Administration of Interleukin-17 Soluble Receptor C Suppresses TH 17 Cells, Oxidative Stress, and Hypertension in Response to Placental Ischemia During Pregnancy

Denise C. Cornelius, James P. Hogg, Jeremy Scott, Kedra Wallace, Florian Herse, Janae Moseley, Gerd Wallukat, Ralf Dechend, Babbette LaMarca

Abstract—Preeclampsia, new onset hypertension with proteinuria during pregnancy, is associated with chronic inflammation and placental oxidative stress (ROS). Chronic interleukin-17 (IL-17) increases blood pressure, autoantibodies (angiotensin II type I receptor [AT1-AA]), and ROS during pregnancy. The objective of this study was to determine whether T-helper 17 (TH17) suppression via IL-17 recombinant receptor C (IL-17RC) decreases pathophysiology associated with placental ischemia (reduced uterine perfusion pressure [RUPP]). On gestation day 14, miniosmotic pumps infusing 100 pg of IL-17RC per day were implanted into pregnant rats undergoing RUPP. On gestation day 18, carotid catheters were inserted. On gestation day 19, mean arterial pressure was recorded and TH17 cells, oxidative stress, and AT1-AA were measured and analyzed via 1-way ANOVA. Mean arterial pressure increased from 101±2 mm Hg in normal pregnant rats (n=19) to 120±1 mm Hg in RUPP rats (n=17) but decreased to 110±2 mm Hg in RUPP+IL-17RC rats (n=22). Pup weight decreased from 2.28±0.2 g in normal pregnant rats to 1.96±0.3 g in RUPP rats but was significantly increased to 2.01±0.1 in RUPP+IL-17RC rats. TH17 cells were 1.77% in RUPP rats but decreased to 0.65% in RUPP+IL-17RC rats. Urinary isoprostanates were normalized in RUPP+IL-17RC rats (52 pg/µg) compared with 89 pg/µg in RUPP controls. Placental ROS was 652 relative light units in RUPP rats but decreased to 337 relative light units in RUPP+IL-17RC rats. AT1-AA was 17.27±0.7 bpm in RUPP rats but decreased to 5.00±0.5 bpm in RUPP+IL-17RC rats. With this study, we show that infusion of IL-17RC blunts TH17s, oxidative stress, AT1-AA, and hypertension in the RUPP model of preeclampsia, indicating that TH17 cells may play an important role in disease pathophysiology. (Hypertension. 2013;62:00-00.)

Key Words: hypertension ■ inflammation ■ oxidative stress ■ pregnancy

Preeclampsia is a disease that affects 5% to 8% of pregnancies in the United States.1,2 Hallmark characteristics of preeclampsia are new onset hypertension with proteinuria, immune activation, imbalances between proangiogenic factors (vascular endothelial growth factor/placental growth factor) and antiangiogenic factor (soluble fms-like tyrosine kinase-1), and oxidative stress (ROS) during pregnancy. The exact pathophysiological mechanisms that lead to the development of preeclampsia have not yet been determined but are thought to involve shallow trophoblasts invasion and early alteration of natural killer cells and T cells.3-4 This shallow trophoblasts invasion is thought to lead to decreased vasuclogenesis in the growing uteroplacental unit.3 The lack of vascular remodeling leads to lower oxygen and nutrient delivery to the placenta, thereby contributing to the development of placental ischemia.

Placental ischemia/hypoxia is implicated as an important mechanism in the development of preeclampsia. Placental ischemia induces oxidative stress, an imbalance in proangiogenic and antiangiogenic factors, and immune activation and endothelial dysfunction in the maternal vasculature.5,6 It has been proposed that the imbalance between the 2 CD4+ T-cell types, T regulatory and T-helper 17 (TH17), is involved in the pathophysiology of preeclampsia.6 We have previously shown that placental ischemia stimulates this CD4+ T-helper cell imbalance, which contributes to excess proinflammatory cytokine production and a shift in angiogenic factors and leads to hypertension during pregnancy.7 TH17 cells are a subclass of CD4+ T cells, characterized by their secretion of the cytokine interleukin-17 (IL-17), which has also been implicated in several autoimmune disorders including psoriasis, multiple sclerosis, rheumatoid arthritis, and irritable bowel syndrome.8,9 Recent studies have shown these cells to be elevated in the circulation of patients with preeclampsia compared with those with normal pregnancies.6,10 Other autoimmune activities during preeclampsia, such as the production of autoantibodies to the angiotensin II type I receptor (AT1-AA) and increased circulating IL-17, indicate many similarities between preeclampsia and autoimmune diseases.5,11 We have previously shown that both TH17 cells and IL-17 are significantly elevated in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia.7 Importantly,
we recently demonstrated the role of the cytokine IL-17, which is predominately secreted by Th17 cells, in causing placental oxidative stress and hypertension during pregnancy. By infusing IL-17 into normal pregnant (NP) rats, we found Th17 cells to be elevated in NP rats, along with urinary isoprostanes, and oxidative stress, AT1-AA, and blood pressure, compared with the NP control rats. Administering a superoxide dismutase mimetic, Tempol, attenuated the hypertension and placental oxidative stress and significantly blunted the AT1-AA in response to IL-17 infusion. Therefore, these data suggested that generated reactive oxygen species seemed to behave as signaling molecules to stimulate the production of autoantibodies that have been shown in our laboratory and other laboratories to play an important role in the pathogenesis of preeclampsia. In this current study, we investigated the IL-17 function and could be a potential treatment for autoimmune disease. In addition, this led us to question a role of endogenous Th17 cells stimulated in the RUPP rat to mediate the pathophysiology of preeclampsia. There are no specifically defined inhibitors of Th17 cells used routinely, and we have shown that infusion of IL-17 stimulated circulating Th17 cells in NP rats. Therefore, we turned to inhibitors of the IL-17 pathway.

IL-17 receptor C (IL-17RC) functions as a receptor for IL-17 and the bioactive heterodimer of IL-17, that is suspected to play a pathophysiological role in preeclampsia and autoimmune disease. The stoichiometry is unclear, and heterodimers or trimers of these molecules are thought to be essential for complete inhibition of the actions of IL-17. It has been suggested that the soluble version of IL-17RC could be an effective inhibitor of IL-17 function and could be a potential treatment for autoimmune-type diseases. In this current study, we investigated the role of endogenously stimulated IL-17 and Th17 cells in mediating hypertension in response to placental ischemia. We tested our hypothesis by administering IL-17RC to block the IL-17 signaling cascade and thereby reduce circulating Th17 cells, oxidative stress, AT1-AA, and hypertension associated with placental ischemia in the RUPP rat model of preeclampsia.

Materials and Methods

Pregnant Sprague-Dawley rats purchased from Harlan Sprague Dawley Inc (Indianapolis, IN) were used in this study. The animals were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle. All experimental procedures executed in this study were in accordance with the National Institutes of Health (NIH) guidelines for use and care of animals. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

Administration of IL-17RC to RUPP Rats

On gestational day 14, under isoflurane anesthesia, NP rats underwent a reduction in uterine perfusion pressure (RUPP) with the application of a constrictive silver clip (0.203 mm) to the aorta superior to the iliac bifurcation performed, whereas ovarian collateral circulation to the uterus was reduced with restrictive clips (0.100 mm) to the bilateral uterine arcades at the ovarian end. Recombinant mouse IL-17RC (100 pg/day; RnD Systems, Minneapolis, MN) was infused intraperitoneally from day 14 through day 19 of gestation through mini–osmotic pumps (model 2002; Alzet Scientific Corporation) in 22 RUPP rats. Murine IL-17RC has 87% homology and 86% identity to rat IL-17RC, indicating high biological similarity to the naturally occurring rat protein. The dose was determined on the basis of the binding ability of the soluble receptor to IL-17A–F as performed by the manufacturer. The groups of rats examined in this study were NP (n=19), RUPP (n=17), and IL-17RC-infused RUPP (RUPP+IL-17RC; n=22).

Measurement of Mean Arterial Pressure In Chronically Instrumented Conscious Rats

Under isoflurane anesthesia, on day 18 of gestation, carotid arterial catheters were inserted for blood pressure measurements. The catheters inserted are V3 tubing (Scientific Commodities, Inc, Lake Havasu City, AZ), 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 Transducer CDX Sema) and recorded continuously for 1 hour after a 1-hour stabilization period. Subsequently, blood and urine samples were collected; kidneys, placenta, and spleens were harvested; and litter size and pup weights were recorded under anesthesia.

Placental Uterine Artery Resistive Index in Response to IL-17RC in RUPP Rats

In a method described by Tam Tam, Power Doppler velocimetry measurements were performed on anesthetized pregnant dams on gestational day 18 at an imaging station with a Vevo 770 unit (VisualSonics) using a 30-Hz transducer and an insonating angle <30°. The peak systolic flow velocity and end diastolic flow velocity were recorded using the uterine artery Doppler waveform. The uterine artery resistive index (UARI) was calculated using the following formula: UARI=peak systolic flow velocity/end diastolic flow velocity/peak systolic flow velocity. UARI was determined for the uterine artery bilaterally at 3 levels, and the mean UARI was calculated for control RUPPs and compared with that of RUPPs+IL-17RC.

Determination of Circulating T-Helper Th17 Lymphocytes

Circulating Th17 T helper cell population was determined from peripheral blood leukocytes collected at day 19 of gestation from NP, RUPP, and RUPP+IL-17RC rats. We used flow cytometric analysis to detect specific CD4+ T-cell populations; CD4+CD25 RORγt (retinoic acid receptor–related orphan receptorγt) was isolated from NP, RUPP, and RUPP+IL-17RC rats. At the time of harvest, plasma was collected, and peripheral blood leukocytes were isolated from plasma by centrifugation on a cushion of Ficoll–Hypaque (Lymphoprep; Accurate Chemical & Scientific Corp, Westbury, NY) according to the manufacturer’s instructions. For flow cytometric analysis, equal number of leukocytes (1×10⁶) were incubated for 30 minutes at 4°C with antibodies against rat CD4 and rat CD25 (BD Biosciences, San Jose, CA). After washing, cells were labeled with secondary fluorocin isothiocyanate (Southern Biotech, Birmingham, AL) and phycoerythrin with cyanin-5 (Santa Cruz Biotechnology Inc, Santa Cruz, CA) antibody for 30 minutes at 4°C. Cells were washed, permeabilized, and stained with rabbit polyclonal RORγt (Santa Cruz Biotechnology Inc, Santa Cruz, CA) for 30 minutes at 4°C. After washing, cells were labeled with secondary phycoerythrin (Southern Biotech, Birmingham, AL) for 30 minutes at 4°C. As a negative control for each individual rat, cells were treated exactly as described above except that they were incubated with anti–fluorescin isothiocyanate, anti–phycoerythrin with cyanin-4, and antitymphoerythrin secondary antibodies alone. Subsequently, cells were washed and resuspended in 500 μL of Roswell Park Memorial Institute medium and analyzed for single and double staining on a Gallios flow cytometer (Beckman Coulter, Brea, CA). The percentage of positive staining cells above the negative control was collected for each individual rat, and mean values for each experimental group (NP [n=10], RUPP [n=12], RUPP+IL-17RC [n=17]) were calculated.

Determination of Urinary Isoprostanate

On day 19 of gestation, urine was collected from NP, RUPP, and RUPP+IL-17RC rats for determination of excreted isoprostanes measured via ELISA from Oxford Biomedical Research (Oxford, MI). The assay displayed a sensitivity of 0.05 ng/mL. The interassay variability was 4.2%, and intraassay variability was 4.7%.

Determination of Placental ROS

Superoxide production in the placenta was measured by using the lucigenin technique we have described previously. Rat placenta...
from NP, RUPP, and RUPP+IL-17RC rats were snap-frozen in liquid nitrogen directly after collection and stored at −80°C until further processing. Placentas were removed and homogenized in RIPA (radio-immunoprecipitation assay) buffer (phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor cocktail; Santa Cruz, CA) as described previously. The samples were centrifuged at 16,000 g for 30 minutes, and the supernatant was aspirated, and the remaining cellular debris was discarded. The supernatant was incubated with lucigenin at a final concentration of 5 μmol/L. The samples were allowed to equilibrate for 15 minutes in the dark, and luminescence was measured every second for 10 seconds with a luminometer (Berthold, Oak Ridge, TN). Luminescence was recorded as relative light units (RLU) per minute. An assay blank with no homogenate but containing lucigenin was subtracted from the reading before transformation of the data. Each sample was repeated 5×, and the average was used for data transformation. The protein concentration was measured using a protein assay with BSA standards (Pierce, Rockford, IL). The data are expressed as RLU/min per mg protein.

**Determination of Circulating AT1-AA**

On day 18 of gestation, blood was collected and immunoglobulin was isolated from 1 mL of serum by ammonium sulfate precipitation. This IgG fraction was used in a bioassay. The AT1-AA activity was measured using spontaneously beating neonatal rat cardiomyocytes and was characterized and antagonized specifically using AT1 receptor antagonists. The results express the difference between the basal beating rate of the cardiomyocytes and the beating rate measured after the addition of the AT1-AA (increase in number of beats/min or Δbeats/min). AT1-AAs were assessed in NP rats, RUPP controls, and RUPP+IL-17RC rats.

**Statistical Analysis**

All of the data are expressed as mean±SEM. Comparisons of control with experimental groups were analyzed by ANOVA with Student t test as post hoc analysis. A value of P<0.05 was considered statistically significant.

**Results**

**IL-17RC Infusion Significantly Blunted Hypertension in RUPP Rats**

Mean arterial pressure was measured on day 19 of gestation in NP, RUPP, and RUPP+IL-17RC rats. The mean arterial pressure increased significantly from 101±2 mm Hg in NP rats to 120±1 mm Hg in RUPP rats (P<0.0001; Figure 1). This increase in mean arterial pressure in RUPP rats was blunted significantly to 110±2 mm Hg in RUPP+IL-17RC rats compared with RUPP rats (P=0.004; Figure 1).

**IL-17RC Infusion Significantly Increased Pup and Placenta Weight and Improved Uterine Artery Resistance in RUPP Rats**

In Figure 2, the pup weight of litters from RUPP rats (1.96±0.3 g) was significantly lower than the pup weight from NP rats (2.28±0.2 g; P=0.028). However, IL-17RC infusion into RUPP rats offset the decreased pup weight. The average pup weight in RUPP+IL-17RC rats was significantly increased compared with that in RUPP rats (2.01±0.1 g; P=0.05) but did not reach that of a NP rat offspring. Moreover, the average placenta weight significantly decreased from 0.5±0.02 g in NP rats to 0.47±0.02 g in RUPP rats (P=0.008), whereas infusion of IL-17RC into RUPP rats normalized placenta weight (0.52±0.2 g; P=0.04). Importantly, maternal body weight was not significantly increased by infusion of IL-17RC into RUPP rats, indicating that the effect of IL-17RC to increase pup weight may be specific to the placental fetal unit (data not shown). Furthermore,
UARI was improved in RUPP rats treated with IL-17RC compared with control RUPP rats. We have shown that UARI increases with placental ischemia from 0.60±0.03 (n=4) in NP rats to 0.71±0.04 (n=7) in RUPP rats. In this study, administration of IL-17RC improves UARI in RUPP rats to 0.64±0.063 in RUPP+IL-17RC (n=5). However, this did not reach statistical significance but does indicate a potential improvement in blood and nutrient supply to the growing utero placental unit.

**IL-17RC Infusion Blunts T<sub>H17</sub> Cells, Oxidative Stress, and AT1-AA in RUPP Rats**

Circulating T<sub>H17</sub> T-helper cells were 0.13±0.09% of gated cells in NP rats, which increased to 6.29±2.64% in RUPP rats. IL-17RC infusion into RUPP rats decreased the T<sub>H17</sub> population to 0.69±0.95% (Figure 3).

Urinary isoprostane levels were determined via ELISA. Isoprostane levels in RUPP rats increased to 89.03 pg/µg protein compared with the increase in NP rats to 56.41 pg/µg (Figure 4, top). However, this was ablated by IL-17RC infusion into RUPP rats. Urinary isoprostane was normalized to 52.23 pg/µg protein in RUPP+IL-17RC rats (Figure 4, top).

Figure 4 (bottom) shows that placental reactive oxygen species was significantly increased in RUPP rats compared with NP rats (652 RLUs versus 390 RLUs; P=0.021). Infusion of IL-17RC into RUPP rats significantly reduced and normalized placental oxidative stress. Placental ROS was 337 RLUs in RUPP+IL-17RC rats (P=0.013).

AT1-AA levels in NP rats were 0.40±0.27 bpm. Autoantibody levels increased significantly in RUPP rats (Figure 5; P<0.0001). However, infusion of IL-17RC into RUPP rats decreased the production of the AT1-AA to 5.99±0.5 bpm from 17.27±0.7 bpm in RUPP rats (Figure 5; P<0.001).

**Discussion**

It has been established that IL-17–producing T<sub>H17</sub> cells are mediators of several autoimmune diseases including psoriasis, rheumatoid arthritis, multiple sclerosis, and irritable bowel syndrome. Recent research also proposes roles for IL-17 and T<sub>H17</sub> cells as mediators of preeclamptic pathophysiology. We have recently published a study showing that chronic IL-17 infusion into pregnant rats increased blood pressure, circulating T<sub>H17</sub> cells, placental ROS, and AT1-AA. Therefore, in this study, we sought to determine whether infusion of soluble IL-17RC would decrease circulating T<sub>H17</sub> cells, oxidative stress, and hypertension in RUPP rat model of preeclampsia. IL-17 soluble receptor C binds the most active forms of IL-17 superfamily, that is, IL-17A and IL-17F. The stoichiometry of this receptor is unclear because it may form a heterodimer in its active form, which would be important to biological activity because IL-17 cytokine is now considered a heterodimer between IL-17A and IL-17F.

RUPP in rats has been shown to increase blood pressure, whereas it has been shown to decrease maternal and pup litter weight. We have recently shown IL-17 and TH17 cells to be increased in RUPP rats compared with NP rat controls. In this study, we demonstrate that IL-17RC infusion into pregnant RUPP rats significantly decreased blood pressure, AT1-AA production, and ROS in the placenta, whereas it increased pup and placenta weight and improved UARI (Figures 1–5). These data further support the role of IL-17 and T<sub>H17</sub> cells in...
the pathophysiology of preeclampsia. Given that infusion of IL-17RC was initiated at the time of placental insult (gestational day 14), it could be that early suppression of TH17 could normalize the placental inflammation to some degree, thereby correcting fetal demise. Although we have administered various anti-inflammatories to RUPP rats and have demonstrated an important role of suppression of inflammation in decreased blood pressure response in this model, this is the first of our studies to demonstrate a fetal protective mechanism that occurs by inhibiting a specific immune pathway in RUPP rats. Therefore, these data are important to demonstrate the importance of immune suppression to improve both maternal and fetal health in response to placental ischemia.

IL-17 is produced by several cell types including γδ T cells, natural killer T cell, neutrophils, eosinophils, and monocytes. Furthermore, expression of cellular receptors is found almost ubiquitously in all tissues. IL-17R is found on endothelial cells, fibroblast cells, and many immune cells. Infusion of soluble IL-17RC could therefore interfere with various cellular immune reactions that are stimulated in response to placental ischemia of pregnancy. Importantly, we show that IL-17RC attenuates the rise in circulating TH17 cells observed in response to placental ischemia.

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The most well-noted physiological role of IL-17 and IL-17–producing TH17 cells is to recruit host defense cells, such as neutrophils, to sites of infection. IL-17 stimulates the production of other cytokines by neutrophils for cell-to-cell communication, and more importantly, it stimulates these cells to release antimicrobial substances. Reactive oxygen species production is a defense mechanism used by neutrophils and macrophages against bacterial infection. However, production of ROS by activated neutrophils can also damage normal host tissue. Here, we demonstrate that blockade of IL-17 decreases urinary isoprostane and significantly decreases placental ROS that results from placental ischemia in RUPP rats (Figure 4). Therefore, the IL-17RC may be inhibiting the recruitment of neutrophils to the placental unit and therefore neutrophilic ROS production.

AT1-AAs have been shown to play an important role in hypertension during pregnancy. We recently showed that IL-17–induced hypertension was associated with AT1-AA and oxidative stress. Administration of the superoxide dismutase mimetic, Tempol, decreased blood pressure, placental ROS, and surprisingly AT1-AA in response to IL-17 infusion during pregnancy. These data indicated the importance of ROS as signaling molecules in the pathophysiology of hypertension during pregnancy. In the current study, we show decreasing...
T<sub>1</sub>7 cells in RUPP rats and decreased oxidative stress, autoantibodies, and blood pressure in response to placental ischemia (Figure 5). Importantly, pup weight was significantly improved in RUPP rats with lower placental oxidative stress and AT1-AA, not only indicating the importance of ROS and AT1-AA in fetal demise but also supporting our hypothesis that T<sub>1</sub>7 cells play an important role in the hypertension and intrauterine growth restriction during preeclampsia. It is important to continue future research investigating the role of immune alterations to mediate the pathophysiology of preeclampsia to develop novel and innovative treatment strategies that can improve both maternal and fetal health in the face of this devastating disease.

**Perspective**

This study demonstrates an important role of IL-17 in mediating the pathophysiology of hypertension and intrauterine growth restriction in the preeclamptic RUPP rat model. The data presented suggest that IL-17 may be a therapeutic target in preeclamptic women. Inhibition of IL-17 may help to regulate the angiotensin system and significantly reduce blood pressure and placental oxidative stress, as well as lead to an increase in fetal weight. These perceived benefits could result in a longer, healthier pregnancy, therefore leading to a decrease in maternal and infant morbidity that is associated with preeclampsia.

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**Disclosures**

None.

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