Chemokines and Leukocyte Activation in the Fetal Circulation During Preeclampsia

Jan Roar Mellembakken, Pål Aukrust, Kjetil Hestdal, Thor Ueland, Thomas Åbyholm, Vibeke Videm

Abstract—Preeclampsia is a potentially life-threatening disease for both mother and fetus. Endothelial dysfunction is pivotal in the pathogenesis of this disorder, possibly reflecting a state of persistent inflammation. In the present study, we examined whether signs of inflammation with production of chemokines and leukocyte activation were present in the fetal circulation during preeclampsia. Venous cord blood was sampled during cesarean sections from 36 neonates born after uncomplicated pregnancies and from 35 born after severe preeclamptic pregnancies with premature newborns. The expression of adhesion molecules on neutrophils and monocytes was analyzed by flow cytometry, and plasma levels of chemokines and soluble adhesion molecules were analyzed by enzyme immunoassay. Newborns of preeclamptic mothers had increased expression of CD15s (P<0.001), CD49d/CD29 (P=0.01/0.005), and CD31 (P=0.007) on neutrophils and CD15s (P<0.001), CD11c (P=0.009), and CD54 (P=0.001) on monocytes. This activation of neutrophils and monocytes was accompanied by raised plasma levels of the CXC chemokines interleukin-8 (P=0.007) and growth-related oncogene-α (P=0.01) and decreased plasma levels of soluble E-selectin (P=0.001) and L-selectin (P=0.002). Although raised levels of adhesion molecules on leukocytes or decreased levels of soluble adhesion molecules in plasma were not related to prematurity or the degree of preeclampsia, raised interleukin-8 levels were found only in neonates of preeclamptic mothers with the highest blood pressures. Our findings suggest the activation of neutrophils and monocytes in the fetus during preeclampsia involving enhanced chemokine activation, possibly contributing to the fetal morbidity of this disorder. (Hypertension. 2001;38:394-398.)

Key Words: preeclampsia ■ circulation ■ leukocytes ■ peptides

In developed nations, preeclampsia accounts for 10% of preterm births, ranks seventh in importance of perinatal death,1 and is a major cause of maternal morbidity and mortality.2 It is defined by the development of hypertension and proteinuria appearing after 20 weeks of gestation.3 Endothelial dysfunction appears to be central in the pathophysiology of preeclampsia4 and has also been reported in the fetus.5,6 but the mechanisms leading to this dysfunction have not been clarified.

In preeclampsia, there is a faulty shallow placentation, ie, a reduced invasion of the trophoblast into the uterus and its spiral arteries,7 resulting in a significant reduction in the uteroplacental blood flow,8 with a chronic prenatal hypoxia of the human umbilical artery and vein, where abnormal Doppler velocity waveforms indicate an increased vascular resistance9 and hypoxia10; (2) directly, by increased plasma adenosine11 and erythropoietin levels,12 reduced pH and PO2 levels, and increased Pco2 levels in the human umbilical artery and human umbilical vein13,14; and (3) clinically, by increased frequency of placental infarcts.15 Long-range uteroplacental hypoxemia may induce inflammatory changes in the placenta with the release of inflammatory chemokines, leading to activation of fetal neutrophils and monocytes, which, in turn, may release additional inflammatory mediators. This may make a vicious circle, leading to enhanced endothelial cell activation. To elucidate whether such an activation occurs, we have examined in neonates from women with severe preeclamptic and uncomplicated pregnancies the expression of a large number of surface adhesion molecules that would increase if neutrophils and monocytes were activated. These molecules represent different steps in endothelial cell/leukocyte interaction, ie, capture, rolling, firm adhesion, and extravasation. Plasma levels of soluble adhesion molecules, further reflecting endothelial cell/leukocyte activation, and of chemokines, influencing neutrophil and monocyte chemotaxis and activation, were also examined.
Subjects
Surface adhesion molecules were analyzed in venous cord blood from 36 newborns from uncomplicated pregnancies and from 35 newborns from preeclamptic pregnancies (Table 1). Plasma samples for analyses of chemokines and soluble adhesion molecules were available in 20 neonates from preeclamptic pregnancies and from 19 neonates from normal pregnancies.

All women were delivered by a cesarean section because vaginal birth in itself may induce acidosis and neutrophil activation in the fetal circulation. Because few patients with mild preeclampsia are delivered by a cesarean section in our hospital, a group of patients with severe preeclampsia was chosen to avoid vaginal birth. Severe preeclampsia was defined as follows: (1) blood pressure of $150/100$ mm Hg measured at 2 occasions 6 hours apart with the patient at bed rest, and (2) $\geq 5$ g per 24-hour urinary protein excretion or 3+ on a semiquantitative assay. These women were all admitted for observation because of preeclampsia. The day a fetal or maternal indication for terminating the pregnancy occurred, a cesarean section was scheduled, and the operations were performed 3 to 24 hours later, with a median of 10 hours. The women in the normal group had uncomplicated pregnancies. All cesarean sections were elective and random selected from those who delivered by a cesarean section in our hospital, a group of patients with severe preeclampsia was chosen to avoid vaginal birth. Severe preeclampsia was defined as follows: (1) blood pressure of $\geq 160/110$ mm Hg measured at 2 occasions 6 hours apart with the patient at bed rest, and (2) $\geq 3$ g per 24-hour urinary protein excretion or 3+ on a semiquantitative assay. These women were all admitted for observation because of preeclampsia. The day a fetal or maternal indication for terminating the pregnancy occurred, a cesarean section was scheduled, and the operations were performed 3 to 24 hours later, with a median of 10 hours. The women in the normal group had uncomplicated pregnancies. All cesarean sections were elective and randomly selected from those who delivered by a cesarean section because of cephalopelvic disproportion, breech presentation, or fear of giving vaginal birth. The study was approved by the Regional Committee of Ethics. Written informed consent was obtained from all women, and the procedures followed were in accordance with the institutional guidelines.

Blood Sampling Protocol
During cesarean sections, blood was collected before stasis from the umbilical cord vein in sterile EDTA-containing Vacutainer tubes (Becton Dickinson). The sampling equipment was surface-sterilized for use in the operation field by 32-kGy $\gamma$-irradiation. No women were in labor or had ruptured membranes. The tubes were placed on melting ice, and platelet-free plasma was prepared within 15 minutes, as previously described. Plasma was stored at $-80^\circ$C.

Staining and Flow Cytometry Analysis
Monoclonal antibody labeling was conducted by a direct immuno-fluorescence technique, except for CD15s and CD88, for which a 2-step labeling method was used. Analyses of the neutrophils and monocytes were performed on a FACSort flow cytometer (Becton Dickinson). Neutrophils and monocytes were identified by linear forward and side scatter and by the expression of CD49D for monocytes. Mean fluorescence intensity was computed for each population.

Enzyme Immunoassays
Concentrations of the CC chemokines RANTES, monocyte chemotactic protein-1, and macrophage inflammatory protein-1$\alpha$, the CXC chemokines interleukin (IL)-8, epithelial cell–derived neutrophil-activating peptide-78, and growth-related oncogene (GRO)-$\alpha$, and the soluble adhesion molecules soluble vascular cell adhesion molecule (VCAM)-1, soluble intercellular adhesion molecule (ICAM)-1, soluble E-selectin, soluble L-selectin, and soluble P-selectin were determined in platelet-free plasma by enzyme immunoassays (R&D Systems). Samples with high levels were diluted and reassayed. In our laboratory, the intra-assay and interassay coefficients of variation were <10% for all enzyme immunoassays.

Statistical Analysis
The results are presented as medians with 95% confidence intervals (CIs) if not otherwise stated. Statistical analyses were performed by the Mann-Whitney U test. Because of multiple comparisons, only values of $P \leq 0.01$ were considered significant. The calculations were performed using the MINITAB statistical software package.

An extended Methods section can be accessed online at http://www.hypertensionaha.org.

Results
There were no significant differences between the mothers in terms of age, but the mean gestational length was shorter ($P<0.001$) and the mean birth weight was lower ($P<0.001$) in the preeclamptic group. In addition, both the initial and final blood pressures were significantly higher in the preeclamptic group (Table 1).

Expression of Adhesion Molecules on Neutrophils and Monocytes
Of the 12 adhesion molecules examined, 8 tended to be increased on neutrophils in newborns from preeclamptic mothers with significantly increased surface expression of CD15s (sialyl-Lewis$^a$), CD49d/CD29, (very late antigen [VLA]-4), and CD31 (platelet and endothelial cell adhesion molecule-1) compared with neutrophils in neonates from healthy mothers (Table 2). The monocytes in newborns from mothers with preeclampsia also showed signs of enhanced activation with increased expression of CD15s, CD11c

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n=36)</th>
<th>Preeclampsia (n=35)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>31.5 (30–33)</td>
<td>31 (29–32.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure, initial, mm Hg</td>
<td>110/68 (108/63–115/70)</td>
<td>120/75 (118/70–123/78)</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood pressure at delivery, mm Hg</td>
<td>115/70 (108/68–120/75)</td>
<td>175/113 (170/110–180/115)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria, g/L</td>
<td>0+</td>
<td>7.56 (5.35–11.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.4 (11.0–11.9)</td>
<td>12.5 (12.1–13.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet count, $\times 10^9$/L</td>
<td>205 (183–236)</td>
<td>178 (161–209)</td>
<td>0.13</td>
</tr>
<tr>
<td>Uric acid, mmol/L</td>
<td>257 (204–315)</td>
<td>390 (361–417)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age, wk+d</td>
<td>38±3 (38±2–38±4)</td>
<td>31±6 (29±3–32±4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3,500 (3,260–3,705)</td>
<td>1,355 (1,115–1,678)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal weight before pregnancy, kg</td>
<td>66 (60–76.5)</td>
<td>68.7 (63.5–74.7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are shown as median and 95% CI.
(p150.95), and CD54 (ICAM-1) compared with monocytes in newborns from healthy mothers (Table 2).

**Plasma Levels of Chemokines and Soluble Adhesion Molecules**

In 20 of the newborns from preeclamptic pregnancies and 19 of the newborns from normal pregnancies, we also examined plasma levels of chemokines and soluble adhesion molecules.

As can be seen in the Figure, upregulation of adhesion molecules on neutrophils and monocytes in the preeclamptic group was accompanied by a significant elevation in plasma levels of the CXC chemokines IL-8 and GRO-α. In contrast, plasma levels of epithelial cell–derived neutrophil-activating peptide-78 and the CC chemokines monocyte chemotactic protein-1, macrophage inflammatory protein-1α, and RANTES in these newborns were not different from the levels in

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**Table 2. Adhesion Molecules on Neutrophils and Monocytes From Venous Cord Blood in Uncomplicated (n=36) and Preeclamptic (n=35) Pregnancies, as Determined by Flow Cytometry**

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Mean Fluorescence Intensity</th>
<th>Normal</th>
<th>Preeclampsia</th>
<th>P</th>
<th>Normal</th>
<th>Preeclampsia</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selectins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>L-selectin</td>
<td>23.6 (20.4–26.5)</td>
<td>29.0 (26.4–30.5)</td>
<td>0.01</td>
<td>10.9 (9.7–11.9)</td>
<td>12.9 (11.4–14.9)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>P-selectin</td>
<td>0.0 (0.0–0.5)</td>
<td>0.3 (0.0–0.8)</td>
<td>0.32</td>
<td>0.0 (0.0–0.7)</td>
<td>0.2 (0.0–2.2)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td><strong>Mucins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sialyl-Lewis*</td>
<td>80 (60–190)</td>
<td>261 (147–395)</td>
<td>0.003</td>
<td>37.0 (20.7–67)</td>
<td>101 (62–203)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>PSGL-1</td>
<td>94 (84–114)</td>
<td>87 (67–96)</td>
<td>0.14</td>
<td>219 (150–263)</td>
<td>213 (176–271)</td>
<td>0.98</td>
<td></td>
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<tr>
<td><strong>Integrins</strong></td>
<td></td>
<td></td>
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<tr>
<td>CD11a</td>
<td>11.0 (7.1–14.3)</td>
<td>13.3 (9.2–15.6)</td>
<td>0.06</td>
<td>35.8 (28.1–41.6)</td>
<td>38.8 (29.3–49.6)</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>CD11b</td>
<td>157 (122–235)</td>
<td>198 (148–253)</td>
<td>0.37</td>
<td>138 (131–206)</td>
<td>175 (157–248)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>CD11c</td>
<td>36.6 (27.1–40.7)</td>
<td>39.7 (33.8–48.3)</td>
<td>0.18</td>
<td>81 (64–95)</td>
<td>106 (97–126)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>CD49d</td>
<td>2.3 (1.2–3.3)</td>
<td>3.3 (2.6–7.0)</td>
<td>0.01</td>
<td>49.3 (39.3–55)</td>
<td>46.9 (42.4–68)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>CD29/VLA-4</td>
<td>16.6 (14.5–20.3)</td>
<td>22.9 (17.5–26.9)</td>
<td>0.005</td>
<td>87 (77–94)</td>
<td>94 (82–121)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td><strong>Immunoglobulin gene superfamily</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ICAM-1</td>
<td>10.4 (7.7–11.2)</td>
<td>12.8 (10.4–14.8)</td>
<td>0.03</td>
<td>43 (35.0–49.9)</td>
<td>61 (52–68)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>ICAM-3</td>
<td>473 (368–520)</td>
<td>592 (545–646)</td>
<td>0.03</td>
<td>456 (374–541)</td>
<td>478 (445–634)</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>CD 31</td>
<td>14.6 (13.0–16.5)</td>
<td>17.9 (15.1–20.1)</td>
<td>0.007</td>
<td>30.6 (28.8–37.5)</td>
<td>35.2 (32.0–39.1)</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

PSGL-1 indicates P-selectin glycoprotein ligand-1. Mean fluorescence intensity is expressed as median and 95% CI.

Plasma levels of the CXC chemokines IL-8 (A) and GRO-α (B) and the soluble (prefix s) adhesion molecules sL-selectin (C) and sE-selectin (D) in 20 newborns from normal pregnancies and 19 newborns from preeclamptic pregnancies.
newborns from healthy mothers (data not shown). Moreover, although plasma levels of CXC chemokines were elevated, plasma levels of soluble L-selectin and soluble E-selectin were significantly decreased in the preeclamptic group (Figure). As for levels of soluble P-selectin, soluble VCAM-1, and soluble ICAM-1, there were no significant differences between the 2 groups of neonates (data not shown).

### Parameters of Leukocyte Activation in Relation to Blood Pressure, Gestational Age, and Prematurity

When newborns in the preeclamptic group were compared according to blood pressure of their mothers (160/110 mm Hg \(n=13\) and \(>160/110\) mm Hg [median, 175/115 mm Hg; \(n=22\)], no differences were found between these 2 groups, except for a moderate increased expression of CD54 on neutrophils in the former group \((P=0.04)\). Furthermore, when newborns in the preeclamptic group were compared according to birth weight \((<1.340\) kg: median, 0.923 kg \([n=17]\); and \(>1.340\) kg: median, 2.000 kg \([n=18]\)), no differences were found between these 2 groups of neonates, except for a moderate increased expression of CD11a \((P=0.02)\) and CD31 \((P=0.04)\) on neutrophils in the former group. Similar findings were revealed when newborns were compared according to gestational age \((<31\) weeks: median, 27.3 weeks \([n=17]\); and \(>31\) weeks: median, 34.3 weeks \([n=18]\)).

When similar comparisons were performed for soluble adhesion molecules, no differences were found, except for an increased level of CD62P in those with highest blood pressure \((P=0.05)\), in those with lowest birth weight \((P=0.03)\), and in those with the lowest gestational age \((P=0.03)\). In contrast, when newborns were compared according to the blood pressures of their mothers (see above), neonates of mothers with the highest blood pressures had significantly raised plasma levels of IL-8 \((4.5\) [1 to 17] pg/mL versus 24.5 [6 to 97] pg/mL; \(P=0.006)\), but not the other chemokines, compared with the plasma levels of the other newborns in the preeclamptic group. In fact, only neonates of mothers with the highest blood pressures had significantly raised IL-8 levels compared with levels in newborns of mothers with normal pregnancies. When chemokine levels in newborns of preeclamptic women were compared according to birth weight or gestational age (see above), no differences were found.

### Discussion

It is uncertain what causes the disturbed circulation in the fetus during preeclampsia. In the present study, we demonstrate increased expression of adhesion molecules on neutrophils and monocytes in neonates from mothers with severe preeclampsia, accompanied by elevated CXC chemokine levels in the circulation. Although there is considerable overlap between the preeclamptic group and the control group, we suggest that these findings may reflect a state of persistent inflammation, which at least partly contributes to the pathogenesis of endothelial dysfunction in these newborns.

The upregulation of different adhesion molecules on neutrophils and monocytes in preeclamptic neonates suggests that these cells are activated and may have a capacity to perform all steps in leukocyte/endothelial interaction, i.e., capture, rolling, firm adhesion, and extravasation in the fetal circulation during preeclampsia. In particular, because the neonates from preeclamptic pregnancies had increased expression of CD15s on neutrophils and monocytes and the fetal endothelium is activated with upregulated P-selectin in preeclampsia, the initial step of capture and rolling of leukocytes on the endothelium may potentially start by adhesion between the CD15s and P-selectin molecules. VLA-4 (CD49d/CD29) was also increased on neutrophils from newborns of preeclamptic mothers, and interestingly, IL-8, which was elevated in these infants, may upregulate the surface expression of VLA-4 integrins on these cells.

Furthermore, monocytes from newborns of preeclamptic mothers showed increased expression of CD11c and CD54. Because CD11c and CD54 are each the ligand of the other, the monocytes may potentially form cellular aggregates in the fetal circulation, which, in turn, could "plug" the microcirculation in the placenta. The hypoxic condition in the placenta during preeclampsia may further activate the circulating monocytes, rendering the placenta more susceptible to such "plugging," possibly representing a vicious circle leading to enhanced leukocyte and endothelial cell activation in preeclampsia.

Although several membrane-bound adhesion molecules were upregulated on leukocytes in the preeclamptic group, plasma levels of soluble L-selectin and soluble E-selectin were significantly decreased in these newborns. These findings may possibly reflect prematurity, inasmuch as lower soluble L-selectin and soluble E-selectin levels have been shown in premature infants, although in the present study, we could not reveal any association between levels of these adhesion molecules and the degree of prematurity. However, it may also reflect enhanced activation of endothelial cells and leukocytes in these infants. Thus, although acute endothelial cell activation may result in enhanced shedding of E-selectin from these cells, a persistent activation of these cells may lead to downregulation of this process. Moreover, it has been suggested that soluble L-selectin may prevent interaction between leukocytes and endothelium; therefore, decreased levels may potentially enhance the interaction between leukocytes and endothelial cells.

A major finding in the present study was the significantly increased plasma levels of the CXC chemokines IL-8 and GRO-α in the preeclamptic group. CXC chemokines are potent chemotactic agents for neutrophils, but they may also activate monocytes and T cells. Moreover, IL-8 and GRO-α may attract leukocytes into sites of inflammation and further activate these cells in inflamed tissues by inducing enhanced generation of reactive oxygen species (ROS) and proliferation as well as increased secretion of matrix-degrading enzymes. Both IL-8 and GRO-α have been shown to delay neutrophil apoptosis, and an extended functional half-life of these cells may further enhance their inflammatory potential. In particular, oxidative stress is through activation of nuclear factor-κB, a transcriptional factor that is a potent inducer of both IL-8 and GRO-α, and these chemokines may, in turn, further enhance ROS generation in neutrophils. Thus, it is possible that the increased levels of these CXC chemokines may reflect a pathogenic loop in newborns of preeclamptic mothers involving enhanced oxidative stress.
In the present study, only infants from severe preeclamptic pregnancies were studied, and we cannot exclude that our findings may reflect an extreme stress to fetuses and not something specific to preeclampsia. However, although there were some indications that enhanced leukocyte activation might be related to the degree of preeclampsia and prematurity, our results suggest that the enhanced expression of adhesion molecules on neutrophils and monocytes at least partly may be a characteristic of preeclampsia, per se. In contrast, only newborns of mothers with the most severe preeclampsia had significantly elevated IL-8 levels, and this chemokine may be a potentially interesting parameter, possibly reflecting the degree of preeclampsia. However, new studies also examining newborns of mothers with mild preeclampsia will have to be performed to further elucidate these issues. Moreover, caution is needed when interpreting “correlation studies,” because the present studies and follow-up studies including more mechanistic experiments and longitudinal testing will have to be performed before any firm conclusion can be drawn.

Hypoxia and oxidative stress are found in the fetoplacental circulation and placenta in preeclampsia, and this may enhance the release of chemokines from various leukocyte subsets. Production of these inflammatory chemokines may lead to chemotaxis of neutrophils and monocytes, initiating transformation into an activated state. Indeed, our findings in the present study suggest activation of neutrophils and monocytes in the fetus in preeclampsia involving enhanced chemokine activation. Adhesion to the endothelium of activated leukocytes may release cytotoxic agents, such as ROS and proteases, and further contribute to activation of both the endothelium and other leukocytes. This may make up a vicious circle for the fetus in preeclampsia, possibly contributing to the increased fetal vascular resistance, intrauterine growth retardation, and mortality seen in preeclampsia.

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