Cotreatment With Interleukin 4 and Interleukin 10 Modulates Immune Cells and Prevents Hypertension in Pregnant Mice

Piyali Chatterjee,1 Valorie L. Chiasson,1 Geetha Seerangan,1 Richard P. Tobin,2 Shelley E. Kopriva,1 M. Karen Newell-Rogers,2 and Brett M. Mitchell1

BACKGROUND

Excessive maternal immune system activation plays a central role in the development of the hypertensive disorder of pregnancy preeclampsia (PE). The immunomodulatory cytokines interleukin 4 (IL-4) and interleukin 10 (IL-10) are dysregulated during PE; therefore we hypothesized that treatment with both recombinant IL-4 and IL-10 during pregnancy could prevent the development of PE in mice.

METHODS

Using our mouse model of PE in which immune system activation is induced by the double-stranded RNA receptor agonist poly I:C, we gave daily injections of IL-4, IL-10, or both on days 13–17 of pregnancy. Mice were then killed on day 18.

RESULTS

Poly I:C caused a significant increase in systolic blood pressure in pregnant (P-PIC) mice compared with vehicle-treated pregnant (P) mice. All 3 treatments significantly decreased blood pressure in P-PIC mice to P levels, ameliorated the endothelial dysfunction, and decreased placental TLR3 levels in P-PIC mice. However, only IL-4/IL-10 cotreatment prevented the proteinuria and increased incidence of fetal demise in P-PIC mice; IL-4 or IL-10 alone had no effect. Additionally, only IL-4/IL-10 cotreatment prevented the significant increase in CD3+γδ T cells and CD11c+ dendritic cells and significant decrease in CD11b+CD14+ suppressor monocytes, as well as completely prevented placental necrosis, in P-PIC mice. Importantly, IL-4/IL-10 cotreatment in P mice had no detrimental effects.

CONCLUSIONS

Taken together, these data demonstrate that exogenous IL-4 and IL-10 administration concurrently during pregnancy can normalize immune cell subsets and prevent PE induced by maternal immune system activation.

Keywords: blood pressure; endothelial dysfunction; hypertension; interleukin; polynosinic polycytidylic acid; preeclampsia; proteinuria.

doi:10.1093/ajh/hpu100

Preeclampsia (PE) is a multisystem pregnancy disorder affecting 5%–8% of women worldwide.1 Hypertension and proteinuria or end-organ damage are the clinical symptoms for diagnosis of the disease, with symptoms appearing at or after the 20th week of gestation.2 Although recent studies have shed light on the underlying mechanisms of PE, the exact etiology of the disease is unknown; thus treatment options for PE are still elusive.

A complex network of cytokines regulates the maternal immune response and inflammation, which prevents the fetus from being rejected. Although inflammation aids in various stages of embryo implantation, placentation, and parturition, it is tightly controlled. Both enzyme-linked immunosorbent assay and immunohistochemical studies suggest that anti-inflammatory cytokines are predominant in the first and second trimester in normal pregnant women.3 Because PE is characterized by maternal immune system dysfunction and excessive inflammation, it is possible that timely administration of anti-inflammatory cytokines will be beneficial in women with PE.

Interleukin 4 (IL-4) and interleukin 10 (IL-10) are important mediators of anti-inflammatory immune responses. IL-4 is a pleiotropic cytokine that is produced by T and B cells and stimulates the production of immunoglobulin E and immunoglobulin G1 antibody production. IL-4 is also known to increase major histocompatibility complex class II and CD23 and inhibit proinflammatory cytokines.4 IL-4 is also detectable at the feto-maternal interface.5 Women suffering from unexplained recurrent abortion show a defect in IL-4 production by decidual CD4+ T cells.5 Several clinical studies have also reported a reduction of serum IL-4 levels in women with PE.5–9

IL-10 is produced by immune cells, such as T-helper (Th) 2, Th1, and Th17, and nonimmune cells that include keratinocytes and epithelial cells.10 IL-10 mediates its...
anti-inflammatory effect by inhibiting proinflammatory cytokines such as interleukin 1, interleukin 6 (IL-6), interleukin 12, and tumor necrosis factor (TNF). Proinflammatory chemokines such as CC and CXC are also inhibited by IL-10. IL-10 inhibits major histocompatibility complex class II expression as well as costimulatory molecules. Interestingly, IL-10 is constitutively expressed in placental cytotrophoblasts. The lack of IL-10 is associated with adverse pregnancy outcomes, including recurrent spontaneous abortion, preterm birth, and PE. Whether or not treatment with IL-4 or IL-10 alone or a combination of IL-4 and IL-10 can prevent PE-like symptoms in mice is unknown.

Excessive inflammation induced by pathogens leads to adverse pregnancy outcomes. Invading pathogens or products from necrotic tissue serve as ligands for pattern recognition receptors such as the Toll-like receptors (TLRs), which lead to activation of signaling cascades and the production of proinflammatory cytokines. A low dose of endotoxin, a TLR4 agonist, administered to pregnant rats causes PE-like symptoms. Kim et al. demonstrated that TLR4 expression is increased in placental interstitial trophoblasts of patients with PE compared with normal pregnant women. Activation of TLR3 (a double-stranded RNA receptor) with the agonist poly I-C on gestation day 6.5 in abortion-prone CBA/J female mice mated with male DBA/2J mice leads to increased fetal loss. We have reported that poly I-C administered to pregnant C57Bl/6J female mice beginning at gestation day 13 induces PE-like symptoms, including hypertension, endothelial dysfunction, proteinuria, and fetal demise. Serum levels of IL-4 fail to increase in mice treated with poly I-C compared with normal pregnant mice, whereas IL-10 levels increase to try to limit inflammation. Furthermore, either IL-4 or IL-10 deficiency in pregnant mice induces mild PE-like symptoms. Because IL-4 and IL-10 play important immunomodulatory roles, we hypothesized that administration of recombinant (r) IL-4, IL-10, or a combination of IL-4 and IL-10 would prevent PE-like symptoms in mice by beneficially modulating immune cell subsets.

METHODS

Animals and treatments

Male breeder and female mice (C57BL/6J, stock No. 002253) were obtained from the Jackson Laboratory (Bar Harbor, ME). Adult female mice (aged 8-12 weeks) were mated and grouped either as pregnant (P) or pregnant treated with poly I:C (P-PIC). P mice received intraperitoneal injections of saline (vehicle), whereas P-PIC mice received intraperitoneal injections of poly I:C (20 mg/kg) on days 13, 15, and 17. Both P and P-PIC mice received daily intraperitoneal injections of either rIL-4 (10 µg/kg), rIL-10 (10 µg/kg), or a combination of rIL-4 (10 µg/kg) and rIL-10 (10 µg/kg) from gestational day 13 to gestational day 17. Mice were killed on gestational day 18, and organs were harvested, frozen in liquid nitrogen, and stored at -80 °C for subsequent studies. All experimental procedures were approved by the Texas A&M Health Science Center/Baylor Scott & White Health Institutional Animal Care and Use Committee.

Blood pressure

Systolic blood pressure (SBP) was measured in conscious animals using noninvasive, automated tail-cuff plethysmography as described previously. The average of 3 measurements was used for data analyses.

Urinary protein excretion

Urine was collected from all groups of mice on gestational day 18, and urinary protein and creatinine concentrations were measured as described previously. Results are expressed as the ratio of urinary protein to creatinine concentration.

Endothelial function

Endothelial function as measured by aortic vascular reactivity was performed as described previously. Endothelium-dependent relaxation to acetylcholine (1 µmol/L – 100 µmol/L) and endothelium-independent relaxation to sodium nitroprusside (0.1 µmol/L – 0.1 µmol/L) were measured after contraction with phenylephrine (1 µmol/L).

Flow Cytometry

Splenocytes from each spleen were stained with fluorescence-conjugated antibodies for different cell types or isotype controls for 30 minutes at 4 °C in the dark. Flow cytometry (BD FACS Canto II, BD Biosciences, San Diego, CA) was performed to identify the following cell surface markers: CD3+/γδ+ T cells, CD3+/CD4+ T cells, CD3+/CD8+ T cells, CD3+/γδ+ T cells, CD11c+ dendritic cells (DCs), and CD11b+/CD14+ myeloid-derived suppressor cells (MDSCs). The fluorochrome-conjugated antibodies were obtained from either BD Biosciences (San Diego, CA) or ebioscience (San Diego, CA). Data are expressed as percentages of total splenocytes.

Serum cytokines

Mouse Th1/Th2/Th17 Cytokine MultiAnalyte ELISAs (SA Biosciences, Valencia, CA) were performed using 50 µl of serum per the manufacturer’s protocol.

Western blots

Western blot analyses were performed using a primary antibody for TLR3 (Imgenex, San Diego, CA) at 1:1,000 dilution. Beta-actin (Sigma, St. Louis, MO) at 1:10,000 dilution was used as a loading control. The secondary antibodies consisted of antimouse and antirabbit immunoglobulin Gs conjugated to Alexa-Fluor 680 or IRDye 800 (LI-COR Biosciences, Lincoln, NE). Infrared visualization was used followed by densitometric analyses using the provided software (Odyssey System; LI-COR Biosciences).

Hematoxylin and eosin staining

Placentas from all groups were harvested on gestational day 18, and placental tissue was processed for
histopathologic analyses using hematoxylin and eosin staining. Placentas were immediately fixed in 10% formaldehyde, dehydrated, and embedded in paraffin. Paraffin-embedded tissues were then sectioned (4 μm), mounted on glass slides, and deparaffinized. The hydrated sections were stained with hematoxylin and eosin using standard procedures.

**Statistics**

Data are presented as means ± SEMs and analyzed using SigmaStat 12 (Systat Software, San Jose, CA). An analysis of variance was used for comparisons between the groups for all measures, and if statistical significance was obtained, then it was followed by the Student–Newman–Keuls post hoc test. The significance level was 0.05.

**RESULTS**

**Effect of IL-4, IL-10, or IL-4/IL-10 treatment on SBP, urinary protein excretion, aortic relaxation responses, and fetal development**

To determine whether administration of either IL-4 or IL-10 alone or in combination prevents the development of hypertension in our TLR3-induced PE mouse model (P-PIC), we measured SBP at baseline, gestational day 13, and gestational day 17. P-PIC mice exhibited significantly increased SBP at gestational day 17 compared with vehicle-treated P mice (P-PIC: 139 ± 5 mm Hg vs. P: 103 ± 3 mm Hg, P < 0.05) (Figure 1a), which is consistent with our previous findings.21 IL-4, IL-10, or IL-4/IL-10 had no effect on SBP in P mice compared with vehicle-treated P mice (Figure 1a). However, IL-4, IL-10, or IL-4/IL-10 treatment all prevented the increase in SBP in P-PIC mice (gestational day 17 SBP: P-PIC + IL-4: 104 ± 1 mm Hg, P-PIC + IL-10: 100 ± 2 mm Hg, P-PIC + IL-4 + IL-10: 99 ± 2 mm Hg; all P < 0.05 vs. P-PIC) (Figure 1b).

Increased urinary protein excretion is characteristic of PE. We confirmed that P-PIC mice develop proteinuria (Figure 1c); however, there were no significant changes in urinary protein to creatinine ratio in P mice treated with IL-4, IL-10, or IL-4/IL-10 compared with vehicle-treated P mice (Figure 1c). IL-4 or IL-10 treatment alone had no effect on the development of proteinuria in P-PIC mice (urinary protein/creatinine ratio: P-PIC: 5.19 ± 0.42, P-PIC + IL-4: 5.04 ± 0.83, P-PIC + IL-10: 6.40 ± 1.32) (Figure 1d). In contrast, only treatment with IL-4/IL-10 prevented the proteinuria in P-PIC mice (P-PIC + IL-4 + IL-10: 2.82 ± 0.46; P < 0.05 vs. P-PIC) (Figure 1d).

PE is characterized by endothelial dysfunction.23 We confirmed that P-PIC mice exhibit a significant decrease in aortic acetylcholine-mediated relaxation responses compared with P mice (Figure 1e).21 IL-4, IL-10, or IL-4/IL-10 treatment had no effect on acetylcholine-mediated relaxation responses in P mice; however, all 3 treatments prevented the decrease in acetylcholine-mediated relaxation responses in P-PIC mice (Figure 1f). There were no differences in aortic phenylephrine-induced contractions or endothelium-independent relaxation responses to sodium nitroprusside in any of the groups (data not shown).

PE is also associated with intrauterine growth restriction in women. P and P-PIC mice treated with either IL-4 or IL-10 alone or IL-4/IL-10 cotreatment did not exhibit significant changes in the mean number of pups per litter compared with vehicle-treated P mice. P-PIC mice had an increased incidence of fetal demise compared with P mice. There was no significant change in fetal demise in P mice treated with IL-4, IL-10, or IL-4/IL-10 (data not shown). Treatment with IL-4 or IL-10 alone did not decrease the high incidence of fetal demise in P-PIC mice (P-PIC: 1.55 ± 0.37, P-PIC + IL-4: 2.55 ± 0.94, P-PIC + IL-10: 1.43 ± 0.24); however, IL-4/IL-10 cotreatment prevented the increase in fetal demise in P-PIC mice (P-PIC + IL-4 + IL-10: 0.30 ± 0.15; P < 0.05 vs. P-PIC).

**Effect of IL-4, IL-10, or IL-4/IL-10 treatment on immune cell subsets**

PE is associated with excessive immune system activation. Therefore, we first evaluated immune system activation by measuring spleen weight to body weight ratios. P-PIC mice exhibited splenomegaly (P-PIC: 6.2 ± 1.0 mg/g vs. P: 2.3 ± 0.2 mg/g; P < 0.05). Treatment with IL-4, IL-10, or IL-4/IL-10 did not prevent the development of splenomegaly in P-PIC mice (P-PIC + IL-4: 6.9 ± 0.6 mg/g, P-PIC + IL-10: 6.2 ± 0.8 mg/g, P-PIC + IL-4 + IL-10: 5.7 ± 0.7 mg/g).

PE is associated with changes in T cell subsets; therefore we determined the percentage of splenocytes for CD3+ T cells, CD3+/CD4+ T cells, and CD3+/CD8+ T cells. No differences were observed among any of these groups (Table 1). However, the percentage of splenic CD3+/γδ+ T cells, which are a small subset of unconventional CD3+ T cells that act as a bridge between innate and adaptive immune responses during infections, increased significantly in P-PIC mice compared with vehicle-treated P mice (P-PIC: 2.37 ± 0.18 vs. P: 1.16 ± 0.21; P < 0.05) (Figure 2a). The percentage of splenic CD3+/γδ+ T cells were significantly increased in both IL-10–treated P and P-PIC mice compared with vehicle-treated P mice (P + IL-10: 1.89 ± 0.09, P-PIC + IL-10: 1.81 ± 0.28; P < 0.05 vs. P) (Figure 2a). Treatment with either IL-4 alone or IL-4/IL-10 prevented the increase in splenic CD3+/γδ+ T cells in P-PIC mice (P-PIC + IL-4: 1.26 ± 0.02, P-PIC + IL-4 + IL-10: 1.56 ± 0.05; P < 0.05 vs. P-PIC) (Figure 2a).

We also compared the percentage of splenic CD11c+ DCs which act as antigen-presenting cells against microbial infection that activate innate immune responses. Figure 2b demonstrates that the percentage of splenic CD11c+ DCs was significantly increased in P-PIC mice compared with P mice (P-PIC: 5.8 ± 0.2 vs. P: 4.2 ± 0.2; P < 0.05). Additionally, daily injections of IL-4 to P mice from gestational day 13 to gestational day 17 significantly decreased CD11c+ DCs compared with vehicle-treated P mice (P-PIC + IL-4: 2.1 ± 0.2) (Figure 2b). IL-10 treatment failed to prevent an increase in CD11c+ DCs in P-PIC mice compared with vehicle-treated P mice. Either IL-4 treatment or IL-4/IL-10 treatment in P-PIC mice prevented the increase in CD11c+ DCs (P-PIC + IL-4: 4.3 ± 0.3, P-PIC + IL-4 + IL-10: 4.1 ± 0.2; P < 0.05 vs. P-PIC) (Figure 2b).

The percentage of splenic CD11b+/CD14− MDSCs was decreased significantly in P-PIC mice compared with P mice (P-PIC: 3.0 ± 0.2 vs. P: 5.1 ± 0.2; P < 0.05) (Figure 2c). Although IL-10 treatment in P-PIC mice did not prevent the decrease in CD11b+/CD14− MDSCs, treatment with
either IL-4 or IL-4/IL-10 prevented the decrease in CD11b+/CD14− MDSCs in P-PIC mice (P-PIC + IL-4: 4.6 ± 0.5, P-PIC + IL-10: 2.8 ± 0.2, P-PIC + IL-4 + IL10: 5.6 ± 0.4; *P < 0.05 vs. P-PIC) (Figure 2c). There were no significant differences in CD11b+ monocytes between all groups (Table 1).

**Effect of IL-4/IL-10 treatment on proinflammatory serum cytokine levels**

Several clinical studies report increased serum levels of proinflammatory cytokines such as IL-6, interferon γ (IFNγ), and TNFα in patients with PE.26 There was a significant increase in serum levels of IL-6, IFNγ, TNFα, and transforming growth factor β (TGFβ) in P-PIC mice compared with P mice, which was restored to normal pregnant levels after cotreatment with IL-4 and IL-10 (Figure 3a–d).

**Effect of IL-4, IL-10, or IL4/IL-10 treatment on placental TLR3 levels and morphology**

We determined TLR3 levels in the placentas of P, P-PIC, and P-PIC mice treated with IL-4, IL-10, and IL-4/IL-10...
IL-4 and IL-10 Treatment in Preeclampsia

Table 1. Splenic immune cell subsets in control and treated pregnant mice

<table>
<thead>
<tr>
<th>Immune cell subset</th>
<th>P</th>
<th>P-PIC</th>
<th>P+ IL-4</th>
<th>P-PIC+ IL-4</th>
<th>P+ IL-10</th>
<th>P-PIC+ IL-10</th>
<th>P+ IL-4+ IL-10</th>
<th>P-PIC+ IL-4+ IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+ T cells</td>
<td>19.81</td>
<td>14.39</td>
<td>28.50</td>
<td>15.06</td>
<td>16.96</td>
<td>15.42</td>
<td>22.32</td>
<td>16.61</td>
</tr>
<tr>
<td>± SEM</td>
<td>0.54</td>
<td>1.29</td>
<td>0.79</td>
<td>1.01</td>
<td>0.79</td>
<td>1.46</td>
<td>1.17</td>
<td>0.95</td>
</tr>
<tr>
<td>CD3+CD8+ T cells</td>
<td>13.13</td>
<td>12.59</td>
<td>13.96</td>
<td>12.06</td>
<td>11.75</td>
<td>11.43</td>
<td>14.61</td>
<td>12.92</td>
</tr>
<tr>
<td>± SEM</td>
<td>0.42</td>
<td>0.54</td>
<td>0.65</td>
<td>0.57</td>
<td>0.89</td>
<td>0.58</td>
<td>0.58</td>
<td>1.14</td>
</tr>
<tr>
<td>± SEM</td>
<td>0.56</td>
<td>1.06</td>
<td>1.05</td>
<td>0.91</td>
<td>1.71</td>
<td>1.85</td>
<td>1.73</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Percentage of total splenocytes for CD3+/CD4+ T cells, CD3+/CD8+ T cells, and CD11b+ monocytes presented as mean ± SEM. Abbreviations: IL-4, interleukin 4; IL-10, interleukin 10; P, pregnant mice; P-PIC, poly I:C–treated pregnant mice.

Figure 2. Effect of interleukin 4 (IL-4), interleukin 10 (IL-10), or IL-4/IL-10 treatment on CD3+/γδ T cells, CD11c+ dendritic cells, and CD11b+/CD14− suppressor monocytes. (a) Splenocytes were obtained from pregnant (P) and poly I:C–treated pregnant (P-PIC) mice and their respective treatment groups and stained, and CD3+/γδ T cells were analyzed by flow cytometry. Results are expressed as means ± SEMs (n = 4–9 mice in each group). *P < 0.05 vs. P mice. (b) CD11c+ dendritic cells were measured by flow cytometry in spleens from P and P-PIC mice and their respective treatment groups. Results are expressed as means ± SEMs (n = 4–9 mice in each group). *P < 0.05 vs. P. (c) CD11b+/CD14− myeloid-derived suppressor cells were measured by flow cytometry in spleens from P and P-PIC mice and their respective treatment groups. Results are expressed as means ± SEMs (n = 4–9 mice in each group). *P < 0.05 vs. P mice.

to evaluate whether treatment with these cytokines had any effect on placental TLR3 levels. Consistent with our previous findings, we found that treatment with poly I:C increased TLR3 levels (2.3-fold; P < 0.05). However, TLR3 levels decreased in P-PIC mice treated with IL-4, IL-10, and IL-4/IL-10 compared with P-PIC mice (Figure 4a,b).

Next, our aim was to determine whether morphologic changes occur in the placenta after poly I:C administration and whether treatments with these cytokines were able to reverse these effects. Poly I:C activation in pregnant mice induced morphologic changes assessed by hematoxylin and eosin staining at the feto-maternal interface compared with P mice. Necrosis was evident in the maternal decidua of P-PIC mice. Treatment with either IL-4 or IL-10 reduced the amount of necrosis; however, necrosis was only completely abolished in P-PIC mice treated with both IL-4 and IL-10 (Figure 4c).

DISCUSSION

Although efforts are underway to decipher the precise causes of PE, treatment options are very limited. The only available effective treatment is delivery of the baby and the
Thus there is a need to design effective strategies to reduce the symptoms of PE. Several studies have reported a predominant Th2-type immunity and a suppressed Th1-type immunity during normal pregnancy. Adoptive transfer of activated Th1-like splenocytes into pregnant mice elicits PE-like symptoms, suggesting that Th2-type immunity may prevent the development of PE. Therefore, this study was designed to test the hypothesis that administration of the anti-inflammatory cytokines IL-4 or IL-10 alone or in combination is sufficient to attenuate PE-like symptoms in a TLR3-induced PE mouse model. The important findings from our study are the following: (i) administration of IL-4 alone, IL-10 alone, or IL-4/IL-10 cotreatment during gestation normalized blood pressure and endothelial function in P-PIC mice; (ii) IL-4/IL-10 cotreatment was able to prevent proteinuria and had the most beneficial effect on fetal development, and it decreased the levels of IL-6, IFNγ, TNFα, and TGFβ; and (iii) only IL-4 and IL-4/IL-10 cotreatment decreased CD3ε⁺/γδ⁺ T cells and CD11c⁺ DCs while increasing CD11b⁺/CD14⁻ MDSCs to normal pregnancy levels.

We have previously reported that hypertension and endothelial dysfunction are induced in a pregnancy-dependent manner in our TLR3-induced PE mouse model and further exacerbated by IL-4 or IL-10 deficiency. A recent study by Zemse et al. in isolated mouse aortas demonstrated that IL-10 could prevent endothelial dysfunction caused by TNFα. No such direct experimental evidence is available for IL-4 treatment on endothelial function in vitro. Lai et al. reported that administration of IL-10 ameliorates hypoxia-induced PE-like symptoms in IL-10 knockout mice. Here we report that in our P-PIC mice, treatment with IL-4, IL-10, or both normalized SBP and endothelial function, which was associated with favorable alterations in immune cell subsets. Hypertensive pregnancy disorders are associated with inflammation and changes in several immune cell subsets. Maternal systemic TLR3 activation either by pathogens or necrotic tissue initiates activation of the innate immune system, which ultimately leads to the activation of adaptive immune responses. Excessive release of proinflammatory cytokines and/or inhibition of anti-inflammatory cytokines by innate immune cells results in endothelial dysfunction leading to hypertension. Although the role of the adaptive immune system in the development of hypertension is well established, the role of innate immune cells is poorly understood. Therefore, we evaluated immune cell subsets involved in both innate and adaptive response. We did not observe any significant changes in T cell subsets; therefore we evaluated changes in innate immune cells. DCs are considered to be guardians that activate innate immune responses to tissue damage or microbial invasion, and their role in hypertension is only beginning to emerge. Moreover, CD11c⁺ precursors appear to be an important source of type

Figure 3. Effect of interleukin 4 (IL-4), interleukin 10 (IL-10), or IL-4/IL-10 treatment on proinflammatory cytokine levels. Levels of (a) interleukin 6 (IL-6), (b) interferon γ (IFNγ), (c) tumor necrosis factor α (TNFα), and (d) transforming growth factor β (TGFβ) were measured by enzyme-linked immunosorbent assay in serum from pregnant (P) and poly I:C–treated pregnant (P-PIC) mice and IL-4/IL-10–treated P and P-PIC groups. Results are expressed as means + SEMs as a percentage of P (n = 3 mice in each group).
I IFNs in response to viral and other stimuli. Recent evidence suggests an increase in activated DCs in the spleen and lymph nodes in hypertensive mice. DC activation occurs during antigen uptake and presentation and is likely to be involved in the uptake and presentation of antigens to T cells in hypertension. In this respect, we observed that the percentage of splenic DCs was significantly increased in P-PIC mice, suggesting they may play a role in the development of hypertension. Both IL-4 alone and IL-4/IL-10 cotreatment decreased DCs in P-PIC mice. Currently, less is known about the roles that CD3+/γδ+T cells and MDSCs play in hypertension. CD3+/γδ+ T cell modulation by TLR ligands can act directly in combination with T cell receptor stimulation to enhance cytokine/chemokine production, which may explain the excessive levels of the proinflammatory cytokine interleukin 17 seen in women with PE. MDSCs, on the other hand, exhibit immunosuppressive functions that may be beneficial during pregnancy. In our study, P-PIC mice exhibited an increased percentage of CD3+/γδ+ T cells and a decreased percentage of MDSCs compared with normal pregnant mice, and treatment with IL-4 alone or cotreatment with IL-4 and IL-10 normalized these cells to normal pregnant levels.

Deficiencies of IL-4 or IL-10 have been associated with several diseases; thus administration of either of these cytokines is a viable treatment option. Angiotensin II–induced hypertensive rats have been shown to exhibit reduced levels of IL-4 in both the spleen and kidney. Treatment with an angiotensin receptor blocker increased IL-4 levels in these rats and reduced blood pressure, proteinuria, and tubulointerstitial damage. Deficiencies in IL-10 accelerate the development of atherosclerosis in mice and increase allograft rejection in various experimental transplant models. Treatment with either rIL-4 or rIL-10 has been shown to prolong allograft survival in these models. Additionally, both IL-4 and IL-10 decrease renal injury and proteinuria in experimental models of renal disease (i.e., 5/6 nephrectomy). Exogenous administration of IL-4 has been shown to have beneficial effects on rheumatoid arthritis, diabetes, and psoriasis. Similarly, IL-10 administration in preclinical trials has been shown to improve autoimmune diseases.

In this article, we provide evidence that cotreatment of IL-4 and IL-10 had the most beneficial effects; thus a combination of IL-4 and IL-10 may be the best treatment option for women with PE.

ACKNOWLEDGMENTS

This work was supported by American Heart Association SouthWest Affiliate Grant-in-Aid 4480033 (to B.M.M.). Opinions and assertions contained herein are solely that of the authors and do not reflect the views of the Texas A&M Health Science Center/Scott & White Healthcare. P. Chatterjee and V. L. Chiasson contributed equally to this work.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES


26. Chatterjee et al. 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-line 5-