Blockade of CD40 ligand for intercellular communication reduces hypertension, placental oxidative stress, and AT$_1$-AA in response to adoptive transfer of CD4$^+$ T lymphocytes from RUPP rats

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Cornelius DC, Castillo J, Porter J, Amaral LM, Campbell N, Paige A, Thomas AJ, Harmon A, Cunningham MW Jr, Wallace K, Herse F, Wallukat G, Dechend R, LaMarca B. Blockade of CD40 ligand for intercellular communication reduces hypertension, placental oxidative stress, and AT$_1$-AA in response to adoptive transfer of CD4$^+$ T lymphocytes from RUPP rats. Am J Physiol Regul Integr Comp Physiol 309: R1243–R1250, 2015. First published August 26, 2015; doi:10.1152/ajpregu.00273.2015.—Preeclampsia (PE) is a major cause of maternal and perinatal morbidity and mortality worldwide (10, 33, 43). Chronic inflammation and immune activation are major factors mediating pathophysiology associated with PE (40, 41, 46). Women with PE have an increase in circulating and placental levels of inflammatory cytokines, such as interleukin (IL)-6, IL-17, and tumor necrosis factor-α (TNF-α), as well as an increase in activation of immune cells, including CD4-positive (CD4$^+$)-T-helper cells and B cells secreting agonistic autoantibody to the angiotensin II (ANG II) type I receptor (AT$_1$-AA) (5, 18, 32, 44, 48). The reduced uterine perfusion pressure (RUPP) model of placental ischemia recapitulates the characteristics of PE, including hypertension, intrauterine growth restriction (IUGR), chronic inflammation, and immune activation. We have previously shown that adoptive transfer of CD4$^+$ T cells from RUPP rats into NP rats results in the development of hypertension, mediated by AT$_1$-AA, endothelin-1 (ET-1) system, and oxidative stress (ROS) (34, 35, 52, 53). Furthermore, this hypertension is associated with elevated soluble fms-like tyrosine kinase-1 (sFlt-1) and inflammatory cytokines (35, 51–53). In addition, we have shown that infusion of purified rat AT$_1$-AA into normal pregnant rats causes hypertension mediated by ROS, sFlt-1, and the ET-1 system (20, 21, 38, 39). Therefore, we believe that T-cell-mediated AT$_1$-AA is an important mechanism causing hypertension through stimulating ET-1, ROS, and sFlt-1 in response to placental ischemia or during PE. T-helper-dependent activation of B cells occurs via interaction with multiple receptors. One interaction that is essential for long-term B-cell activation is between CD40 on B cells and the CD40 ligand on activated platelets. Exaggerated platelet activation and inflammation result from endothelial damage in PE and HELLP syndrome is associated with increased shedding of the CD40 ligand (sCD40L) (2). One of the consequences of increased sCD40L is increased activation of CD40 on the surface of
multiple immune cells that are implicated in the pathophysiology of this disease. Therefore, we hypothesized that communication of activated T cells with endogenous immune and/or vascular cells via CD40-CD40L interactions during PE mediates pathophysiology in response to placental ischemia-stimulated CD4+ T cells and is crucial to stimulating production of the AT1-AA.

The AT1-AA activates the AT1 receptor similar to ANG II and contributes to endothelial dysfunction during PE (14, 24). Previous studies have shown that AT1-AA-mediated AT1 signaling activates the MAPK/ERK pathway and NADPH oxidase leading to NF-kB activation, tissue factor expression, decreased trophoblast invasion, and increased ROS production, all of which have been implicated in the pathophysiology of PE (7, 29, 54). Long-term B-cell antibody responses and differentiation into memory cells are T-cell-dependent mechanisms, therefore suggesting an important role for T-helper cells in B-cell production of AT1-AA. Therefore, the objective of this study was to determine if blockade of communication between placental ischemia-stimulated CD4+ T cells with endogenous cells in normal pregnant (NP) recipient rats at the CD40-CD40L interaction would attenuate AT1-AA production and therefore reduce the hypertension, inflammation, and oxidative stress previously observed with adoptive transfer of RUPP CD4+ T cells into NP recipient rats.

MATERIALS AND METHODS

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

Measurement of mean arterial pressure into chronically instrumented conscious rats. Carotid arterial catheters were inserted on day 18 of gestation for blood pressure measurements that were assessed on day 19 of gestation. 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 the rats were placed in individual restraining cages. Arterial pressure was monitored with a pressure transducer (Cobe III Transducer CDX Sema) and recorded continuously for 1 h after a 30-min 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.

Reduction of uterine perfusion pressure. The reduction of uterine perfusion pressure (RUPP) model is a well-established model of placental ischemia in pregnant rats and has been described in detail previously (11, 12, 17). On gestational day 14, while under isoflurane anesthesia, NP rats underwent a RUPP with the application of a constrictive silver clip (0.203 mm) to the aorta superior to the iliac bifurcation while ovarian collateral circulation to the uterus was reduced with restrictive clips (0.100 mm) to the bilateral uterine arteries at the ovarian end. Rats were excluded from the study when the clipping procedure resulted in total reabsorption of all fetuses.

Separating splenic CD4+ T-cell lymphocytes. At the time of harvest (gestational day 19), spleens were collected from RUPP rats and lymphocytes were isolated from spleens by centrifugation on a cushion of Ficoll-Hypaque (Lymphoprep; Accurate Chemical & Scientific, Westbury, NY) according to the manufacturer’s instructions. Anti-CD4 antibodies (BD Biosciences, San Jose, CA) were biotinylated using the DSB-X Biotin Protein Labeling Kit (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. Isolated lymphocytes were incubated with biotinylated anti-CD4 antibody. The CD4+ population was isolated using FlowCyt Dynabeads (Invitrogen, Oslo, Norway) according to the manufacturer’s protocol. The isolated CD4+ T lymphocytes were incubated overnight in RPMI containing HEPE (25 Mn), glutamine (2 mM), Pen/Strep (100 U/ml), 1.022 ng/ml IL-2, and 4 ng/ml IL-12 with or without 2.5 μg/ml anti-CD40 ligand (eCD40L) at 5% CO2, 37°C in a humidified atmosphere. RUPP CD4+ T cells or RUPP CD4+ T cells+CD40L were diluted in sterile saline at a concentration of 2 × 106 cells/ml, and 1 × 106 cells were injected intraperitoneally into gestation day 12 NP rats. Recipients of RUPP CD4+ T cells were designated NP+RUPP CD4+ T cells, and recipients of RUPP CD4+ T cells incubated with eCD40L were designated NP+RUPP CD4+ T+anti-CD40L. The groups of rats examined in this study were NP (n = 13), NP+RUPP CD4+ T cells (n = 7), and NP+RUPP CD4+ T+anti-CD40L (n = 24).

Determination of CD40L binding efficiency. RUPP CD4+ T lymphocytes incubated with or without eCD40L were analyzed for binding efficiency using flow cytometry. After incubation, 1 × 106 cells were labeled with secondary fluorescein isothiocyanate (FITC; Southern Biotech, Birmingham, AL) antibody for 30 min at 4°C. As a negative control for each individual rat, cells incubated without eCD40L were also labeled with FITC secondary antibodies alone. Subsequently, cells were washed and suspended in 500 μl of Roswell Park Memorial Institute medium (RPMI) and analyzed for single staining on a Gallios flow cytometer (Beckman Coulter, Brea, CA). The percentage of positive staining cells above the negative control was collected for three separate cultures.

Determination of placental ROS. Superoxide production in the placenta was measured by using the lucigenin technique as we have previously described (25, 39). Rat placentas from NP, NP+RUPP CD4+ T cell, and NP+RUPP CD4+ T+anti-CD40L 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 buffer (phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor cocktail; Santa Cruz Biotechnology, Santa Cruz, CA) as described previously (25, 39). The samples were centrifuged at 16,000 g for 30 min, and the supernatant aspirated and remaining cellular debris discarded. The supernatant was incubated with lucigenin at a final concentration of 5 μM/l. The samples were allowed to equilibrate for 15 min in the dark, and luminescence was measured every second for 10 s 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 five times, 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 per minute per milligrams of protein.

Determination of placental preproendothelin mRNA levels. The placenta of the rats pups were separated, weighed, and immediately snap frozen in liquid nitrogen and stored at −80°C. Total RNA was extracted using the RNAeasy Protect Mini kit supplied by Qiagen kit after the tissue was crushed in liquid nitrogen with a mortar and pestle. The isolation procedure was then performed as outlined in the instructions provided by the manufacturer. Real-time PCR was utilized, as previously described, to determine tissue preproendothelin-1 levels (26, 28). cDNA was synthesized from 1 μg of RNA with Bio-Rad iScript cDNA reverse transcription, and real-time PCR was performed using the Bio-Rad iQ SYBR Green Supermix and iCycler. The following primer sequences provided by Life technologies were PPET-1 as previously described: forward 1, CTAAGTTCAAGGCATCCTTG, and reverse 1, TCCTTTGTCTGCTTG GC (27). Invitrogen’s reverse-transcription PCR primer control kit was used to amplify B-actin transcripts as control. Levels of mRNA were calculated using the

**Determination of cytokine production.** Plasma collected from all pregnant rats were measured for IL-6, TNF-α, and sFlt-1 concentrations using commercial ELISA kits (Quantikine) available from R&D Systems according to the manufacturer’s protocol.

**Determination of circulating AT1-AA.** On day 19 of gestation blood was collected and immunoglobulin was isolated from 200 µl of serum by protein G-Sepharose protein purification system (Knauer, Germany). This IgG fraction was used in a bioassay. The AT1-AA activity was measured using spontaneously beating neonatal rat cardiomyocytes 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) (16, 24, 38). AT1-AAs were assessed in NP, NP + RUPP CD4 + T cell, and NP + RUPP CD4 + T + anti-CD40L rats.

**Statistical analysis.** All of the data are expressed as means ± SE. Comparisons of control with experimental groups were analyzed by ANOVA with Tukey’s multiple comparisons test as post hoc analysis. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Blockade of CD40L attenuates hypertension in NP rat recipients of placental ischemia-induced CD4 + T lymphocytes.** CD4 + T lymphocytes were isolated from RUPP spleens and cultured as described above. Lymphocytes cultured with or without antibody to CD40L were injected intraperitoneally into gestation day 12 NP rats at a concentration of $2 \times 10^6$ cells/ml. Flow cytometry was performed to determine binding efficiency of anti-CD40L antibody to RUPP CD4 + T cells. Eighty-eight percent of the cells cultured with antibody to CD40L stained positive to for anti-CD40L-FITC (Fig. 1), thereby inhibiting this receptor from intercellular communication with endogenous cells in recipient rats. Mean arterial pressure was measured on day 19 of gestation in NP, NP + RUPP CD4 + T cell, and NP + RUPP CD4 + T + anti-CD40L rats (Fig. 2). The MAP increased significantly from 99 ± 2 in NP rats ($n = 13$) to 116 ± 4.3 in NP + RUPP CD4 + T cell rats ($n = 7$; $P < 0.01$). Antibody-mediated neutralization of CD40L on RUPP CD4 + T lymphocytes before adoptive transfer caused a significant decrease in blood pressure to 104 ± 1.9 in NP + RUPP CD4 + T + anti-CD40L rats ($n = 24$; $P < 0.05$ vs. NP + RUPP T cells). These results indicate the increase in blood pressure in response to placental ischemia-induced CD4 + T lymphocytes is in part mediated by T-cell communication with endogenous cells through CD40-CD40L interactions.

**Inhibition of CD40-CD40L between RUPP CD4 + T cells and endogenous B cells attenuates the increased AT1-AA in response to adoptive transfer.** AT1-AA was 1.9 ± 0.3 in NP ($n = 9$), was 7 ± 1.3 in NP + RUPP CD4 + T cells ($n = 7$; $P < 0.001$ vs. NP), and was only 1.5 ± 0.6 pg/ml in NP + RUPP CD4 + T + anti-CD40L ($n = 17$; $P < 0.0001$ vs. NP + RUPP CD4 + T cells; Fig. 3). This therefore suggests that B-cell production of AT1-AA occurs in a T-cell-dependent manner that requires CD40 and CD40L interactions between B cells and CD4 + T cells.

**Inhibition of CD40-CD40L interactions blunts activation of the ET-1 system in response to adoptive transfer of RUPP CD4 + T cells.** We have previously shown that the placental endothelin-1 system is activated in response to placental ischemia-induced CD4 + T lymphocytes and that B-cell production of AT1-AA occurs in a T-cell-dependent manner that requires CD40 and CD40L interactions between B cells and CD4 + T cells.
Inhibition of CD40-CD40L interactions blunts sflt-1 in response to adoptive transfer of RUPP CD4<sup>+</sup> T cell to NP rats. Plasma sFLT-1 increased from 79 ± 14 in NP (n = 4) to 107 ± 9 in NP+RUPP CD4<sup>+</sup> T cells (n = 4, nonsignificant) and was only 74 ± 21 pg/ml in NP+RUPP CD4<sup>+</sup> T+anti-CD40L (n = 4; Fig. 6C). However, these differences did not reach statistical significance.

**DISCUSSION**

Altered immune activation and vascular dysfunction are key mediators of pathophysiology in PE. Clinical studies have shown that T-lymphocyte numbers and activation are increased in women with PE compared with women with normal pregnancies (5, 8, 32). Preclinical studies in our RUPP model mirror these findings. We have previously determined a role for the activated CD4<sup>+</sup> T-helper cell population in mediating hypertension, ET-1 activation, inflammation, oxidative stress, and AT<sub>1</sub>-AA production in response to placental ischemia; all of which are mediators of PE pathophysiology (34, 51–53). In addition we have shown an important role for AT<sub>1</sub>-AA to stimulate ET-1, ROS, and sFlt-1 as mechanisms of increasing blood pressure during pregnancy. Inhibition of AT<sub>1</sub>-AA secretion with Rituximab lowered AT<sub>1</sub>-AA and blood pressure in the RUPP rat model of placental ischemia (25). Furthermore, we have previously demonstrated that inhibition of T-cell activation attenuates such mediators and lowers blood pressure in response to placental ischemia (34). However, inhibition of T or B cells and temporarily leaving the mother immunocompromised is not a plausible treatment option for PE because a controlled inflammatory response and immune protection from outside pathogens is absolutely required for a healthy, successful pregnancy.

Much research concerning the stimulus for and the route of production of the AT<sub>1</sub>-AA has been performed in recent years. Based on the autoantibodies’ ability to bind and activate the

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**Fig. 2.** Hypertension in response to placental ischemia-stimulated CD4<sup>+</sup> T cells is attenuated with CD40L neutralization. Mean arterial pressure (MAP) was measured on day 19 in normal pregnant (NP; n = 13), NP+RUPP CD4<sup>+</sup> T cells (n = 7), and NP+RUPP CD4<sup>+</sup> T+anti-CD40L (n = 24) rats. Hypertension in response to adoptive transfer of placental ischemia-stimulated CD4<sup>+</sup> T cells is attenuated after CD40L binding with neutralizing antibody (***P < 0.001, ****P < 0.0001).

Inhibition of CD40-CD40L interactions blunts placental ROS in response to adoptive transfer of RUPP CD4<sup>+</sup> T cell to NP rats. One mechanism by which CD40-CD40L causes endothelial dysfunction is through its mediation of ROS production (1, 4, 42, 49). Placental ROS increased from 143.4 ± 22 in NP (n = 6) to 318.6 ± 89 in NP+RUPP CD4<sup>+</sup> T cells (n = 3; P < 0.05). CD40L binding reduced placental ROS to 118.7 ± 24 in NP+RUPP CD4<sup>+</sup> T+anti-CD40L (n = 6; P < 0.001 vs. NP+RUPP CD4<sup>+</sup> T cells; Fig. 5).

Inhibition of CD40-CD40L interactions blunts TNF-α and IL-6 in response to adoptive transfer of RUPP CD4<sup>+</sup> T cell to NP rats. Plasma IL-6 increased from 35.52 ± 6 in NP (n = 7) to 84.8 ± 12 in NP+RUPP CD4<sup>+</sup> T cells (n = 5; P < 0.05 vs. NP) and was significantly decreased to 46.9 ± 2.9 pg/ml in NP+RUPP CD4<sup>+</sup> T+anti-CD40L (n = 5, P < 0.05 vs. NP+RUPP CD4<sup>+</sup> T cells; Fig. 6A). Additionally, TNF-α was 7.8 ± 2 in NP, was 18.8 ± 8 in NP+RUPP CD4<sup>+</sup> T cells, and was only 13.9 ± 4 pg/ml in NP+RUPP CD4<sup>+</sup> T+anti-CD40L (Fig. 6B). These data demonstrate that while blockade of CD40-CD40L interactions does not inhibit increases in circulating and TNF-α, it does inhibit increases in circulating IL-6 in response to RUPP CD4<sup>+</sup> T cells. CD40-expressing B cells are known to secrete IL-6 (6); therefore, neutralization of CD40L may have decreased CD40-activated B-cell production of IL-6. The lack of inhibition of TNF-α expression and the presence of some residual IL-6 is not surprising as activated T cells express these cytokines and blockade of CD40L with antibody is not expected to reverse the activation of the adoptively transferred CD4<sup>+</sup> T cells.

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**Fig. 3.** Angiotensin II type I receptor (AT<sub>1</sub>-AA) production is attenuated after αCD40L binding. AT<sub>1</sub>-AA levels was measured via chronotropic events in cardiomyocytes in culture. AT<sub>1</sub>-AAs are significantly increased in NP+RUPP CD4<sup>+</sup> T cells (n = 7) rats compared with NP rats (n = 9). Blockade of CD40L significantly decreased circulating AT<sub>1</sub>-AA in NP+RUPP CD4<sup>+</sup> T+anti-CD40L rats (n = 17) (***P < 0.001, ****P < 0.0001).
AT$_1$ receptor, one may assume that AT$_1$-AAs are derived from a very specific epitope region on the AT$_1$-receptor. However, it is important to note that autoantibodies can also be generated by molecular mimicry (36, 47). The seven amino acid epitope region on the second extracellular loop of the AT$_1$ receptor, which binds to the AT$_1$-AA, shares a six amino acid homology with capsid proteins of the human parvovirus B19 (PVB19), which is associated with several autoimmune diseases as well as PE (15, 47). Herse et al. (15) showed that antibodies generated against the VP2 capsid protein of the PVB19 increase the beating rate of rat neonatal cardiomyocytes to the same level as observed after incubation with AT$_1$-AAs isolated from PE patients. Furthermore, the increase in beats per minute stimulated by autoantibodies against PVB19 was suppressed by losartan and the specific seven amino acid sequence, which neutralizes AT$_1$-AA activation of the AT$_1$ receptor. These experiments suggest the VP2 capsid protein of PVB19 could serve as the antigen for AT$_1$-AA generation. An earlier study by Stephan et al. (47) examined women with abnormal uterine perfusion and with normal uterine perfusion during the second and third trimester pregnancy and found no correlation between PVB19 positive patients and the presence of AT$_1$-AAs in the maternal circulation of normal and abnormal pregnant women. They also showed no differences in AT$_1$-AA activity in PVB19 positive or negative patients. Thus Stephan et al. (47) concluded that AT$_1$-AA generation is independent of epitope mimicry of the AT$_1$ receptor by PVB19. Whether or not PVB19 is an epitope that mimics the AT$_1$ receptor for AT$_1$-AA production is still unclear. Future studies administering the PVB19 capsid protein antibodies into pregnant rodents are warranted to determine whether or not these antibodies generate PE symptoms in rodents similar to AT$_1$-AA infusion.

Another recent theory of AT$_1$-AA generation arises from posttranslational modification of the AT$_1$ receptor by tissue transglutaminase (TG2) (30, 31). TG2 can modify the AT$_1$ receptor to create an antigen for AT$_1$-AA production, and placentas from women with PE have an increase in TG2 activity. Cystamine, an endogenous inhibitor of TG2, is decreased in cultured explants of PE placentas. Furthermore, the administration of cystamine to pregnant mice inhibits AT$_1$-AA induced hypertension and proteinuria (30). Administration of LIGHT, a new TNF-$\alpha$ superfamily member also known as tumor necrosis factor superfamily member 14, was injected into pregnant mice and elicited all the hallmarks of PE, including increased circulating AT$_1$-AAs during pregnancy. In these mice TG2 activity was increased in the placenta and cystamine administration (via the drinking water) decreased TG2 activity, circulating AT$_1$-AAs, hypertension, and proteinuria (31). Data from these studies suggest that posttranslational modifications to the AT$_1$ receptor may also have a role in AT$_1$-AA production, but more studies are needed to verify these conclusions.

With the route of production still unknown, we hypothesized that CD4$^+$ T cells are necessary for the production of the AT$_1$-AA. To answer this question, we inhibited communication through CD40L, one of the mechanisms for T-cell communication to B cells, necessary for B-cell proliferation. Therefore the objective of this study was to determine if inhibition of T-cell to B-cell communication via CD40-CD40L interaction would attenuate AT$_1$-AA and other pathophysiology observed in response to adoptive transfer of CD4$^+$ T cells from the RUPP rat model of placental ischemia.

In the current study, we demonstrate that antibody-mediated neutralization of CD40L on placental ischemia-stimulated CD4$^+$ T cells, before adoptive transfer, attenuates the hypertension, placental oxidative stress, and AT$_1$-AA production in NP recipient rats. This suggests that communication of acti-

Fig. 4. $\alpha$CD40L binding blunts activation of the placental endothelin-1 (ET-1) system. mRNA of preproendothelin was measured in the placentas of NP ($n = 6$), NP+RUPP CD4$^+$ T cells ($n = 6$), and NP+RUPP CD4$^+$ T+anti-CD40L ($n = 6$) rats. Preproendothelin is increased in placenta of NP+RUPP CD4$^+$ T cells compared with NP rats. CD40L binding blunted the increased transcription of preproendothelin in NP+RUPP CD4$^+$ T+anti-CD40L rat placentas ($^{***}p < 0.001$, $^{****}p < 0.0001$).

Fig. 5. Placental reactive oxygen species (ROS) in response to RUPP-stimulated CD4$^+$ T cells is mediated by CD40-CD40L interactions. Placental oxidative stress was measured using chemiluminescence. In response to adoptive transfer of placental ischemia-stimulated CD4$^+$ T cells, placental ROS was elevated compared with NP animals. Increased ROS production was attenuated after CD40L binding to RUPP CD4$^+$ T cells ($^{*}p < 0.05$).
vated CD4+ T cell with endogenous cells including B cells via the CD40-CD40L signal plays a role to mediate these pathophysologies in PE. CD40 is expressed on immune cells and vascular cells, including ECs and smooth muscle cells. Activation of CD40 on B cells by CD40L on T cells not only activates B cells to produce antibodies but also mediates differentiation into memory B cells and facilitates class switching to IgG. It is important to note that AT1-AA is detected as IgG antibody in PE women and in the RUPP rat. Therefore, it could be that this interaction plays a central role in the production of AT1-AA. AT1-AA and mediates its pathophysiological effects, including endothelial dysfunction and oxidative stress, through activation of the AT1 receptor (7, 14, 24).

We have previously shown that placental ischemia, RUPP CD4+ T cells and AT1-AA lead to increased placental PPET-1 mRNA expression and secretion of ET-1 by cultured human umbilical endothelial vascular cells in culture (20, 22), suggesting that placental activation of the ET-1 system may be important in the pathology of the disease. We believe this shows the importance of T cells and AT1-AA to impact the placenta thereby leading to increased ET-1 which could ultimately play a role in the hypertension. We previously showed that NP recipients of RUPP CD4+ T cells had lower blood pressure response when treated with an ETA receptor antagonist, thus emphasizing the importance of ETA in response to RUPP CD4+ T cells. In this study, RUPP CD4+ T-cell mediated increased placental PPET-1 mRNA levels which could be a contributing factor to hypertension in this study. Importantly, blockade of CD40L resulted in decreased oxidative stress and ET-1 production leading to lower blood pressures in this study.

However, because CD40 is also expressed on ECs and VSMCs, the vascular dysfunction and increase in ROS production could be a result of direct interactions between CD40L on the activated CD4+ T cells and CD40 on vascular cells. The CD40-CD40L signaling axis has a role to induce ET-1 expression in ECs directly and indirectly by inducing expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) (45). Furthermore, Chen, et al. (3) demonstrated that treatment of ECs with recombinant CD40L resulted in increased superoxide production. It has been shown that activated platelets express CD40L and their interaction with vascular cells can lead to endothelial dysfunction in cardiovascular disease. However, Isler et al. (17) previously provided evidence that activated platelets are not present in the RUPP rat model of placental ischemia. Therefore, we conclude that in this preclinical PE model, CD40L on activated T cells, not activated platelets, has a role to directly contribute to endothelial dysfunction and oxidative stress through CD40-CD40L interactions on vascular cells.

Although there was a downward trend, blockade of CD40L on CD4+ T cells did not have profound effects to decrease plasma sFlt-1 or TNF-α in response to RUPP CD4 + T cells. This is not necessarily surprising, as T cells only exhibit...
CD40L surface expression after they are activated. Binding CD40L with neutralizing antibody would not be expected to reverse activation of the T cell and would therefore not have an effect to decrease secretion of sFlt-1 or inflammatory cytokines, which we have shown to be secreted by RUPP CD4+ T cells (53). Importantly, blockade of CD40L on CD4+ T cells significantly decreased circulating IL-6. This may be due to decreased CD40 mediated activation of B cells, as CD40-activated B cells have been shown to secrete IL-6 (6). Therefore, the decrease in IL-6 may also be due to decreased B-cell activation by the RUPP CD4+ T cells which would account for less AT1-AA and support our hypothesis that AT1-AA is a CD4+ T-cell-mediated event.

The impact of CD40L antibody binding on proper maternal immune and vascular function is an important factor to consider in determining the feasibility of this approach as a therapy for women with PE. Furthermore, in assessing the effects of this treatment on fetal outcomes, it is important to weigh the benefits against any risks associated with use of this biologic. TNF-α inhibitors have been well studied during pregnancy and were found to have no teratogenic effects on the fetus. However, an increased risk of infection after birth was associated with administration during late pregnancy (37). Limited clinical studies have shown that inhibition of T-cell activation with abatacept during pregnancy had no pattern of negative effects on the mother or fetus (19). Even fewer studies are available on the use of other biologics, such as rituximab, anakinra, tocilizumab, and belimumab during pregnancy (37). Therefore, further studies are required to assess the viability of neutralization of CD40L as a therapy in the treatment of PE.

Perspectives and Significance

In this study, we have demonstrated that interaction between endogenous cells and placental ischemia-stimulated CD4+ T cells via CD40-CD40L binding is one important mechanism that leads to much of the pathophysiology of PE. Inhibition of this intercellular communication through blockade of CD40L on CD4+ T cells attenuated hypertension, activation of the ET-1 system, placental oxidative stress, and AT1-AA production. Data presented in this study highlight the consequences of improper immune activation during pregnancy and identify activated CD4+ T cells as a potential therapeutic target for the management/treatment of PE.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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