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The role of inflammation in the pathology of preeclampsia

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Abstract

Preeclampsia (PE) affects 5–7% of all pregnancies in the U.S. and is the leading cause of maternal and prenatal morbidity. PE is associated with hypertension after week 20 of gestation, decreased renal function, and small-for-gestational-age babies. Women with PE exhibit chronic inflammation and production of autoantibodies. It is hypothesized that during PE, placental ischemia occurs as a result of shallow trophoblast invasion which is associated with an immune imbalance where pro-inflammatory $CD4^+$ T cells are increased and T regulatory cells (Tregs) are decreased. This imbalance leads to chronic inflammation characterized by oxidative stress, pro-inflammatory cytokines, and autoantibodies. Studies conducted in our laboratory have demonstrated the importance of this immune imbalance to cause hypertension in response to placental ischemia in pregnant rats. These studies confirm that increased $CD4^+$ T cells and decreased Tregs during pregnancy leads to elevated inflammatory cytokines, endothelin (ET-1), reactive oxygen species (ROS), and agonistic autoantibodies to the Angiotensin II (Ang II), type 1 receptor (AT1-AA). All of these factors taken together play an important role in increasing the blood pressure during pregnancy. Specifically, this review focuses on the decrease in Tregs, and their associated regulatory cytokine IL-10, which is seen in response to placental ischemia during pregnancy. This study will also examine the effect of regulatory immune cell repopulation on the pathophysiology of preeclampsia. These studies show that restoring the balance of the immune system through increasing Tregs, either by adoptive transfer or by infusing IL-10, reduces the blood pressure and pathophysiology associated with placental ischemia in pregnant rats.

Preeclampsia: Hypertension During Pregnancy

Preeclampsia (PE) affects 5–10% of pregnant women each year worldwide, while in the United States it accounts for 18% of maternal deaths each year and is the number one cause of premature births (1). PE is diagnosed by hypertension after week 20 of pregnancy and maybe accompanied by proteinuria and/or edema. According to the National High Blood Pressure Education Program (2), blood pressure of 140/90 mmHg during pregnancy in women who were not previously hypertensive constitutes a preeclamptic pregnancy (3).

Preeclampsia is a systemic disease that affects the function and health of multiple organs and leads to pathophysiology during pregnancy that affects both the mother and baby. While there is no cure for PE, except for early delivery of the feto-placental unit. The severity of

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the pathophysiology associated with PE varies depending the presence of other conditions associated with PE, such as eclampsia, edema, renal failure, liver failure, and HELLP (hemolysis elevated liver low platelet) syndrome (4–6). Women with PE are at high-risk for eclamptic seizures and therefore are given magnesium sulfate for its prevention (7). In severe PE where the systolic blood pressure of the mother is 160–180 mmHg or above and diastolic blood pressure is 105–110 mmHg or higher, intravenous antihypertensive therapy such as hydralazine (vasodilator) or labetalol (beta-blocker) is used to manage the blood pressure(2, 8). Other classes of antihypertensive drugs like angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) cannot be used during pregnancy due to their potential teratogenic effects on the fetus (9). Since current treatments for PE are not always effective to improve maternal and fetal outcomes, a greater understanding of the pathogenesis of the disease is necessary so that more effective treatment regimens can be developed.

Much research has been devoted to understanding the causes and mechanisms leading to the development of PE. Despite the many attempts to understand the origins of PE, it remains unknown. However, the importance of the placenta in the development of PE has been well established (10). Evidence supporting the role of the placenta in PE has been presented numerous times, although the exact pathogenesis responsible for altering the placental function is not known (11). Additionally, placentas from preeclamptic women also show vascular abnormalities and inflammation compared to placentas from healthy pregnancies, suggesting a role for inflammation in the disease (12, 13).

Placental Ischemia and Immune Imbalance During Preeclampsia

While the specific mechanisms leading to the development of PE have yet to be elucidated, the proposed mechanism suggests shallow invasion of trophoblast cells leads to decrease in spiral artery remodeling and vascularity of the placenta. The process of trophoblast invasion and spiral artery remodeling during a normal pregnancy, rely heavily on the maternal immune system to properly regulate this important transformation (14, 15). During trophoblast invasion, the decidua, which is the lining of the uterus and forms the maternal portion of the placenta, contains a high number of immune cells that are necessary for the proper migration of trophoblast cells (16–18). Macrophages, natural killer (NK) cells, dendritic cells (DCs), T cells, and T regulatory cells (Tregs) are present in the decidua and are required for the normal invasion of trophoblast cells during placentation. These cells infiltrate the decidua and congregate around the trophoblast cells allowing them to reach the endometrium through controlled removal of native cells in the spiral artery (19–22). Uterine NK cells (uNK), macrophages, and DCs together play an integral role in trophoblast invasion and decidual formation (23, 24). On the other hand Tregs and regulatory cytokines ensure the proper control and function of pro-inflammatory cells and their actions to ensure proper invasion (18, 25, 26). In addition, DCs present in the decidua are thought to function to promote a Th2 dominate state in the uterus and placenta in order to induce immunotolerance of the mother to the fetus (27). Together, the immune cells present in the decidua during pregnancy work to ensure proper implantation and to promote trophoblast invasion that is neither shallow nor overly invasive (14, 15). This is accomplished through their production of cytokines and angiogenic factors, which are necessary for a normal

pregnancy. The balance in the number of immune cells present and the factors they produce are crucial for a healthy pregnancy and any imbalance in these local immune responses could potentially result in an malformation of the placenta. Decreases or impaired placental vascularization is a risk factor for complications during pregnancy, such as PE or even loss of the pregnancy (28, 29).

During a normal pregnancy there is an increase in the innate immune cell activation in the periphery (30). Cells involved in innate immunity, such as monocytes and granulocytes, are increased in the circulation and show an increase in cell activation during pregnancy (31, 32). Other inflammatory cells, such as DCs and NK cells, are decreased in the periphery during normal pregnancy (33, 34). The function of DCs has recently been studied and it was noted that co-stimulatory molecules responsible for DC tolerance are increased during normal pregnancy (35, 36).

In contrast to normal pregnancies, pregnancies affected by PE are associated with an improper immune response and milieu of alternately activated immune cells and cytokines (37, 38). Placental ischemia, resulting from the insufficient trophoblast invasion seen in PE, has been shown to produce an imbalance in immune function that leads to chronic inflammation and presents similarly to an autoimmune disease (28, 39, 40). This immune imbalance consists of increased pro-inflammatory immune cells and cytokines, and decreased regulatory immune cells and cytokines, which create a chronic and uncontrolled state of inflammation (38, 41–43). This alteration in immune balance is believed to contribute to the overall pathophysiology associated with PE, which includes the production of reactive oxygen species (ROS) (44, 45), increased endothelin-1 expression (10, 46–48), and B cell production of autoantibodies to the Angiotensin II (AngII) type 1 receptor (AT1-AA), which all culminate in the development of hypertension during pregnancy (1, 40, 49–52).

Pathophysiology of Preeclampsia

Inflammatory Cytokines

Preeclampsia is associated with chronic immune activation that leads to an increased production of inflammatory cytokines by pro-inflammatory T cells, and a decrease in regulatory and anti-inflammatory cytokines, which further promotes an inflammatory state during PE (53–56). This imbalance between pro-inflammatory and regulatory cytokines is associated with the placental ischemia that occurs during a preeclamptic pregnancy. This imbalance worsens as the pregnancy progresses (57).

Anti-inflammatory cytokines that help regulate the immune response, like interleukin (IL)-10 and IL-4, play important roles in a normal, successful pregnancy by providing a balance to the immune system (56). Other cytokines, such as tumor necrosis factor alpha (TNF-α), IL-6, and IL-17 which are pro-inflammatory in nature and are usually secreted from activated Th1 and Th17 cells during immunological challenges, promote cytotoxic and inflammatory responses (47, 54, 58–61). During PE, increased TNF-α and IL-6 are present in the circulation and in the trophoblast cells of the placenta, while IL-10 and IL-4 are

decreased (59, 62, 63). This imbalance leads to chronic peripheral and placental inflammation, which plays a role to further complicate the pregnancy.

In the vasculature, increased TNF-α and IL-6 both contribute to endothelial dysfunction, which is a hallmark feature of PE and is characterized by increased adhesion molecules and endothelial cell permeability (64, 65). TNF-α activates endothelial cells, decreases nitric oxide synthase mRNA, and increases the production of the potent vasoconstrictor endothelin-1 by increasing the expression of preproendothelin-1 mRNA (47, 66). Endothelin-1 has been shown in some studies to be increased by 2–3 fold in the circulation of women with PE; expression of its precursor, preproendothelin-1, in tissues of preeclamptic patients is also increased compared to normal pregnant women (67, 68). While endothelin-1 levels seen in preeclamptic patients may only be increased around 2–3 times that of normal pregnant patients, the highest levels of endothelin-1 are usually seen during the end of PE, which indicates that the role of endothelin-1 is to promote a further increase in blood pressure rather than initiate hypertension during pregnancy (10, 69). The role of endothelin-1 to increase blood pressure during pregnancy was demonstrated in a rat model of PE where a selective endothelin-1 type A (ET_A) receptor antagonist was administered and resulted in a significant decrease in blood pressure (70).

Oxidative stress occurs as an imbalance in pro- and anti-oxidant factors in the vasculature when the pro-oxidant factors surpass the anti-oxidant factors and leads to the production of ROS. Although usually considered harmful, ROS are generated during normal pregnancy and are required for the progression and development of a healthy pregnancy (71). During this time, antioxidant defenses that scavenge ROS are present to keep a balance between the pro- and anti-oxidant factors involved, which leads to a controlled host defense that protects the mother from the harmful effects of oxidative stress (71). During PE, however, this delicate balance is shifted toward pro-oxidant species and the anti-oxidant defenses are decreased and are insufficient in controlling these pro-oxidant factors, thus resulting in oxidative stress (72–75). This oxidative stress in the placenta contributes to the development of inflammation in the vasculature, which includes the production of inflammatory cytokines TNF-α, IL-6, and IL-17 ultimately leading to a state of endothelial dysfunction through recruitment of immune cells that release oxidative stress molecules (76).

Decreases in nitric oxide (NO) bioavailability is also associated with endothelial dysfunction due to the loss of vasodilator actions. Normal pregnancies are associated with increased nitric oxide production, which leads to changes in the vasculature and vascular/renal hemodynamics necessary for a healthy pregnancy (77–79). In addition, studies have shown that chronic nitric oxide synthase inhibition during pregnancy leads to an increase in total peripheral resistance, which culminates in the development of hypertension and intrauterine growth restriction (80). Thus, it is likely that a decrease in nitric oxide availability due to increased ROS production during PE is contributing to the increased blood pressure seen during the disease.

Expression of adhesion molecules in the vasculature is also increased by TNF-α, and both TNF-α and IL-6 lead to increased vascular permeability (81). Placental increases in TNF-α and IL-6, as seen during PE, have been shown to excessively increase the death of

trophoblast cells and increase endothelial activation (82). Ultimately, these inflammatory cytokines lead to endothelial dysfunction and the vasoconstrictor, endothelin-1, ROS and decreased NO which contribute to the hypertension present during PE (83, 84).

Agonistic Autoantibody to the Angiotensin II Receptor

Another role of TNF-α and IL-6 and IL-17 that contributes to hypertension during pregnancy is stimulating B cell production of agonistic autoantibody to the Ang II, type 1 receptor (AT1-AA). AT1-AA is produced by stimulated B cells during PE, whereas normal pregnancies have little to no AT1-AA detectable (50–52, 83–87). The renin-angiotensin system (RAS) and its ability to increase blood pressure have been investigated in preeclamptic pregnancies (88, 89). The components of the renin-angiotensin system (RAS) are increased during normal pregnancy, but the responsiveness of the vasculature to Ang II is decreased (89). During a preeclamptic pregnancy, women have decreased components of the RAS, and display an increase responsiveness to Ang II (89, 90). Heterodimerization of the AngII, type 1 (AT1) receptor occurs more frequently in women with PE and they also have an increased presence of the AT1 receptor in the placenta (91, 92).

The presence of AT1-AAs in women with PE has been previously reported (87). This autoantibody was discovered in the sera of preeclamptic patients and found to interact with the Ang II type 1receptor through binding of a seven amino acid stretch of the receptor, which activates the receptor and causes an increase in chronotropic response (87). Purified AT1-AA was shown to activate the AT1 receptor and elicit many effects that are associated with the pathophysiology seen during PE. Activation of the AT1 receptor by the autoantibody has been shown to increase the activation of pro-inflammatory transcription factors in the vasculature and trophoblast cells, which increase the production of TNF-α and ROS (1, 49, 85). AT1-AA activation of the AT1 receptor also increases vasoconstriction of the renal artery, expression of ET-1, and soluble anti-angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sflt-1), that are associated with PE (50, 93). Other studies have demonstrated a role for the AT1-AA in the contribution of hypertension during pregnancy by using an AT1 receptor blocker and B cell depletion to decrease the blood pressure in a rat model of PE (94).

T Lymphocytes During Preeclampsia

One population of immune cells that is altered and is believed to play a very important role in mediating preeclampsia is T lymphocytes (95–97). Increased production of inflammatory T cells is accompanied by a reduction in Tregs (98). This imbalance is seen in the circulation of women with PE as well as at the level of the placenta and creates a state of chronic inflammation during preeclamptic pregnancy, which is believed to contribute to the pathogenesis of the disease (41, 42). Several subclasses of $CD4^+$ T cells are involved in promoting an inflammatory state during PE. The Th1 and Th17 subclasses are responsible for promoting inflammation during PE, while Tregs and Th2 cells are decreased and therefore unable to properly control the inflammation associated with increased inflammatory T cell populations (99).

Studies have shown that a healthy pregnancy is associated with a decreased Th1/Th2 profile where Th2 cells are favored in the maternal peripheral circulation for the purpose of immunotolerance to the fetus (29, 56, 100–102). While this balance is important for pregnancy, other T lymphocyte subpopulations play a crucial role in pregnancy as well such as the pro-inflammatory Th17 cells (103). Th17 cells are present throughout normal pregnancy and studies have shown that this population of cells increases during late pregnancy and may have a role in contributing to the initiation of labor at term during normal pregnancy (104). Tregs are essential during pregnancy because of their role in promoting immune tolerance through regulation of Th1 from naïve T cells, along with their ability to secrete IL-10 (41, 95, 105, 106). Both Tregs and IL-10 decrease Th1 cells, which in turn decreases inflammatory cytokines such as IL-6 and TNF-α (103, 105). Low levels of indoleamine 2,3-dioxygenase (IDO) and diminished numbers of Tregs have been reported in the PE placenta (107, 108). Indoleamine 2,3-dioxygenase (IDO), an enzyme that mediates the conversion of tryptophan to kynurenine, is known to regulate T-cell activity and an endothelial-derived relaxing factor (107). In a recent study by Santillan et al, mice deficient for IDO (IDO-KO) were generated and evaluated for preeclampsia phenotypes (108). Pregnant IDO-KO mice exhibited renal glomerular endotheliosis, proteinuria, endothelial dysfunction, intrauterine growth restriction, and mildly elevated blood pressure compared to wild-type mice. Collectively these clinical and basic science studies indicate an important role for IDO and TREGS in normal renal function and blood pressure control during pregnancy. Furthermore, these studies demonstrate the importance of maintain a delicate balance between inflammatory and anti-inflammatory cells and cytokines in order to maintain a successful, healthy pregnancy.

A role of inflammatory T cells in the pathogenesis of PE has been established through adoptive transfer studies using Th1-like splenocytes. Zenclussen et al. (2004) demonstrated the importance of inflammatory T cells in a study where splenocytes were isolated from normal pregnant mice then cultured to promote differentiation of Th1-like cells that secreted Th1 specific cytokines (109). Adoptive transfer of these Th1-like cells into normal pregnant mice provoked symptoms of PE including an increase in blood pressure, alterations in kidney function, and increased markers of inflammation in the decidua. Adoptive transfer of the activated Th1-like splenocytes in non-pregnant mice did not result in any changes in immune composition or blood pressure response, demonstrating that a heighted vascular response to Th1-like cells is specific to pregnancy (109).

Reduced Uterine Perfusion Pressure (RUPP) Model of Preeclampsia

As previously discussed, PE results from improper placentation and trophoblast invasion during pregnancy, which leads to decreased blood flow to the placenta and fetus, creating a state of placental ischemia (28, 110). Placental ischemia in pregnant rats, promotes a chronic inflammatory response in the mother which contributes to the pathophysiology of PE, including increased mean arterial blood pressure (MAP), endothelial dysfunction, AT1-AA (87) (111), ET-1, chronic inflammation and oxidative stress and intrauterine growth restriction (46, 112–115). Development of an animal model that recapitulates the disease is vital. The reduced uterine perfusion pressure (RUPP) rat model of PE uses a combination of aortic constriction and occlusion of the uterine-ovarian arteries to decrease blood flow to the

uterus and create a state of placental ischemia in pregnant rats (116). Although with the RUPP rat we cannot examine mechanisms that lead to impaired trophoblast invasion, we can investigate pathways stimulated in response to placental ischemia that lead to the development of hypertension during pregnancy, since these rats develop a similar pathophysiology as women with PE. Mechanical induction of placental ischemia is performed in pregnant Sprague-Dawley rats. These rats are anesthetized on day 14 of gestation and a surgical procedure is performed to place restrictive silver clips on the abdominal aorta above the iliac bifurcation, and, in order to prevent compensatory blood flow through the ovaries, silver clips are also placed on branches of the ovarian arteries (116, 117). This procedure reduces uteroplacental blood flow by approximately 40% and increases blood pressure by approximately 20–30 mmHg versus normal pregnant Sprague-Dawley rats (116). These rats also demonstrate characteristics of endothelial dysfunction with a reduction in NO bioavailability (116) and increased contractility of vascular smooth muscle cells (118). Similarly to women with PE, the increase in Ang II receptor activity leads to an increased production of endothelin-1 in renal cortices, placentas, and in the circulation (119). ROS in both placenta and in circulation is increased in RUPP rats versus normal pregnant animals (120). In addition, antiangiogenic factors like sFlt-1 and soluble endoglin (sEng) are increased in RUPP animals, which further lead to decreases in blood supply to the placenta and fetus (121, 122).

Placental ischemia in preeclamptic patients leads to increases in inflammatory T cells and inflammatory cytokines accompanied by decreases in regulatory cells and regulatory cytokines, resulting in a chronic state of inflammation. This imbalance is the basis for the development of PE and we believe it to be responsible for some of the pathophysiology seen during the disease. Therefore, immune alterations associated with PE were validated in RUPP rats to demonstrate that these same imbalances occur in their immune repertoire as a result of placental ischemia. In comparison to normal pregnant rats, RUPP rats exhibit a chronic inflammatory state. An increase in inflammatory $CD4^+$ T cells is seen in these rats along with a decrease in Tregs. Moreover, increased inflammatory cytokines such as TNF-α and IL-6 and IL-17 are seen in the circulation of RUPP rats, while regulatory cytokines, IL-10 and IL-4, are decreased (47, 58).

The Role of $CD4^+$ T cells stimulated in RUPP rats to cause pathophysiology of PE

A study done in our lab was designed to test the impact of placental ischemia on $CD4^+$ T cells and the downstream effects of $CD4^+$T cells activation during pregnancy. In order to do so $CD4^+$ T cells from RUPP rats were adoptively transferred into normal pregnant rats which resulted in the development of many characteristics of PE. Normal pregnant rats that received RUPP $CD4^+$ T cells had 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, ruling out an immune rejection response (43). Adoptive transfer of RUPP $CD4^+$ T cells into normal pregnant rats also led to an increase in circulating inflammatory cytokines TNF-α, IL-6, and IL-17, and sFlt-1, AT1-AAs, ET-1 and ROS. Blood pressure increase in response to RUPP $CD4^+$ T cells was blocked by Tempol,

endothelin type A (ETA) receptor blockade and Losartan or Rituximab, for B cell depletion (94, 123). These adoptive transfer studies, along with the previously described Th1-like splenocyte study, indicate a strong role for preeclamptic inflammatory T cells in the development of hypertension during pregnancy, and in promoting the pathophysiology that accompanies the blood pressure increase.

The Effect of Decreased Tregs and IL-10 in Preeclampsia

Along with increased levels of inflammatory T cells, Tregs are decreased during PE in response to placental ischemia. These cells are identified by the expression of cell surface markers CD4 and CD25 and the specific internal transcription factor forkhead box protein 3 ($Foxp3^+$) (124). Tregs are responsible for suppression of responses by the adaptive and innate immune system and control unwanted immune responses through various mechanisms. Loss of Treg function has been shown to lead to autoimmune diseases and other immunopathology, including maternal loss of tolerance for the fetus during pregnancy (124). Tregs are increased very early in normal pregnancy and reach their highest levels during the second trimester before decreasing back to normal (125). The peak times for Tregs coincides with important processes like vascular remodeling and trophoblast invasion, which are integral to a healthy pregnancy. Without Treg function, immune processes would be chronically shifted towards uncontrolled activation and stimulation of inflammatory T cells and their secretion of inflammatory cytokines. Therefore, in diseases such as PE, where Tregs and their secretion of IL-10 and TGF-β are reduced (126), thereby leaving the stimulation and proliferation of inflammatory T cells unchecked.

Evidence for reduced Tregs to contribute to PE has been provided by many clinical studies. Tregs have been shown to be decreased both in the circulation and decidua of women with PE, and the decrease in Tregs is directly proportional to the severity of the disease (127). Decreased circulating Tregs has also been seen in women who have had multiple miscarriages (106). Studies have shown that decreases in decidual Tregs causes increased apoptosis in trophoblast cells, preventing sufficient invasion of trophoblasts into the decidua (128, 129).

Interleukin-10 is an important anti-inflammatory cytokine that is secreted by Tregs, but the cytokine also stimulates the production of Tregs from naïve T cells (130–132). IL-10 mediates inhibition of Th1 secreted inflammatory cytokines and provides an important balance for inflammation at the fetal-maternal interface (133). An important feedback mechanism exists between IL-10 and Tregs where IL-10 can induce the expression of transcription factor Foxp3, which is necessary for the production of Tregs (134). Other studies have also shown that IL-10 can increase differentiation of Tregs. In one study, naïve $CD4^+$ T cells were incubated with IL-10-producing dendritic cells that promoted the differentiation of IL-10 producing Tregs, highlighting the important feedback mechanism that exists between IL-10 and Tregs (135).

Pro-inflammatory cytokines down-regulated by IL-10 include IFN-y, IL-2, and TNF-α (136, 137). Levels of IL-10 are increased throughout normal pregnancy and first begin to drop when labor begins (138). In addition to Tregs, villous cytotrophoblasts also secrete IL-10

during pregnancy and the expression of IL-10 (139) and its receptor has been identified on a number of cell types found in the decidua such as trophoblasts, stromal cells, macrophages and uterine NK cells, but it is not clear how IL-10 may influence trophoblast invasion (140). However, the presence of IL-10 at the fetal-maternal interface and its strong anti-inflammatory properties are suspected to contribute to allogeneic tolerance of the fetus during normal pregnancy (63, 141–145).

During PE, reduced levels of IL-10 have been observed in circulation and in the placenta (143, 146, 147). Previous studies have demonstrated IL-10 to be a potent cytokine that regulates vasculature function and prevents vascular dysfunction (131, 146). IL-10 decreases inflammatory cytokines that are linked to oxidative stress, while promoting vascular healing, a necessary process for spiral artery remodeling and placental perfusion. These functions of IL-10 were shown to lead to a restoration of endothelin-dependent relaxation and increased eNOS expression in endothelin-1 treated aortic rings (148). IL-10 has also been shown to improve characteristics of PE in pregnant, DOCA/saline rats where investigators noted a decrease in circulating endothelin-1 and IFN-γ, restoration of aortic relaxation responses, decreased proteinuria and an improvement in litter size following IL-10 treatment (149).

Our most recent studies demonstrated that adoptive transfer of Tregs from normal pregnant rats into RUPP rats lowered blood pressure, minimized intrauterine growth restriction (IUGR), reduced the inflammatory response with a corresponding increase in anti-inflammatory cytokines, significantly decreased ET-1 expression, and placental oxidative stress. Importantly, Tregs also attenuated the production of agonistic AT1-AA (150). These data support a role for decreased Tregs in the pathophysiology of PE. In addition, the supplementation of normal pregnant Tregs prior to the time of induced placental ischemia (gestational day 12) suggests the importance of normal pregnant immune regulation in maintaining lower levels of inflammation, oxidative stress, AT1-AA, and ET-1, all of which are responsible for increasing blood pressure, and may ultimately result in a more normal fetal weight and safer blood pressures in response to placental ischemia.

Based on these studies, it appears that the significantly different cytokine profile seen in RUPP rats can be somewhat improved by increasing the Treg population. Restoring Tregs in RUPP animals also inhibited effector T cell activation, which may be the mechanism by which inflammation and oxidative stress were lowered (150). Without T-cell activation, inflammatory cytokine production would be decreased. This in turn would result in fewer inflammatory cells and less production of ROS. Again, a very important finding from this study was that adoptive transfer of Tregs inhibited the production of AT1-AA (150), which also could be occurring through prevention of T-cell mediated activation of B cells. Tregs also secrete immunosuppressive factors such as IL-10, which has been suggested by other studies to have direct effects in improving vascular function as well as inhibiting the inflammatory function of effector $CD4^+$ T cells. While the exact beneficial mechanism of Treg function needs to be further investigated, it is clear that Tregs play an important role in promoting a normal, healthy pregnancy and may prove to be a useful target for the treatment of PE.

In order to examine ways to safely increase Tregs during pregnancy we turned to IL-10 supplementation. Therefore, in a separate study, we supplemented IL-10 via osmotic mini pumps to RUPP rats to restore the circulating levels to that of normal pregnant rats (151). IL-10 supplementation lowered the overall number of circulating $CD4^+$ T cells while increasing the population of circulating Tregs to that of normal pregnant rats. In addition to normalizing Tregs, IL-10 supplementation also normalized pro-inflammatory cytokines TNF-α and IL-6 and, importantly, supplementation of IL-10 to RUPP rats significantly lowered AT1-AA, placental ROS and ET-1, which proved beneficial in decreasing blood pressure during pregnancy. Normal pregnant rats supplemented with IL-10 exhibited no changes in circulating inflammatory cytokines nor blood pressure (151).

In addition to these recent findings, our laboratory has previously shown that placental explants from preeclamptic patients secreted lower levels of IL-10 in culture under normal oxygen and hypoxic conditions compared to placental explants from normal pregnant patients (152). Lower levels of IL-10 during preeclampsia would allow for increased T cell activation and differentiation into a pro-inflammatory phenotype, thus, leading to an increased number of inflammatory T helper cells, cytokines and possibly B cell stimulation and thus AT1-AAs. In addition, B cell depletion in RUPP rats caused lower AT1-AA and blood pressure in response to placental ischemia, however this technique is not ideal for a growing fetus (153). In our recent study, IL-10 supplementation normalized Tregs and caused significant reductions in AT1-AA in response to placental ischemia. Furthermore, when treated with IL-10, RUPP rats had a significantly lower blood pressure than the untreated RUPP group, although not fully normalized to the degree of normal pregnant rats (151). We have previously shown that two mechanisms of hypertension stimulated by the AT1-AA activation of the AT1 receptor are ET-1 production and placental oxidative stress (85), both of which were reduced in RUPP rats treated with IL-10 (151).

Summary and conclusion

While the exact mechanisms by which PE occurs still remain unknown, studies investigating the immune imbalance that accompanies and contributes to the progression of the disease are beginning to identify possible targets for treatment. Knowing how different components of the immune system individually affect the progression and development of PE will be useful in developing drugs that are necessary to improve the outcomes of mothers and babies affected by the disease. Not only have studies demonstrated that regulatory immune factors IL-10 and Tregs decrease the pathophysiology of PE, but these studies also suggest that this decrease is largely mediated through AT1-AA reduction, ROS and ET-1. In addition, IL-10 and Tregs also improve the pathophysiology of PE through Th1 regulation and by exhibiting anti-inflammatory effects in general. While it may not be feasible to simply supplement Tregs and IL-10 to mothers with PE due to the immunosuppressive actions, the identification of these two important immune factors and their mechanisms does offer a starting point for developing new therapeutic targets for treatment of this disease.

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Summary Statement

Not only have studies demonstrated that regulatory immune factors IL-10 and Tregs decrease the pathophysiology of PE through reducing AT1-AA, ROS and ET-1, but they suggest that boosting these factors during pregnancy can improve the outcome of placental ischemia.

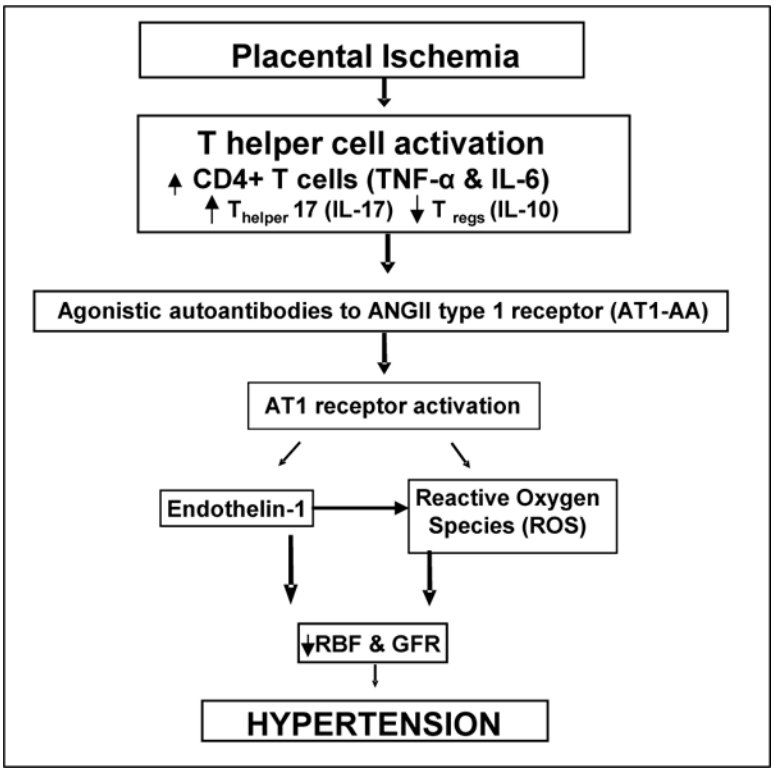

Figure.

CD4+ Tcells stimulated in response to placental ischemia exhibit an elevated TH17, decreased TREG ratio. Studies from our lab have shown that adoptive transfer of this ratio of CD4+ T cells is instrumental in causing AT1-AA, ET-1, ROS, and decreased renal excretory function, all important mediators of hypertension during PE,