Elevated Maternal IL-16 Levels, Enhanced IL-16 Expressions in Endothelium and Leukocytes, and Increased IL-16 Production by Placental Trophoblasts in Women with Preeclampsia

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Cytokine IL-16 plays an important role in innate immune responses. However, little information is available about IL-16 function in human pregnancy. In this study, we collected maternal blood samples from 125 pregnant women between 26 and 41 wk of gestation, 63 from normal pregnant women and 62 from women with preeclampsia (PE). Serum IL-16C levels were measured by ELISA. We also examined IL-16C and IL-16N immunostaining in placental tissues in PE, suggesting that IL-16 could be an important cytokine engaged in the altered immune system and exaggerated inflammatory response in PE syndrome. The Journal of Immunology, 2008, 181: 4418–4422.

Interleukin-16 is one of the first characterized cytokines with chemoattractant activity for human T cells and is originally designed as lymphocyte chemoattractant factor (1). It can be synthesized by both immune and nonimmune cells including T cells, mast cells, dendritic cells, fibroblasts, and epithelial cells, etc. (1). IL-16 plays an important role in innate immune responses. This cytokine can initiate and amplify an inflammatory response by releasing cell-signal molecules via binding to its receptor CD4 on CD4+ T cells. For example, increased intracellular Ca2+ and IP3 were detectable in lymphocytes and monocytes after IL-16 stimulation (1). This cytokine could also induce phosphorylation of c-Jun and p38 in macrophages (2). IL-16 is constitutively present in peripheral blood leukocytes and spontaneously released during apoptotic process (3). Preeclampsia (PE) syndrome is a leading cause of maternal and fetal morbidity and mortality in human pregnancy. Although defective placental trophoblast invasion and dysfunction of maternal vascular endothelium are significant in the pathogenesis of PE, maladaptive or altered immune response is also believed to play an important role in this pregnancy disorder. Exaggerated inflammatory response (4), imbalance of Th1/Th2 cytokine generation (5–7), and aberrant interaction of maternal leukocytes and placental trophoblasts at the maternal-fetal interface (8) are all considered to be the crucial components of the altered immune response during PE.

Leukocyte activation and increased endothelial inflammatory response have been demonstrated in women complicated by PE (9–11). Several inflammatory cytokines including IL-6, IL-8, IL-10, TNF-α, and leptin were found to be either elevated or decreased in women with PE compared with the normal pregnant controls (5, 12–14). However, little is known about the potential role of IL-16 in PE. IL-16 gene produces two functional proteins, one from C terminus and one from N terminus. The C-terminal product exclusively attributes to cytokine function, and the N-terminal product plays a role in cell cycle control. To characterize IL-16 profiles in normal pregnancy and PE, in this study we determined: 1) IL-16C levels in maternal serum; 2) IL-16C and IL-16N immunostaining in maternal systemic vessels; 3) IL-16C and IL-16N protein expressions in maternal leukocytes; and 4) IL-16C production by placental trophoblasts. Comparisons between normal and preeclamptic groups were made.

Materials and Methods

Patient information and blood sample collection

Normal pregnant women were recruited during their routine prenatal visit at perinatal center or when they were admitted to the Labor and Delivery Unit at Louisiana State University Health Sciences Center-Shreveport, LA. Women diagnosed with PE were recruited when they were admitted to the Labor and Delivery Unit. Normal pregnancy is defined as pregnancy with normal blood pressure (<140/90 mmHg), no proteinuria, and absence of
Table I. Demographic data for study subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal Pregnancy</th>
<th>Mild PE</th>
<th>Severe PE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group (wk)</strong></td>
<td>&lt;34 (n = 20)</td>
<td>34–37 (n = 13)</td>
<td>37 (n = 30)</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>20</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td><strong>Maternal age</strong></td>
<td>22 ± 5</td>
<td>22 ± 4</td>
<td>22 ± 3</td>
</tr>
<tr>
<td><strong>Racial status</strong></td>
<td>Black 16 (n = 1)</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>White 4 (n = 2)</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Other 0 (n = 0)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>29 ± 8</td>
<td>32 ± 6</td>
<td>34 ± 10</td>
</tr>
<tr>
<td><strong>Gestational age</strong></td>
<td>31 ± 2</td>
<td>36 ± 1</td>
<td>40 ± 1</td>
</tr>
<tr>
<td><strong>Primagravida (%)</strong></td>
<td>55 ± 3</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td><strong>Blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Systolic</strong></td>
<td>118 ± 12</td>
<td>125 ± 11</td>
<td>123 ± 13</td>
</tr>
<tr>
<td><strong>Diastolic</strong></td>
<td>70 ± 9</td>
<td>73 ± 8</td>
<td>74 ± 14</td>
</tr>
<tr>
<td><strong>Mode of delivery (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cesarean 30 (n = 15)</td>
<td>33</td>
<td>55</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.

obstetrical and medical complications. Diagnosis of mild PE was defined as follows: sustained systolic blood pressure of ≥140 mmHg or a sustained diastolic blood pressure of ≥90 mmHg on two separate readings; proteinuria measurement of 1+ or more on dipstick, or 24-h urine protein collection with ≥300 mg in specimen. PE was defined severe if one or more of the following criteria is present: maternal blood pressure ≥160/110 mmHg; proteinuria ≥3+ or ≥5 gm/24 h; oliguria of less than 500 ml in 24 h; intraterine growth restriction, and presence of persistent headache or visual disturbances. Venous blood was drawn after signed consent was obtained. Serum was isolated and aliquots were stored in a −70°C freezer until assay. In this study, a total of 125 serum samples were used, 63 from normal pregnancies and 62 from women with PE. For the normal pregnant group, all women, whose blood was drawn at perinatal clinics, were followed after delivery and a normal pregnancy was confirmed by chart review. Smokers and patients with infections were excluded. The clinical characteristics of the study population are shown in Table I. This study was approved by the Institutional Review Board for Human Research at Louisiana State University Health Sciences Center-Shreveport, LA.

Measurement of IL-16

Maternal serum IL-16 concentrations and placental trophoblast IL-16 production were measured by ELISA. The DuoSet IL-16 ELISA Development kit (DY316) was purchased from R&D Systems. The assay procedures followed the manufacturer’s instruction. The range of a standard curve was 15.6–1000 pg/ml. An aliquot of 50-μl samples was measured in duplicate for all samples. Within and between assays, variations were less than 5 and 8%, respectively. In this ELISA kit, the IL-16 standard (recombinant hu-

Leukocyte IL-16 protein expression

Leukocytes were extracted from maternal blood as previously described (15), and total cellular protein was obtained by using an ice-cold lysis buffer containing 50 mmol/l Tris-HCl (pH 7.6), 1% Triton X-100, 0.5% Nonidet P-40, 1 mmol/l PMSF, and 0.5% mmol/l DTT. An aliquot of total leukocyte protein extract (15 μg per sample) was subjected to electrophoresis on 12% polyacrylamide gels using a Miniprotein 3 gel running system (Bio-Rad) and then transferred to nitrocellulose mem-

Placental trophoblast isolation and culture

Placentas were obtained immediately after delivery, and trophoblasts were isolated as previously described (16, 17). In brief, placental tissue was separated by sterile dissection from different cotyledons, excluding chorionic and basal plates. Piece tissues were washed repeatedly with ice-cold PBS to remove blood from the intervilous space. Villous tissues were digested with an equal volume of Dispase (BD Biosciences) at 37°C for 20 min. The digestion was stopped by inactivation of Dispase with EDTA. After centrifugation, tissue pellets were mixed well with DMEM (Sigma-Aldrich) and incubated on ice for 15–20 min. The supernatant containing trophoblast cells were collected and centrifuged. Isolated trophoblast cells were further purified by Percoll density gradient centrifugation (Sigma-

Table II. Maternal serum IL-16 levels in normal and preeclamptic pregnant women

<table>
<thead>
<tr>
<th></th>
<th>Normal Pregnancy</th>
<th>Mild PE</th>
<th>Severe PE</th>
</tr>
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<tbody>
<tr>
<td><strong>IL-16 (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;34 wk (n = 20)</td>
<td>162 ± 12</td>
<td>144 ± 17</td>
<td>172 ± 15</td>
</tr>
<tr>
<td>34–37 wk (n = 13)</td>
<td>172 ± 15</td>
<td>157 ± 24</td>
<td>171 ± 12</td>
</tr>
<tr>
<td>&gt;37 wk (n = 30)</td>
<td>287 ± 46</td>
<td>551 ± 77**</td>
<td>471 ± 126**</td>
</tr>
<tr>
<td><strong>Mean ± SE (range)</strong></td>
<td>(57–334)</td>
<td>(68–299)</td>
<td>(131–1962)</td>
</tr>
<tr>
<td></td>
<td>(28–395)</td>
<td>(64–468)</td>
<td>(145–1792)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SE. *p < 0.05; **p < 0.01; severe PE vs. normal pregnancy at the same gestational age.

b *p < 0.05: severe PE vs. mild PE, respectively.
Maternal serum IL-16C levels in normal pregnancy and in PE. Results for clinical characteristics of the study population are presented as mean ± SD, and data for IL-16C concentrations are presented as mean ± SE. Data were analyzed by analysis of variances (ANOVA), nonparametric Mann-Whitney U test, or unpaired t test using StatView computer software. Student-Newman-Keuls test was used as a post hoc test. A probability level of less than 0.05 was considered statistically significant.

**Results**

**Maternal serum IL-16C levels**

Maternal serum IL-16C levels in normal and preeclamptic pregnancies are shown in Table II. IL-16C levels were relatively consistent in normal pregnant women throughout the third trimester. The levels were increased in mild PE (n = 11) and significantly increased in severe PE (n = 51) compared with the normal pregnant controls (n = 63), 287 ± 46 and 515 ± 58 (p < 0.01) vs 163 ± 9 pg/ml, respectively. In severe PE, IL-16C levels were significantly higher than that in mild PE (p < 0.05) and in gestational matched normal pregnant controls, p < 0.01, respectively (Table I). These results suggest that in PE increased IL-16C levels are associated with the severity of the disease.

Fig. 1 shows the correlations of serum IL-16C levels with gestational age (upper panel) and maternal body mass index (BMI) (lower panel) in normal pregnancies and in women with PE. Our data indicates that neither gestational age nor maternal BMI is correlated with serum IL-16C levels during pregnancy.

**Statistical analysis**

Results for clinical characteristics of the study population are presented as mean ± SD, and data for IL-16C concentrations are presented as mean ± SE. Data were analyzed by analysis of variances (ANOVA), nonparametric Mann-Whitney U test, or unpaired t test using StatView computer software. Student-Newman-Keuls test was used as a post hoc test. A probability level of less than 0.05 was considered statistically significant.

**IL-16 immunostaining in maternal vessel endothelium**

Maternal vessel IL-16 expression was examined by immunostaining of s.c. fat tissue sections. It is known that IL-16 gene yields two functional proteins, one from C terminus and one from N terminus. Thus, Abs against both IL-16C terminus and IL-16N terminus were used. As shown in Fig. 2, a strong vessel endothelium staining of IL-16C Ab was observed in tissue sections from women

**FIGURE 1.** Correlations of serum IL-16C levels with gestational age and maternal BMI in normal pregnancy and in PE. **Upper panel, Correlation of IL-16C levels with gestational age, normal pregnancy: y = 2.318x + 64.885, r = 0.136; and PE: y = -19.491x + 1180.229, r = 0.174. Lower panel, Correlation of IL-16C levels with BMI, normal pregnancy: y = -0.528x + 190.945, r = 0.055; and PE: y = 1.797x + 581.316, r = 0.018, respectively. Open circle: normal; and solid circle: PE, Dash line: normal; and solid line: PE, respectively.

**FIGURE 2.** Representative immunostaining of IL-16C Ab in maternal vessel endothelium. Intensive IL-16C staining was observed in vessel endothelium of tissue sections from PE compared with those from normal pregnant controls. A and B. Normal; C and D: PE, A and C are veins; B and D are arteries. Bar = 10 micron.

Our data also showed that maternal IL-16C levels were not influenced by mode of delivery both in normal pregnant women and in women with PE. In the normal group, the mean level of IL-16C was 177 ± 26 pg/ml in vaginal deliveries and 169 ± 18 pg/ml in cesarean section deliveries. In the preeclamptic group, the mean level of IL-16C was 451 ± 66 pg/ml in vaginal deliveries and 507 ± 78 pg/ml in cesarean section deliveries. These results suggest that labor does not affect maternal IL-16C levels.

**FIGURE 3.** IL-16 protein expression in leukocytes from normal and preeclamptic pregnancies. IL-16C and IL-16N expressions were examined in five normal and four preeclamptic leukocyte samples. β-actin expression was used as an internal control. Leukocytes from PE expressed relative more IL-16C than IL-16N and the ratio of IL-16C to IL-16N was higher in leukocytes from PE than that from normal pregnant controls, p < 0.05.

**FIGURE 4.** Functional protein expression in placental cells. Purified trophoblast cells (5 × 10⁶ cells/well) were placed into a 6-well cluster cell culture plates in 4 ml of DMEM supplemented with 2% FBS and cultured for 48 h. At the end of incubation, culture medium was collected and measured for IL-16C by ELISA. IL-16C production is expressed as pg/ml/1 × 10⁶ cells.
with PE both in veins and arteries (Fig. 2, C and D). In contrast, weak or negative staining was revealed in vessel endothelium of tissue sections from normal pregnant controls (Fig. 2, A and B). In addition, neither normal tissue sections nor preeclamptic tissue sections was stained with the IL-16 Ab against N terminus (data not shown).

**IL-16 protein expression in maternal leukocytes**

Leukocyte IL-16 expressions were determined by Western blot. Again, Abs against IL-16N and IL-16C terminus were used. As shown in Fig. 3, both IL-16N and IL-16C were expressed in leukocytes from normal pregnant women. In contrast, leukocytes from women with PE expressed relatively more IL-16C than IL-16N, and the ratio of IL-16C to IL-16N was higher in leukocytes from PE than that from normal pregnant controls, \( p < 0.05 \).

**IL-16 production by placental trophoblasts**

Placenta releases many hormones, growth factors, and cytokines that could enter the maternal circulation during pregnancy. To further determine whether placental trophoblasts produce IL-16C and whether there is a difference for IL-16C production between normal and preeclamptic placentas, trophoblasts were isolated and IL-16C production was determined. Our results showed that trophoblasts from preeclamptic placentas \(( n = 8)\) produced significantly more IL-16C than the cells from normal placentas \(( n = 9)\), \( 326 \pm 67 \) vs \( 156 \pm 26 \) pg/ml/1 \( \times 10^9 \) cells, \( p < 0.05 \) (Fig. 4).

**Discussion**

It is well known that pregnancy-induced immune tolerance is an important immune adaptation process during normal pregnancy (18). Accumulated evidence has also shown that PE is associated with maladaptation of immune responses, with a prominent role of dysregulation in the innate immune system, probably controlled by T cells and cytokine networks at the maternal-fetal interface resulting in systemic inflammatory response triggered by trophoblast-derived stimuli (8, 18). In this study, we found that maternal serum IL-16C levels were significantly higher in women with PE than those in normal pregnant controls, which is consistent with a previous published work (19). Our data indicates that the increased IL-16C levels in PE may specifically attribute to the disease process. This notion is supported by our data that maternal IL-16C levels are neither associated with gestational age nor delivery mode in both normal and preeclamptic pregnancies. Likewise, IL-16C levels are not associated with BMI in our patient population. In addition, steroid usage seems to not affect circulating IL-16C levels in severe PE, since most of severe preeclamptic patients less than 34 wk of gestation received steroids to promote fetal lung maturity and no significant difference was found in severe PE between the groups of <34, 34–37, and >37 wk of gestation. However, our data did show that in PE, increased IL-16C levels are related to the severity of the disease, i.e., IL-16C levels were significantly higher in severe PE than in mild PE compared with the normal pregnant controls.

To investigate whether maternal vascular endothelium was involved in IL-16-related inflammatory and immune responses during pregnancy and PE, we examined IL-16 expression in maternal systemic vessels. We found a strong IL-16C staining in vessel endothelium both in arteries and veins in tissue sections from women with PE compared with that from normal pregnant women. In contrast, neither normal nor preeclamptic vessels exhibited a positive staining for IL-16N-terminal Ab. Since IL-16/C4D preferentially induces Th1 cell migration (20), our observations not only support increased inflammatory response occurring in the maternal systemic vasculature in PE but also suggest a possible interaction of immune responses between circulating leukocytes and vascular endothelium, perhaps related to recruitment and infiltration of leukocytes into interstitial tissues and delayed apoptosis seen in PE (10, 21).

Leukocytes express IL-16. We found that leukocytes from PE exhibited a strong IL-16C expression compared with IL-16N expression relative to the leukocytes from normal pregnant controls. The C terminus exerts cytokine function, whereas the N terminus plays a role in cell cycle control. The relative strong expression of IL-16C to IL-16N ratio in leukocytes from PE indicates up-regulation of leukocyte cytokine function in PE, which is in line with increased leukocyte activation and increased inflammatory response in this pregnancy disorder. The reason for the phenomenon of down-regulation of IL-16N expression in leukocytes from PE is not known. It could be related to leukocyte apoptotic, which needs further investigation.

The source of IL-16C in the maternal circulation could be derived from multiple bases. IL-16 can be synthesized by both immune and nonimmune cells such as T cells, mast cells, and epithelial cells, etc. (1). Although, it is impossible to measure IL-16C production by maternal vascular endothelium or circulating leukocytes in vivo, enhanced IL-16C immunostaining in vessel endothelium and increased ratio of IL-16C to IL-16N protein expression in maternal leukocytes from PE suggest that both vessel endothelium and circulating leukocytes could be sources of IL-16C in the maternal circulation. Another possible source of IL-16C could be placental trophoblasts. Trophoblasts are directly bathed in the maternal blood in the placental intervillous space. It is well accepted that hormones and growth factors produced by trophoblasts could be released and enter the maternal circulation. Our results of increased IL-16C production by trophoblasts from preeclamptic placentas point out the possibility may exist.

In conclusion, in the present study, we found elevated maternal IL-16C levels in women with PE compared with the normal pregnant controls. We also observed intensive IL-16C immunostaining in maternal vessel endothelium and increased ratio of IL-16C to IL-16N expression in leukocytes from women with PE. In addition, increased IL-16C production was also found by trophoblasts from preeclamptic placentas. These data indicate that IL-16C profile is increased in both the maternal and the placental systems in PE and support the concept that increased inflammatory response occurs in this pregnancy disorder. IL-16 is a multifunctional cytokine and exerts diverse biological activities in regulation of Th1 cell function and immune tolerance. Although the role of IL-16 in PE is largely unknown, the findings of up-regulated IL-16 profile implicate that IL-16 could be an important cytokine engaged in the altered immune system and exaggerated inflammatory response in this pregnancy disorder.
Disclosures
The authors have no financial conflict of interest.

References