Elevated Maternal Soluble Gp130 and IL-6 Levels and Reduced Gp130 and SOCS-3 Expressions in Women Complicated With Preeclampsia

Yuping Wang, David F. Lewis, Yang Gu, Shuang Zhao, Lynn J. Groome

Abstract—Increased inflammatory response plays a significant role in the vascular pathophysiology in preeclampsia. However, the mechanism for increased inflammatory response in preeclampsia is largely unknown. Interleukin (IL)-6 levels are elevated in women with preeclampsia. IL-6 and its receptors, IL-6R and glycoprotein (gp)130, play a critical role in mediating antiinflammatory response via induction of SOCS-3 (suppressor of cytokine signaling-3). However, IL-6 receptor levels and expressions have not been studied in preeclampsia. In this study, we measured IL-6 and its 2 soluble receptors, soluble IL-6R and soluble gp130, in maternal plasma from normal and preeclamptic pregnant women and found that not only IL-6 but also soluble gp130 levels were significantly higher in preeclamptic women than in normotensive pregnant controls. We further examined IL-6R, gp130, and SOCS-3 expressions in maternal vessels and leukocytes and found that gp130 and SOCS-3 expressions were downregulated in both vessel endothelium and leukocytes from preeclampsia. Different patterns for IL-6R and gp130 expressions were found. IL-6R expression was also downregulated in leukocytes from preeclampsia. Our results suggest that increased plasma soluble gp130/soluble IL-6R/IL-6 ratio and reduced membrane transsignaling gp130 expression could contribute to decreased SOCS-3 expression and subsequent reduction in SOCS-3 antiinflammatory activity in women with preeclampsia. Thus, reduced gp130 and SOCS-3 expressions may offer, at least in part, a plausible explanation of reduced antiinflammatory protection in the maternal vascular system in preeclampsia. (Hypertension. 2011;57:00-00.) ● Online Data Supplement

Key Words: IL-6 □ gp130 □ SOCS-3 □ endothelium □ leukocyte □ preeclampsia

Preeclampsia is a multiple-system disorder unique to human pregnancy that is characterized by maternal hypertension and proteinuria. This pregnancy disorder is a leading cause of preterm delivery. It is also a major cause of maternal and fetal morbidity and mortality in human pregnancy. Although the etiology of preeclampsia is not known, exacerbated inflammatory response is believed to play a significant role in the vascular dysfunction in preeclampsia.1,2 Normal pregnancy is an inflammatory state compared to the nonpregnant condition.3 Endothelial dysfunction,4,5 persistent leukocyte and platelet activations,5,6 and elevated inflammatory cytokine interleukin (IL)-6 and tumor necrotic factor (TNF)α levels7–9 are all considered hallmarks of increased inflammatory response in this pregnancy disorder.4,10 Increased inflammatory response not only contributes to oxidative stress and vasoconstriction,11,12 but also leads to metabolic disorders such as increased insulin resistance.13 However, little is known about how inflammatory response is being controlled during normal pregnancy and what is responsible for the exacerbated inflammatory response in preeclampsia.

IL-6 is a key regulator of SOCS-3 (suppressor of cytokine signaling-3) induction via binding to its IL-6 receptors on the cell membrane. IL-6 has 2 receptors. One is the cognate IL-6 receptor (IL-6R) and the other is the transsignal subunit glycoprotein (gp)130. gp130-induced transsignaling is critical for the induction of negative cytokine regulator SOCS-3.14,15 Like many membrane receptors, both IL-6R and gp130 have their soluble forms, soluble (s)IL-6R and soluble (s)gp130, and their levels can be detected in the circulation and extracellular fluids. Different actions of the 2 soluble receptors have been identified. Soluble IL-6R has agonistic effects on IL-6,16,17 whereas sgp130 has been demonstrated to be an antagonist to sIL-6R/IL-6.17,18

We recently reported trophoblast IL-6R and SOCS-3 expressions were reduced from preeclamptic placentas,19 suggesting that altered IL-6 receptor and SOCS-3 expressions may account for the increased inflammatory response in preeclamptic placentas. Several studies have shown that maternal IL-6 levels are elevated in women with preeclampsia.8,20 However, sIL-6R and sgp130 levels, and IL-6R, gp130, and SOCS-3 expressions have never been studied in...
preeclampsia. Thus, in this study, we measured IL-6, sIL-6R, and sgp130 levels in maternal plasma from normal and preeclamptic pregnant women. We also determined IL-6R, gp130, and SOCS-3 expressions in maternal vessel endothelium and leukocytes from women with normal pregnancy and preeclampsia to test our hypothesis that altered IL-6/gp130 receptor expressions may contribute to increased inflammatory response in the maternal vasculature in preeclampsia.

Methods

Patient Information and Sample Collection
Normal pregnant women were recruited during their routine prenatal visit at the perinatal center or when they were admitted to the labor and delivery unit. Normal pregnancy was defined as pregnancy with normal blood pressure (<140/90 mm Hg), no proteinuria, and absence of obstetric and medical complications. Women diagnosed with preeclampsia were recruited when they were admitted to the labor and delivery unit. Diagnosis of preeclampsia was defined as follows: sustained systolic blood pressure of ≥140 mm Hg or a sustained diastolic blood pressure of ≥90 mm Hg on 2 separate readings; proteinuria measurement of 1+ or more on dipstick, or 24-hour urine protein collection with ≥300 mg in the specimen. Smokers and patients with signs of infection were excluded. To avoid clinical phenotypic differences in preeclamptic patients, patients complicated with HELLP syndrome (hemolysis, elevated liver enzyme and low platelet count), diabetes, and/or renal disease were excluded. This study was approved by Institutional Review Board and conducted at Louisiana State University Health Sciences Center-Shreveport. Signed consent was obtained at the time of enrollment. In this study, a total of 136 plasma samples were measured for IL-6, sIL-6R, and sgp130 levels (64 from normal and 72 from preeclamptic pregnancies). Maternal subcutaneous fat tissue from 6 normal and 6 preeclamptic pregnant women were collected during cesarean section delivery and used to examine maternal vessel IL-6R, gp130, and SOCS-3 expressions. Maternal leukocyte samples from 6 normal and 5 preeclamptic pregnant women were used to determine leukocyte protein expressions for IL-6R, gp130, and SOCS-3.

Assays for IL-6, sIL-6R, and sgp130
Maternal venous blood was obtained from 136 pregnant women. Blood sample was collected into sodium heparin tube and plasma was obtained by centrifugation. Plasma samples were aliquoted and stored at −80 °C until assay. Plasma IL-6, sIL-6R, and sgp130 levels were measured by ELISA. DuoSet ELISA development kits of IL-6 (DY206), sIL-6R (DY227), and sgp130 (DY228) were purchased from R&D Systems (Minneapolis, MN). The assay procedures followed the instructions of the manufacturer. The range of a standard curve was 0.5 to 600 pg/mL for IL-6, 1 to 1000 pg/mL for sIL-6R, and 10 pg/mL to 10 ng/mL for sgp130. All samples were measured in duplicate. Within and between assay variations were less than 6% and 8% for all three ELISA assays, respectively.

Immunohistochemistry
Fresh subcutaneous tissue was fixed immediately with 10% formalin and then embedded with paraffin. A standard immunohistochemistry staining procedure was performed. Briefly, a series of deparaffinization was done with xylene and ethanol alcohol. Antigen retrieval was performed by boiling tissue slides with 0.01 mol/L citric buffer. Hydrogen peroxide was used to quench the endogenous peroxidase activity. After blocking, the sections were incubated with primary monoclonal antibodies specific against human IL-6R (C-20) (Santa Cruz Biotechnology, San Diego, CA), gp130 (H-255) (Santa Cruz Biotechnology), and SOCS-3 (ab16030) (Abcam Inc, Cambridge, MA) overnight at 4 °C. Corresponding biotinylated secondary antibodies and ABC staining system (Santa Cruz Biotechnology) were used according to the instructions of the manufacturer. Stained slides were counterstained with Gill’s formulation hematoxylin. Tissue sections stained with isotype IgG were used as controls.
Table 2. Maternal Plasma Levels for IL-6, Soluble IL-6R, and Soluble Gp130 and Ratios of Soluble Gp130/Soluble IL-6R/IL-6 in Normal and Preeclamptic Pregnant Women

<table>
<thead>
<tr>
<th>Assays</th>
<th>&lt;34 Weeks</th>
<th>34–37 Weeks</th>
<th>&gt;37 Weeks</th>
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<tbody>
<tr>
<td>IL-6, pg/mL (range)</td>
<td>1.80±0.43 (0–6)</td>
<td>8.92±1.64* (0–38)</td>
<td>4.44±1.19 (0–18)</td>
</tr>
<tr>
<td>sIL-6R, ng/mL (range)</td>
<td>20.09±1.51 (12–36)</td>
<td>18.86±1.02 (11–32)</td>
<td>17.39±0.93 (13–25)</td>
</tr>
<tr>
<td>sgp130, ng/mL (range)</td>
<td>167.47±9.82 (66–212)</td>
<td>180.41±7.54 (127–258)</td>
<td>145.79±7.12 (67–195)</td>
</tr>
<tr>
<td>sgp130/sIL-6R/IL-6, ratio</td>
<td>15.52±4.02</td>
<td>77.22±14.42†</td>
<td>39.38±10.81</td>
</tr>
</tbody>
</table>

Data are means±SE. *P<0.05, †P<0.01, preeclamptic (PE) vs normal matched with gestational age group.
At least 3 different forms of soluble gp130 were reported in human serum samples with molecular weights of 50, 90, and 130 kDa. We detected the 50- and 90-kDa but not 130-kDa forms of sgp130 in leukocyte samples (Figure 1C). Both 50 and 90-kDa bands could be blocked by gp130 blocking peptide (Santa Cruz Biotechnology, sc-656P, data not shown). These results indicate that leukocytes express soluble form of gp130. Leukocyte gp130 expression is reduced in preeclamptic compared to normal pregnancies (total PE versus normal: 1.38±0.10 versus 2.05±0.25, P<0.05) and the reduced 90-kDa molecular mass (PE versus normal: 0.14±0.07 versus 0.70±0.16, P<0.05) accounts for the total gp130 reduction in leukocytes from preeclampsia, Figure 1C (scatter plots).

**Downregulation of SOCS-3 Expression in Maternal Vessel Endothelium and in Leukocytes From Preeclampsia**

Because the IL-6 receptors IL-6R and gp130 play key roles in the induction of SOCS-3 expression, we examined SOCS-3 expression in maternal vessels and leukocytes from normal and preeclamptic pregnant women. Results are shown in Figure 2. We found intensive SOCS-3 staining in vessel endothelium both in arteries and veins in tissue sections from normal pregnant women. The smooth muscle layer also shows intensive SOCS-3 staining. In contrast, SOCS-3 expression was markedly reduced in vessel endothelium both in arteries and veins in tissues from women with preeclampsia. SOCS-3 expression was also reduced in smooth muscle layers of vessels from preeclampsia (Figure 2A). No staining was seen in sections stained with isotype IgG (data not shown). Consistent with maternal vessels, relative SOCS-3 expression was also reduced in leukocytes from preeclampsia (n=5) compared to those from normal (n=6) pregnant women; 0.27±0.04 versus 0.47±0.05; P<0.05 (Figure 2B).

**Discussion**

In this study, we found that: (1) maternal IL-6 and sgp130, but not sIL-6R, levels were increased in women with preeclampsia compared to normal pregnant controls; (2) IL-6R expression was downregulated in leukocytes from preeclampsia; and (3) gp130 expression was downregulated in both vessel endothelium and leukocytes from preeclampsia. These results may reflect possible mechanisms by which IL-6 plays a role in the pathogenesis of preeclampsia.
We observed that IL-6R was strongly expressed in leukocytes, but undetectable in maternal vessel endothelium in both normal and preeclamptic pregnancies. In contrast, gp130 is intensively expressed in endothelium of vessels from normal pregnant women, but reduced gp130 expression was observed in vessel endothelium in preeclampsia. Leukocytes express both IL-6R and gp130, which is consistent with previous published work.22,30,31 However, both IL-6R and gp130 expressions were significantly reduced in leukocytes from women with preeclampsia. The different patterns of IL-6R and gp130 expressions between vessel endothelium and circulating leukocytes indicate that a distinct IL-6 receptor signaling regulation does exist between endothelial cells and leukocytes.

Both soluble forms of IL-6 receptors, sIL-6R and sgp130, were detected in leukocyte samples. For IL-6R, it seems that increased alternative splicing occurs in leukocytes from preeclampsia because alternative splicing produces 45- to 50-kDa sIL-6R.23 For gp130, the mature form (130 kDa) of gp130 was not shown in leukocyte samples, instead two truncated (soluble) forms, 50 and 90 kDa, were detected. The 90-kDa form was significantly reduced in preeclamptic samples. Previous studies have shown that the 50-kDa soluble form is produced by alternative splicing mRNA,26 and can be detected in human plasma and urine samples.27 The 50-kDa sgp130 probably contains only the hemopoietic domain.27 The reason for increased maternal sgp130 levels in preeclampsia is not known, but decreased gp130 expression in both vessel endothelium and leukocytes clearly indicate that the integrity of gp130 is impaired in endothelial cells and leukocytes in preeclampsia. Chalaris et al reported that increased sIL-6R shedding was associated with reduced cellular IL-6R expression in Ba/F3 cells, a murine pro-B cell line.31 The reason for reduced gp130 expression in preeclamptic leukocytes is not known, but increased plasma sgp130 levels suggest that reduced cellular receptor expressions could be as a result from increased soluble receptor shedding in preeclampsia.

Reduced SOCS-3 expression found in both maternal vessel endothelium and leukocytes in preeclampsia is another significant finding in our study. SOCS-3 belongs to a family of molecules involved in inhibiting the JAK-STAT signaling pathway. SOCS-3 is an antiinflammatory mediator28,32,33 and plays a critical role in inhibiting inflammatory response via
negative regulation of several cytokine pathways, particularly the IL-6 induced receptor-associated JAK/STAT pathway.\textsuperscript{32,34} The finding of reduced SOCS-3 expression in maternal vasculature in preeclampsia supports the concept of reduced antiinflammatory activity and increased inflammatory response in women with preeclampsia.

Increased inflammatory response and adiposity are related to each other. Women with obesity or insulin resistance have a higher incidence of developing preeclampsia during pregnancy. However, in our study population, as shown in Table 1 (subjects for maternal plasma measurement), Table S1A (subjects for maternal vessel IL-6R, gp130 and SOCS-3 staining), and Table S1B (subjects for maternal leukocyte IL-6R, gp130 and SOCS-3 expressions), difference in BMI was only seen in Table S1B leukocyte studies, which only limited subjects were involved. It seems that increased maternal IL-6 and sgp130 levels might not be directly related to the maternal BMI, at least in our study population.

It has been well accepted that placenta-derived factors play a significant role in inducing vessel endothelium and leukocyte activation/dysfunction\textsuperscript{35–37}; however, the mechanism of increased inflammatory response in preeclampsia is not fully understood. Little is known about how the cellular endogenous defense mechanism functions in the vascular system against inflammatory stimulus challenge. Our findings of downregulation of SOCS-3 expression in both vessel endothelium and leukocytes suggest that antiinflammatory activity/function of gp130/STAT/SOCS-3 signaling pathway is impaired in the maternal vascular system in preeclampsia, which, at least in part, offers a plausible explanation of increased inflammatory response in this pregnancy disorder.

**Figure 3.** Proposed mechanisms of IL-6 and its receptor signaling pathway in SOCS-3 induction in cells with or without IL-6R. A, Cells have both IL-6R and gp130 receptors such as in leukocytes. IL-6 binds to IL-6R on the cell membrane and forms a receptor complex with gp130. The binding process induces receptor dimerization and Janus kinase (JAK) phosphorylation of the signal transducing subunit gp130. Phosphorylated JAK further induces STAT3 phosphorylation, and dimeric STAT3 migrates to the nucleus, where it recognizes specific elements in the promoter of SOCS-3 gene and induces SOCS-3 expression. Once induced, SOCS-3 acts back on the JAK/STAT pathway to inhibit signal transduction, attenuate cytokine-activated signal transduction pathway, and suppress cytokine generation and cytokine-induced inflammatory responses. B, Cells have gp130, but not IL-6R, such as in endothelial cells. In the extracellular compartment such as in the blood circulation, IL-6 cannot directly bind to gp130 on the cell membrane. Instead, IL-6 binds sIL-6R and forms receptor/ligand complex, sIL-6R/IL-6. The complex could then bind to gp130 on the cell membrane and induce gp130 phosphorylation and SOCS-3 induction. Therefore, sIL-6R is considered as an agonist to IL-6 and plays a critical role in IL-6–induced gp130 activation, and the integrity of the cell membrane gp130 receptor is critical for signal transduction and SOCS-3 induction. C, Proposed mechanism of reduced antiinflammatory activity in vascular endothelial cells in preeclampsia (PE): a condition of increased sgp130 level and gp130 phosphorylation and SOCS-3 induction, our results do suggest that different regulatory mechanisms exist in response to IL-6 between endothelial cells and leukocytes in the systemic vasculature. In the case of endothelial cells, because they are lacking IL-6R,\textsuperscript{31,32} soluble receptor–ligand complex sIL-6R/IL-6 could be the active form that binds to membrane gp130 and initiates gp130/STAT signaling and SOCS-3 induction, as proposed in Figure 3B, whereas in the case of leukocytes, circulating IL-6 can directly act on leukocytes and initiate the signaling cascade, because leukocytes express IL-6R, as proposed in Figure 3A. Therefore, it is logical to expect that in preeclampsia, elevated levels of IL-6 and sgp130 and increased ratio of sgp130/sIL-6R/IL-6 combined with reduced gp130 expression could result in less SOCS-3 induction and, consequently, aberrant antiinflammatory activity/function in leukocytes and vessel endothelium, as proposed in Figure 3C. Further study of sIL-6R and sgp130 function and status of these soluble receptors, as well as SOCS-3 regulation, are warranted and would provide insight into mechanisms at the cellular and molecular levels as to how inflammatory response is being controlled during normal pregnancy and what failed mechanisms occur that lead to increased inflammatory response in preeclampsia.
Sources of Funding
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Disclosures
None.

References
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Elevated maternal sgp130 and IL-6 levels and reduced gp130 and SOCS-3 expressions in women complicated with preeclampsia

ONLINE SUPPLEMENT

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Short tile: Gp130 and SOCS-3 in pregnancy and preeclampsia

Precis: Increased sgp130 levels, and altered IL-6 receptors and SOCS-3 expressions may associate with increased inflammatory response in women with preeclampsia.
Table S1A. Clinical information of study subjects from which maternal vessels were examined in the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal (n=6)</th>
<th>Preeclampsia (n=6)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Maternal age (years)</td>
<td>24 ± 4 (19-28)</td>
<td>30 ± 6 (23-38)</td>
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<td>Racial Status</td>
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<td>Black</td>
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<td>3</td>
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<td>Other</td>
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<tr>
<td>BMI</td>
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<td>31 ± 6 (27-39)</td>
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<td>Blood Pressure (mmHg)</td>
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<tr>
<td>Systolic</td>
<td>119 ± 6 (111-128)</td>
<td>174 ± 14 (160-193)</td>
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<td>Diastolic</td>
<td>73 ± 6 (67-83)</td>
<td>102 ± 8 (93-110)</td>
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<tr>
<td>Gestational Age at delivery (weeks)</td>
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<tr>
<td>Primigravida</td>
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Data are expressed as Mean ± SD (range)

Table S1B. Clinical information of study subjects from which maternal leukocytes were used in the study

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<tr>
<td>Black</td>
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<td>3</td>
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<tr>
<td>BMI</td>
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<td>41 ± 9 (31-52)</td>
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<tr>
<td>Blood Pressure (mmHg)</td>
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<tr>
<td>Systolic</td>
<td>123 ± 9 (113-136)</td>
<td>183 ± 27 (156-225)</td>
<td>0.006</td>
</tr>
<tr>
<td>Diastolic</td>
<td>74 ± 4 (70-79)</td>
<td>112 ± 15 (103-137)</td>
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<tr>
<td>Gestational Age at delivery (weeks)</td>
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Data are expressed as Mean ± SD (range)