Interleukin-10 reduces inflammation, endothelial dysfunction, and blood pressure in hypertensive pregnant rats

John H. Tinsley, Sanique South, Valorie L. Chiasson, and Brett M. Mitchell

Department of Internal Medicine, Division of Nephrology and Hypertension, Texas A&M Health Science Center College of Medicine/Scott & White Memorial Hospital, Temple, Texas

Submitted 30 October 2009; accepted in final form 2 January 2010

Tinsley JH, South S, Chiasson VL, Mitchell BM. Interleukin-10 reduces inflammation, endothelial dysfunction, and blood pressure in hypertensive pregnant rats. Am J Physiol Regul Integr Comp Physiol 298: R713–R719, 2010. First published January 6, 2010; doi:10.1152/ajpregu.00712.2009.—Hypertensive disorders of pregnancy are characterized by systemic and placental inflammation; however, treatment for these conditions has remained elusive. We tested whether administration of the anti-inflammatory cytokine interleukin-10 (IL-10) during pregnancy would attenuate the hypertension, endothelial dysfunction, proteinuria, and inflammation seen in pregnant DOCA/saline-treated (PDS) rats. Normal pregnant (NP) rats and PDS were given daily intraperitoneal injections of recombinant IL-10 from gestational day 13 until death on day 20. Systolic blood pressure, aortic endothelium-dependent relaxation responses, and urinary protein excretion were measured on days 13 and 20 of gestation. Fetal number and development, plasma endothelin-1 levels, serum and placental levels of IFNγ and IL-10, and aortic and placental levels of platelet endothelial cell adhesion molecule (PECAM) were assessed on gestational day 20. Systolic blood pressure, aortic endothelial dysfunction, and urinary protein excretion were significantly increased at gestational day 13 in PDS rats. However, all of these were restored to NP levels following IL-10 treatment in PDS rats. IL-10 treatment also significantly increased the number of pups per litter in PDS rats and did not further affect fetal development. The beneficial effects of IL-10 in PDS rats were likely mediated by the decreased plasma levels of endothelin-1, decreased levels of circulating and placental IFNγ, as well as decreased aortic and placental expression of PECAM. These data demonstrate that exogenous IL-10 can normalize blood pressure and endothelial function in pregnancy-induced hypertensive rats and may be beneficial in women with hypertensive disorders of pregnancy.

PREECLAMPSIA (PE), diagnosed as de novo hypertension and proteinuria during pregnancy, and other hypertensive disorders of pregnancy affect ~10% of pregnancies in the US and are responsible for 15–20% of maternal deaths worldwide (8). PE is associated with decreased fetal development and increased risk of future maternal heart disease. Although recent scientific findings have greatly aided in explaining the potential mechanisms involved in the development of PE, the exact causes remain unknown and effective treatments for PE remain elusive.

Abnormal maternal immune system responses play a key role in the development of PE (10, 13, 31). A current theory is that women who develop PE have abnormal immunological responses to the fetus and placenta and that the hypertension and proteinuria represent clinical signs of a mild form of fetal rejection, while severe forms of PE result in spontaneous abortion and fetal demise. Consistent with this abnormal immunological response, clinical studies have routinely reported increased serum levels of proinflammatory T helper type 1 (Th1) cytokines in women with PE. Additionally, studies (19–21) performed in pregnant rats made hypertensive by reducing uterine perfusion pressure have also demonstrated increases in proinflammatory cytokines. However, measures of anti-inflammatory T helper type 2 (Th2) cytokines have been shown to increase, decrease, or remain unchanged in women with PE compared with normal pregnant (NP) women (4, 16, 27). Recent reports (26, 27) have shown that proinflammatory cytokines are necessary for successful implantation and placental development, therefore decreasing inflammation following placentation might be a viable therapeutic target.

Interleukin 10 (IL-10) is an immunomodulatory Th2 cytokine that exerts anti-inflammatory effects through several mechanisms (3, 23). These include inhibition of NF-κB, which results in the reduction of the proinflammatory cytokines IL-1β, IL-6, and TNF-α, as well as the inhibition of tissue factor expression, IFN-induced gene transcription, IL-2, chemokines such as regulated on activation, normal T-expressed and secreted (RANTES), and metalloproteinases in monocytes/macrophages and CD4+ T cells (3, 23). IL-10 also suppresses inflammation by inducing suppressor of cytokine synthesis-3 as well as heme oxygenase-1 expression, both of which are decreased in PE (2, 42). Additionally, IL-10 plays a role in the differentiation of T cells into regulatory T cells, which aid in immunoregulation. With respect to pregnancy, serum and placental IL-10 levels have been reported to increase in normal pregnancy (12); however, reports in women with PE show decreased, unchanged, or increased serum and placental levels of IL-10 (12, 14, 33). In an LPS-treated rat model of fetal demise and intrauterine growth restriction (IUGR), administration of IL-10 attenuated the incidence of fetal death as well as growth restriction (29); however, the effects of IL-10 treatment on maternal blood pressure and endothelial function were not reported. Clinically, recombinant IL-10 has been used with moderate success for the treatment of psoriasis and rheumatoid arthritis (3, 18), both immunological diseases characterized by an increased Th1-mediated inflammatory state. Whether or not IL-10 can ameliorate the hypertension, endothelial dysfunction, and proteinuria seen in PE remains unknown.

We (15, 22, 34, 35) have reported previously an experimental model of PE in which pregnant rats treated with low concentrations of DOCA and 0.9% saline exhibit hypertension, proteinuria, endothelial dysfunction, and IUGR, whereas non-pregnant rats receiving identical treatment do not. These DOCA/saline-treated rats have increased vascular oxidative stress due to uncoupled nitric oxide synthesis and elevated
levels of the proinflammatory cytokines/chemokines IL-2, IL-12, IFNγ, and RANTES (22, 34). Furthermore, we (34) reported that excessive maternal immune responses play a major role in the development of PE-like symptoms in these rats as immunosuppression with either azathioprine or mycophenolate mofetil normalized systolic blood pressure, endothelial function, urinary protein excretion, and cytokine profiles. However, detrimental fetal effects were seen, especially with mycophenolate mofetil, a known teratogen, thus limiting their potential clinical use in women with PE.

Therefore, we examined whether increasing levels of the anti-inflammatory cytokine IL-10 could attenuate the PE-like symptoms in pregnant DOCA/saline-treated (PDS) rats yet have no detrimental effects on fetal development. We hypothesized that administration of recombinant IL-10 to PDS rats would restore systolic blood pressure, aortic relaxation responses, urinary protein excretion, and inflammatory cytokines and markers to NP levels, yet have no effect on fetal development when administered after placenta.

METHODS

Animals/treatments. Male breeder and female Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 200–250 g were acclimated for 1 wk and housed in groups on a 6 AM lights on/6 PM lights-off cycle in standard housing conditions. Female PDS rats were made hypertensive as described previously (15, 22, 34). Female PDS rats received weekly intraperitoneal injections of a low concentration of DOCA (week 1 PDS rats received weekly intraperitoneal injections of a low concentration of DOCA (week 1 = 12.5 mg, week 2 = 6.25 mg, and week 3 = 6.25 mg) and given 0.9% saline to drink, while NP rats were given tap water to drink. Standard rat chow and either saline or tap water was given ad libitum. Some NP and PDS rats were killed on day 13 of gestation to examine blood pressure, endothelial function, and proteinuria before IL-10 treatment. Other NP and PDS rats were treated with either 10 mg/kg rat recombinant IL-10 (Invitrogen, Carlsbad, CA) or vehicle by daily intraperitoneal injection beginning on gestational day 13 and formed the NP, NP + IL-10, PDS, and PDS + IL-10 groups, respectively. These injections were given at 11 AM each day and continued until the animals were killed on gestational day 20. All procedures were approved by the Texas A&M Health Science Center/Scott & White Memorial Hospital Institutional Animal Care and Use Committee.

Blood pressure. Systolic arterial blood pressure was measured by tail-cuff plethysmography as described previously, and measurements were taken before any injections on that day (22, 34). Rats were trained for 3 days before data collection, and measures were taken at baseline and on gestational days 13 and 18. Although the rats were killed on day 20, day 18 blood pressures were taken to avoid the effects of metabolic cage housing (described below) on systolic blood pressure.

Endothelial function. Isometric force generation of aortic rings were measured as previously described (22, 34). Aortic relaxation responses were assessed in some NP and PDS rats on gestational day 13, before the initiation of IL-10 treatment, and on gestational day 20 in NP, NP + IL-10, PDS, and PDS + IL-10 rats. Indomethacin (10 μM) was present in all experiments to inhibit prostacyclin production for further examination of nitric oxide-mediated relaxation responses. Endothelium-dependent relaxation to acetylcholine (1 nM to 100 μM) was measured following contraction to an EC50 concentration of phenylephrine and was expressed as percent relaxation from phenylephrine-induced contraction.

Proteinuria. One day before death, urine was collected from rats housed individually in metabolic cages and urinary protein and creatinine concentrations were measured as described previously (22, 34). Results are expressed as the ratio of urinary protein to creatinine (mg/ml).

Fetus, serum, and tissue analyses. Pup number and number of malformed fetuses were noted at death on gestational day 20. Aortic and placental tissue, along with blood obtained through the renal artery, were collected at time of death on gestational day 20. Serum was obtained from clotted blood centrifuged at 10,000 rpm for 10 min. For determination of endothelin-1, EDTA-treated plasma was collected and subjected to an extraction procedure followed by the immunoassay according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN). Rat-specific ELISAs for IFNγ and IL-10 (Pierce, Rockford, IL) were performed on 100 μl of serum in duplicate per the manufacturer’s protocol. Sensitivity of the assays are as follows: endothelin-1: <1.0 pg/ml, IFNγ: <2 pg/ml, and IL-10: <3 pg/ml. For aortic and placental measures, tissues were homogenized in cell lysis buffer (Cell Signaling, Boston, MA) and protein concentrations were determined using the Bradford assay. IFNγ and IL-10 were also measured in placental lysates (100 μg protein) by ELISA (Pierce, Rockford, IL). Immunoblotting was performed using 40 μg of protein and primary antibodies for platelet endothelial cell adhesion molecule (PECAM)-1 (Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (Sigma, St. Louis, MO). Secondary antibodies consisted of anti-goat and anti-mouse IgGs conjugated to IRDye800CW (LI-COR Biosciences, Lincoln, NE). The bands were identified simultaneously using near-infrared visualization (Odyssey System; LI-COR Biosciences). Densitometry was performed using the Odyssey software and is expressed as the ratio of PECAM to β-actin.

Statistics. Data are presented as means ± SE. ANOVA was used for comparisons between the NP, PDS, NP + IL-10, and PDS + IL-10 groups for all measures followed by the Student-Newman-Keuls post hoc test when necessary. SigmaStat 3.5 (Systat Software, San Jose, CA) was used to perform all statistical analyses. The significance level was 0.05.

RESULTS

Blood pressure. PDS rats exhibit hypertension at day 18 of gestation (15, 22, 34, 35). In this study, we found that systolic pressure is also significantly elevated at gestational day 13 in PDS rats (Fig. 1). However, systolic blood pressure in PDS rats receiving daily injections of IL-10 decreased back to baseline and NP levels at day 18 (Fig. 1). IL-10 did not significantly alter systolic blood pressure in NP rats (Fig. 1).

Fig. 1. Effects of IL-10 treatment on systolic blood pressure in normal pregnant (NP) and pregnant rats treated with DOCA + 0.9% saline (PDS) rats. PDS significantly elevated systolic blood pressure at gestational days 13 and 18 in rats compared with NP rats. Daily IL-10 treatment beginning on gestational day 13 reduced systolic blood pressure in PDS rats. Results are means ± SE (n for each group is in parentheses). *P < 0.05 vs NP at the same time point.
**Endothelial function.** Hypertensive disorders of pregnancy are associated with endothelial dysfunction, and we have reported impaired endothelium-dependent relaxation responses in blood vessels from PDS rats previously (22, 34). In the current study, we found that endothelial dysfunction was evident by gestational day 13 before the initiation of IL-10 treatment, as demonstrated by significantly decreased aortic relaxation responses in PDS rats (Fig. 2A). However, IL-10 treatment restored aortic relaxation responses of PDS rats to NP levels (Fig. 2B). IL-10 did not alter acetylcholine-induced relaxation responses in the NP group (Fig. 2B).

**Proteinuria.** Increases in urinary protein output are one of the defining factors associated with PE. There were no significant changes in urinary protein-to-creatinine ratio during gestation in NP rats (Fig. 3). However, PDS rats exhibited a significant increase in proteinuria compared with the NP animals at both gestational days 13 and 20 (Fig. 3). Treatment with IL-10 during gestation normalized the urinary protein-to-creatinine ratio in rats to levels of NP rats and had no significant effect on NP urinary protein excretion (Fig. 3).

![Fig. 2. Effects of IL-10 treatment on aortic relaxation responses in NP and PDS rats. A: PDS significantly decreased aortic endothelium-dependent relaxation responses in rats compared with NP rats on gestational day 13 (P < 0.05 vs. NP). Treatment with IL-10 restored aortic relaxation responses to NP levels in PDS rats at gestational day 20. Results are means ± SE (n = 4–7 in each group). *P < 0.05 vs NP.](http://ajpregu.physiology.org/)

**Fetal development.** IUGR is often associated with hypertensive disorders of pregnancy, and our PDS rats consistently had a smaller number of pups per litter as well as several malformed fetuses per litter (22, 34). The number of pups per litter in NP rats was 14.5 ± 0.3 compared with PDS rats, which had 11.2 ± 0.7 (P < 0.05). Treatment with IL-10 had no effect on the number of pups per litter in NP rats (13.2 ± 0.9; P > 0.05 vs. NP) but significantly increased the number of pups per litter in PDS rats (15.6 ± 0.7; P < 0.05 vs. PDS). With respect to malformed fetuses, NP rats had 0.13 ± 0.13 malformed fetuses per litter; however, PDS rats had 0.57 ± 0.30 malformed fetuses per litter. IL-10 treatment had no significant effect on fetal development, as NP rats treated with IL-10 had 0.50 ± 0.50 malformed fetuses per litter (P > 0.05 vs. NP) and PDS rats treated with IL-10 had 0.56 ± 0.38 malformed fetuses per litter (P > 0.05 vs. PDS).

**Plasma endothelin-1.** Endothelin-1, a potent vasoconstrictor, is produced primarily in the endothelium and is elevated in numerous forms of hypertension. Several rat models exhibiting hypertensive pregnancies, such as the reduced uterine perfusion pressure and nitric oxide synthase inhibition-induced models, all have elevated plasma levels of endothelin-1 (11, 30). In our PDS rats, we found an almost twofold increase in plasma endothelin-1 levels compared with NP rats at gestational day 20 (Fig. 4). This increase is consistent with the elevated systolic pressure and endothelial dysfunction seen in the PDS rats (Figs. 1 and 2B). Treatment with IL-10 completely blocked the PDS-induced increase in endothelin-1 and had no effect on plasma endothelin-1 levels in the NP group (Fig. 4).

**Serum and placental cytokine levels.** To assess the efficacy of recombinant IL-10 administration, we measured serum IL-10 levels by ELISA in NP, PDS, NP + IL-10, and PDS + IL-10 rats. Figure 5A demonstrates a marked increase in serum IL-10 levels in both NP and PDS rats treated with IL-10, confirming delivery of the recombinant cytokine. The significant increase in IL-10 levels in untreated PDS rats is consistent with our previous report (34) and likely represents a compensatory increase in response to excessive maternal immune system...
activation. Serum levels of IFNγ were significantly increased in PDS rats at gestational day 20; however, this was normalized by IL-10 treatment (Fig. 5B). Similarly, placental IFNγ levels were significantly increased in PDS rats at gestational day 20, and these were normalized by IL-10 treatment (Fig. 5C). IL-10 treatment had no effect on serum or placental IFNγ levels in NP rats.

Aortic and placental PECAM-1 levels. It is known that adhesion molecules such as PECAM-1 are elevated in patients with pregnancy-induced hypertension and PE and that PECAM correlates positively with disease severity (39). PECAM-1 levels were increased approximately threefold in the aortas (Fig. 6A) and placentas (Fig. 6B) from PDS rats compared with those from NP animals. However, IL-10 blocked the PDS-induced increase in PECAM-1 expression in both tissues.

**DISCUSSION**

Despite an increase in clinical and basic science research focused on PE, as well as an increasing incidence of PE in women, no treatment options exist besides delivery. In an effort to determine whether IL-10 may be a viable therapeutic in women with PE, we tested the effects of IL-10 treatment on PE-like symptoms in a rat model of PE. Novel findings of the current study include the following: 1) the presence of hypertension, endothelial dysfunction, and proteinuria at gestational day 13 in PDS rats; 2) IL-10 treatment during gestation normalized blood pressure, endothelial function, urinary protein excretion, and pup number per litter; and 3) IL-10 delivered systemically decreased plasma levels of endothelin-1 and serum levels of IFNγ, placental levels of IFNγ, and aortic and placental levels of PECAM expression.

Although PE is strictly a human disease, several animal models have been important in understanding the mechanisms that occur during the development of PE. However, some models produce hypertension, endothelial dysfunction, and proteinuria in nonpregnant animals as well, thus limiting their interpretations. In contrast to other DOCA/saline animal models of hypertension in which uninephrectomies are performed and high concentrations of DOCA are administered, rats treated with low concentrations of DOCA and 0.9% saline only exhibit hypertension, endothelial dysfunction, and proteinuria if pregnant, supporting their usefulness as an animal model of PE (15, 22, 35). These rats also exhibit excessive immune system activation, similar to women with PE, as DOCA/saline...
treatment promotes T-cell activation and TNF-α-mediated detrimental cardiovascular effects (34). While the cardiovascular effects of pregnancy plus DOCA/saline treatment have been reported at the end of gestation (15, 22, 34, 35), it was unknown when these PE-like symptoms develop during pregnancy. Here we report that hypertension, endothelial dysfunction, and proteinuria were all evident by gestational day 13 in PDS rats. These data suggest that following placentation (days 7–10 in rats) the “clinical” symptoms of PE are present, which corresponds well with women who are diagnosed with PE at or after the 20th wk of pregnancy. Thus PDS rats likely represent an animal model of early onset PE (28).

IL-10 exerts its primary anti-inflammatory effects by inhibiting T-cell proinflammatory cytokine production and the antigen-presenting capabilities of macrophages and dendritic cells (3, 23, 32). These actions make it a promising therapeutic for immunological diseases characterized by excessive inflammation. Clinical trials in healthy volunteers have demonstrated that recombinant IL-10 treatment is safe, effective, and well tolerated (9). IL-10 therapy has been tested in patients with various inflammatory disorders including organ transplant recipients, rheumatoid arthritis, Crohn’s disease, and psoriasis (3). Thus IL-10 could potentially be used for the treatment of PE, as PE is also characterized as a Th1-dominant, excessive inflammatory disorder (26). Other actions of IL-10 support its use as a PE therapeutic. IL-10 induces a Th2 cytokine predominance, which has been shown to be prevalent in successful pregnancies (24, 25, 36). IL-10 may also reverse the down-regulation of suppressor of cytokine synthesis-3 as well as heme oxygenase-1 evident in PE, as well as restore endothelial function and nitric oxide bioavailability by preventing oxidative stress (40, 41). All of these effects would be expected to lead to a decrease in blood pressure, endothelial dysfunction, and proteinuria. When we administered recombinant IL-10 to hypertensive pregnant rats following placentation, we saw a normalization of systolic blood pressure, endothelial function, and urinary protein excretion. Furthermore, we saw an increase in the number of pups per litter in PDS rats and, importantly, no further detrimental effects on fetal development in both PDS and NP rats. Thus IL-10 had similar beneficial effects on cardiovascular parameters in this model as immunosuppressive agents (34), but it had better outcomes related to fetal devel-

Fig. 6. Effects of IL-10 treatment on aortic and placental platelet endothelial cell adhesion molecule (PECAM) expression in NP and PDS rats. A: PDS significantly increased aortic PECAM expression in rats compared with NP rats on gestational day 20. IL-10 treatment normalized aortic PECAM expression in PDS rats. B: PECAM expression was significantly increased in placentas from PDS rats compared with NP rats on gestational day 20. IL-10 treatment normalized placental PECAM expression in PDS rats. Results are means ± SE (n = 4–7 in each group). *P < 0.05 vs NP.
opment. Additionally, IL-10 treatment had no cardiovascular effects in NP rats. These data support the examination of IL-10 as a possible treatment option for PE.

We have previously reported that PDS rats exhibit increased serum levels of the proinflammatory cytokines IL-2, IL-12, and IFNγ and the chemokine RANTES on gestational day 20 (34), similar to women with PE compared with NP women (4, 5, 7, 16, 17, 27). Here we report that rats treated with DOCA/saline during pregnancy also exhibit significantly increased serum levels of endothelin-1, which may contribute to the decreased endothelial function and increased systolic blood pressure evident in these rats. Serum levels of endothelin-1 are also increased in women with PE (1, 6). IL-10 has been shown ex vivo to inhibit endothelin-1-mediated endothelial dysfunc-
tion in rodent blood vessels (40). In the current study, we saw normalization of plasma endothelin-1 levels as well as vascular relaxation responses following IL-10 treatment in DOCA/saline-treated rats; however, whether IL-10 directly affects endothelin-1 production by endothelial cells, which contain IL-10 receptors, warrants further investigation. Given the known effect of IL-10 on Th1 cytokine suppression, we ex-
ected serum IFNγ levels to decrease and this was found to be true. However, it was unknown whether exogenous IL-10 could reach and alter placental cytokine production. Placental IFNγ levels were increased significantly in PDS rats; however, IL-10 treatment restored these levels to those of NP rats. This finding suggests that increasing serum levels of IL-10 are sufficient to suppress the proinflammatory state of the placenta during DOCA/saline treatment. PECAM plays a key role in leukocyte adhesion and inflammation, and its expression is positively associated with PE severity (38, 39). Our PDS rats exhibit markedly increased levels of PECAM in both the aorta as well as the placenta, demonstrating the increased inflammatory state of these vascular beds in PDS rats compared with NP rats. IL-10, through its known effects of inhibiting tissue factor and adhesion molecule expression (3, 23), significantly re-
duced aortic and placental PECAM expression in PDS rats. Taken together, these data suggest that the reduction in sys-
temic and placental inflammation by IL-10 is in part mediated by decreased endothelin-1, IFNγ, and PECAM levels and may contribute to the beneficial effects of IL-10 on blood pressure and endothelial function in this experimental model of PE.

Given the predominant anti-inflammatory effects of IL-10 and excessive inflammation in PE, one would expect a defi-
ciency of IL-10 in women with hypertensive pregnancies. Some studies have reported decreased IL-10 levels in women with PE, while others have reported increased levels. This is likely explained by the complex temporal and cell-specific actions of IL-10 and IL-10 release during pregnancy. High levels of IL-10 would be expected to aid in the termination of the inflammation process and increase as a compensatory mechanism. Interestingly, IL-10-deficient mice exhibit normal pregnancies but altered fetal growth (37); however, blood pressure and endothelial function during pregnancy in these mice have not been reported. Thus IL-10 is not mandatory for fetal and placental tolerance yet may modulate the maternal immune response to pregnancy. This is currently under inves-
tigation in our laboratory. In conclusion, DOCA/saline treat-
ment during pregnancy induces hypertension, endothelial dys-
function, and proteinuria that are evident following placenta-
tion, and IL-10 treatment during the latter half of pregnancy is able to normalize these symptoms by decreasing both sys-
temic and placental mediators of inflammation. Addition-
ally, exogenous IL-10 exerts no significant effects on fetal development and may be a potential therapeutic for women diagnosed with PE.

Perspectives and Significance

These data demonstrate that treatment of hypertensive preg-
nant rats with exogenous IL-10 during the latter part of gesta-
tion is effective at lowering systolic blood pressure and nor-
malizing endothelial function, urinary protein excretion, and proinflammatory cytokines both at the systemic and placental level. IL-10, which is used clinically for other inflammation-related diseases, may be beneficial in women with PE or pregnancy-induced hypertension.

ACKNOWLEDGMENTS

We thank Darijana Horvat and Dr. Jules B. Buschett for assistance and use of the Nova electrolyte analyzer.

GRANTS

This work was supported by a Scott & White Research Development Grant.

DISCLOSURES

None of the authors have any financial interests or are associated with any companies that have a financial interest in the information contained within this manuscript.

REFERENCES


