  population. In our most recent studies, 2 approaches were used to investigate therapeutic options to safely increase  $T_{regs}$ . The first approach was through IL-10 supplementation. In this study, we supplemented RUPP rats with IL-10 via osmotic minipumps to achieve circulating levels of IL-10 comparable with normal pregnant rats.<sup>64</sup> Supplementation of RUPP rats with IL-10 led to a decrease in the overall number of circulating CD4<sup>+</sup> T cells, whereas the circulating population of  $T_{regs}$  increased to a level similar to what was observed in normal pregnant rats. Interleukin 10 supplementation also normalized placental ROS and circulating TNF- $\alpha$  and IL-6 levels and markedly improved AT1-AA and ET-1, leading to an overall decrease in blood pressure in response to placental ischemia.<sup>64</sup>

In the second approach, we administered the anti-CD28 superagonistic antibody via intraperitoneal injection to stimulate endogenous  $T_{regs}$  in the RUPP rat.<sup>65</sup> Anti-CD28 administration significantly increased the circulating population of  $T_{regs}$  to a level similar to normal pregnant rats. Furthermore, a significant increase in circulating levels of the anti-inflammatory cytokine IL-10 and the regulatory protein transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) were also observed. In addition, significant decreases were observed in the effector T-cell-stimulating cytokine IL-2, the proinflammatory cytokine IL-6, and AT1-AA. Placental ROS; placental, renal, and aortic ET-1; and blood pressure were significantly lowered in RUPP rats treated with anti-CD28. Importantly, fetal weight in RUPP rats was improved to levels no longer significantly lower than normal pregnant rats after anti-CD28 treatment.<sup>65</sup>

These studies demonstrate the regulatory role of the anti-inflammatory cytokine IL-10 and  $T_{regs}$  to improve maternal and fetal outcomes in a rodent model of PE. Restoring  $T_{regs}$  in RUPP animals inhibited effector T-cell activation which may be the mechanism by which inflammation, oxidative stress, AT1-AA, and ET-1 were lowered.<sup>63–65</sup> In the absence of T-cell activation, inflammatory cytokine secretion would decrease and result in a reduced population of inflammatory cells and lower levels of ROS. Furthermore, inhibition of AT1-AA production could also be occurring through prevention of T-cell-mediated activation of B cells.<sup>63</sup> Regulatory T-cell secretion of IL-10 could have direct effects in improving vascular function as well as inhibiting the inflammatory function of effector CD4<sup>+</sup> T cells. We have previously shown that ET-1 production and placental ROS are 2 mechanisms stimulated by AT1-AA activation of the AT1 receptor to induce hypertension in response to placental ischemia.<sup>66</sup> Both factors are reduced after IL-10 supplementation in RUPP rats.<sup>64</sup>

Regulatory immune factors counterbalance an overactive immune response to prevent chronic inflammation and antibody-mediated responses that result in damage to self. In PE, this counterbalance is compromised leading to chronic immune activation, inflammation, and autoantibody production. Previously, we have demonstrated the efficacy of various

anti-inflammatory agents to decrease inflammatory and vasoactive factors leading to reduced blood pressure in the RUPP rat.<sup>22,67,68</sup> Thus, reestablishment of the immune balance could restore immune tolerance toward the fetus and have positive effects on maternal and fetal outcomes in PE.

## Summary and Conclusions

Preeclampsia is associated with chronic immune activation characterized by persistently higher levels of proinflammatory cytokines and diminished immunoregulatory factors. This immune imbalance promotes an inflammatory state during PE.<sup>9,69–71</sup> While the exact underlying mechanisms that mediate PE development remain unclear, studies investigating the immune imbalance that accompanies and contributes to the progression of the disease are beginning to identify possible targets for treatment. This imbalance between proinflammatory and regulatory cytokines that occurs during a preeclamptic pregnancy suggests that PE is a state of dysregulated immune activation. Development of therapeutic strategies that focus on restoration of the immune balance, which is necessary for a successful pregnancy, may improve maternal and fetal outcomes in the face of placental ischemia. Further studies are needed to identify appropriate therapies to supplement current PE management protocols that are safe for both mother and the fetus. However, the recognition of these 2 regulatory immune factors ( $T_{regs}$  and IL-10) and elucidation of the mechanisms by which they may improve PE offer a starting point in the development of new therapeutic targets for the effective treatment of this disease.

## Author Contributions

DCC authored the manuscript.

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