

# Commentary

## What Causes Endothelial Cell Activation in Preeclamptic Women?

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Preeclampsia is a hypertensive disorder of pregnancy and a leading cause of fetal and maternal morbidity and mortality. It is associated with proteinuria, pathological edema, coagulation abnormalities, reduced uteroplacental blood flow, and intrauterine growth restriction.

The cause of preeclampsia is not known, but there is good evidence that it is associated with endothelial cell activation and dysfunction. Many investigators have demonstrated markers of endothelial cell activation in women with preeclampsia. Indicators of endothelial activation include increased circulating levels of von Willebrand factor, endothelin, soluble vascular cell adhesion molecule, thrombomodulin, and cellular fibronectin as well as increased growth factor activity.<sup>1,2</sup> Perhaps the most important indicator of endothelial cell dysfunction is decreased plasma and urinary levels of prostacyclin, because the endothelium is the main source of prostacyclin.<sup>3-5</sup>

Factors present in the blood of preeclamptic women cause endothelial cell activation. For example, exposure of endothelial cells to plasma or serum from preeclamptic women causes activation of nuclear factor- $\kappa$ B and increased expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 compared with plasma or serum from normal pregnant women.<sup>6,7</sup> Treatment with antioxidants prevents these effects, so oxidative stress appears to mediate the effects of plasma factors. The question remains what factor or factors present in the blood of preeclamptic women causes endothelial cell activation. To date, a number of possible candidates have been suggested.

### *Shed Membrane Particles*

In this issue of *The American Journal of Pathology*, Meziani and colleagues present a novel mechanism for endothelial cell activation in preeclamptic women.<sup>8</sup> They show that shed membrane particles of leukocytes and platelets

that are present in plasma have the ability to induce vascular inflammation and modulate vascular tone. They report that preeclamptic women have significantly more total membrane particles, more leukocyte-derived membrane particles, and more platelet-derived membrane particles than normal pregnant women. Vascular effects were tested by incubating the particles for 24 hours with human omental vessels or mouse aortic rings and then evaluating their responses to serotonin. Membrane particles from preeclamptic but not normal pregnant women reduced serotonin-induced vasoconstriction. Use of a nitric-oxide synthase inhibitor and determination of nitric oxide release suggested that preeclamptic membrane particles antagonized serotonin vasoconstriction by stimulating inducible nitric-oxide synthase to increase nitric oxide. Treatment of vessels with a cyclooxygenase-2 (COX-2) inhibitor reduced serotonin vasoconstriction.

Preeclamptic membrane particles also induced inflammation in treated vessels as evidenced by activation of nuclear factor- $\kappa$ B, expression of COX-2, formation of nitrotyrosine, and production of superoxide. Preeclamptic membrane particles also stimulated production of 8-isoprostane from treated vessels, demonstrating their ability to induce oxidative stress. Separation of platelet membrane particles from leukocyte membrane particles suggested that leukocyte membrane particles were responsible for inducing COX-2, whereas platelet membrane particles were responsible for stimulating nitric oxide. The investigators suggest that platelet membrane particles may have a protective effect in preeclamptic women by counteracting vasoconstriction. The data also suggest that leukocyte membrane particles may be a cause of endothelial inflammation in preeclampsia. Studies showing inflammatory effects of preeclamptic serum or plasma on endothelial cells could be due to leukocyte membrane particles because the centrifugal force used to separate

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serum or plasma is not sufficient to remove membrane particles.

Leukocytes and platelets are not the only source of membrane particles in pregnancy. The placenta is an additional source.<sup>9</sup> The maternal side of the placenta is lined by the multinucleated syncytiotrophoblast cells, which continually break down and are replaced by fusion of the underlying cytotrophoblast cells. Syncytiotrophoblast membrane fragments are an additional source of membrane particles during pregnancy. During preeclamptic pregnancy, the placenta is under oxidative stress with increased production of lipid peroxides and decreased antioxidant protection.<sup>5,10,11</sup> Placental oxidative stress destabilizes the syncytiotrophoblast cells, resulting in an increase in the release of membrane particles containing oxidized lipids. Syncytiotrophoblast membrane particles isolated from plasma of preeclamptic women have also been shown to cause endothelial activation.<sup>9</sup>

### Oxidative Stress

Another proposed cause of endothelial cell activation is oxidative stress, and preeclampsia is associated with oxidative stress.<sup>5,12</sup> Placentas of women with preeclampsia produce significantly more lipid peroxides than placentas of women with normal pregnancy,<sup>5,10</sup> and the lipid peroxides are secreted primarily toward the maternal circulation.<sup>13</sup> Markers of lipid peroxidation are elevated in the maternal circulation. Thiobarbituric reactive substances, which primarily reflect malondialdehyde (a breakdown product of lipid peroxides), and 8-isoprostane are elevated in maternal plasma of preeclamptic women.<sup>5,12</sup> Oxidized lipids present in low-density lipoproteins could cause oxidation of endothelial cell membranes as the low-density lipoproteins are taken up by the cells, and the oxidized lipids are incorporated into the cell membranes. Oxidation of endothelial cell membranes causes them to become leaky to proteins.<sup>14</sup> Thus, oxidation of endothelial cells could explain edema and proteinuria, which are clinical symptoms of preeclampsia.

Maternal circulating oxidized lipids may be the cause of endothelial cell activation, but attempts to directly measure oxidized lipids or oxidized low-density lipoprotein in maternal blood have been inconsistent. Branch et al reported higher titers of autoantibodies to oxidized low-density lipoprotein,<sup>15</sup> but other investigators have not confirmed this.<sup>16</sup> Diedrich et al used a highly specific high-performance lipid chromatographic-chemiluminescence technique to measure lipid peroxides in plasma, but they could not detect an increase in preeclamptic women.<sup>17</sup> They did, however, confirm a significant elevation in thiobarbituric reactive substances. These findings raise the possibility that circulating oxidized lipids per se are not the cause of endothelial cell activation, but rather, the cause is a breakdown product such as malondialdehyde. Malondialdehyde is an aldehyde and is thus capable of inducing toxic effects on endothelial cells.

### Elevated Plasma Lipids

Elevated plasma lipids are another possible cause of endothelial cell activation. Plasma lipid levels increase in pregnancy but increase further in women with preeclampsia.<sup>18</sup> Serum-free fatty acids and triglycerides are increased in women with preeclampsia and are increased before 20 weeks' gestation in women who later develop preeclampsia. Treatment of endothelial cells with sera from preeclamptic women increases the content of triglycerides and reduces the release of prostacyclin. One of the fatty acids elevated in preeclamptic women is linoleic acid. Linoleic acid induces oxidative stress in endothelial cells, and endothelial cells incubated with linoleic acid show a concentration-dependent reduction in the release of prostacyclin.<sup>18</sup>

### Activated Neutrophils

Another possible cause of endothelial cell activation is neutrophils. Neutrophil numbers increase during pregnancy and increase further with preeclampsia. Several reports demonstrate that neutrophils are activated in women with preeclampsia.<sup>19</sup> Neutrophil activation likely occurs as they circulate through the intervillous space and are exposed to oxidized lipids secreted by the placenta.<sup>5</sup>

Plasma factors have been shown to stimulate transendothelial migration of neutrophils *in vitro*. Plasma from preeclamptic women significantly stimulated transendothelial migration of neutrophils compared with plasma from normal pregnant women or normal nonpregnant women.<sup>20</sup> Treatment with antioxidants blocked the response, demonstrating that oxidative stress was involved.

We recently showed extensive infiltration of neutrophils into the systemic vasculature of women with preeclampsia.<sup>21,22</sup> Neutrophils were flattened and adhered to the endothelium and infiltrated into the intimal space. Neutrophil infiltration was associated with markers of inflammation in the endothelium. Nuclear factor- $\kappa$ B was activated, and there was increased expression of intercellular adhesion molecule-1, COX-2, and interleukin-8.<sup>21,22</sup> Although circulating factors might initially activate the endothelium, flattening and adherence of neutrophils to the endothelium would exacerbate the situation through release of reactive oxygen species, tumor necrosis factor- $\alpha$ , and myeloperoxidase.

The endothelium is not the only part of the vasculature that is affected in preeclampsia. The vascular smooth muscle is also dysfunctional, showing activation of nuclear factor- $\kappa$ B and increased expression of intercellular adhesion molecule-1, COX-2, and interleukin-8.<sup>21,22</sup> Although circulating factors in maternal blood, such as membrane particles, oxidized lipids, unoxidized lipids, or malondialdehyde, might activate endothelial cells, they probably would not affect the underlying vascular smooth muscle. Vascular smooth muscle dysfunction requires a further mechanism that may involve neutrophils. Release of reactive oxygen species, tumor necrosis factor- $\alpha$ , and

myeloperoxidase by neutrophils that have infiltrated into the intimal space could be responsible for smooth muscle inflammation in preeclampsia, whereas neutrophil release of thromboxane and thromboxane produced in smooth muscle as a result of the increased expression of COX-2 could be responsible for vasoconstriction leading to hypertension.

### Conclusion

Shed membrane particles from leukocytes are a novel idea for causing endothelial cell activation and inflammation in preeclampsia. Shed membrane particles from the syncytiotrophoblast cells of the placenta are an additional source of membrane particles; however, most of these are trapped in the mother's lungs and do not enter the maternal systemic arterial circulation. Leukocytes, on the other hand, are present in the maternal systemic arterial circulation in large numbers, especially in preeclamptic women, and they are activated. Activated leukocytes would presumably undergo increased rates of apoptosis resulting in increased circulating leukocyte membrane particles. Thus, leukocyte membrane particles might just be the cause of endothelial cell activation in preeclampsia, as proposed by Mezziani and colleagues.<sup>8</sup>

### References

1. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK: Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol* 1989, 161:1200–1204
2. Taylor RN, de Groot CJ, Cho YK, Lim KH: Circulating factors as markers and mediators of endothelial cell dysfunction in preeclampsia. *Semin Reprod Endocrinol* 1998, 16:17–31
3. Chavarria ME, Lara-Gonzalez L, Gonzalez-Gleason A, Garcia-Palata Y, Vital-Reyes VS, Reyes A: Prostacyclin/thromboxane early changes in pregnancies that are complicated by preeclampsia. *Am J Obstet Gynecol* 2003, 188:986–992
4. Mills JL, DerSimonian R, Raymond E, Morrow JD, Roberts 2nd LJ, Clemens JD, Hauth JC, Catalano P, Sibai B, Curet LB, Levine RJ: Prostacyclin and thromboxane changes predating clinical onset of preeclampsia: a multicenter prospective study. *JAMA* 1999, 282:356–362
5. Walsh SW: Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. *Semin Reprod Endocrinol* 1998, 16:93–104
6. Endresen MJ, Morris JM, Nobrega AC, Buckley D, Linton EA, Redman CW: Serum from preeclamptic women induces vascular cell adhesion molecule-1 expression on human endothelial cells in vitro: a possible role of increased circulating levels of free fatty acids. *Am J Obstet Gynecol* 1998, 179:665–670
7. Takacs P, Kauma SW, Sholley MM, Walsh SW, Dinsmoor MJ, Green K: Increased circulating lipid peroxides in severe preeclampsia activate NF-kappaB and upregulate ICAM-1 in vascular endothelial cells. *FASEB J* 2001, 15:279–281
8. Mezziani F, Tesse A, David E, Martinez MC, Wangesten R, Schneider F, Andriantsitohaina R: Shed membrane particles from preeclamptic women generate vascular wall inflammation and blunt vascular contractility. *Am J Pathol* 2006, 169:1473–1483
9. Redman CW, Sargent IL: Placental debris, oxidative stress and preeclampsia. *Placenta* 2000, 21:597–602
10. Walsh SW, Vaughan JE, Wang Y, Roberts 2nd LJ: Placental isoprostanol is significantly increased in preeclampsia. *FASEB J* 2000, 14:1289–1296
11. Wang Y, Walsh SW: Increased superoxide generation is associated with decreased superoxide dismutase activity and mRNA expression in placental trophoblast cells in pre-eclampsia. *Placenta* 2001, 22:206–212
12. Hubel CA: Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med* 1999, 222:222–235
13. Walsh SW, Wang Y: Secretion of lipid peroxides by the human placenta. *Am J Obstet Gynecol* 1993, 169:1462–1466
14. Granger DN, Rutigli G, McCord JM: Superoxide radicals in feline intestinal ischemia. *Gastroenterology* 1981, 81:22–29
15. Branch DW, Mitchell MD, Miller E, Palinski W, Witztum JL: Preeclampsia and serum antibodies to oxidized low-density lipoprotein. *Lancet* 1994, 343:645–646
16. Gratacos E, Casals E, Deulofeu R, Gomez O, Cararach V, Alonso PL, Fortuny A: Serum antibodies to oxidized low-density lipoprotein in pregnant women with preeclampsia and chronic hypertension: lack of correlation with lipid peroxides. *Hypertens Pregnancy* 2001, 20:177–183
17. Diedrich F, Renner A, Rath W, Kuhn W, Wieland E: Lipid hydroperoxides and free radical scavenging enzyme activities in preeclampsia and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome: no evidence for circulating primary products of lipid peroxidation. *Am J Obstet Gynecol* 2001, 185:166–172
18. Lorentzen B, Henriksen T: Plasma lipids and vascular dysfunction in preeclampsia. *Semin Reprod Endocrinol* 1998, 16:33–39
19. Clark P, Boswell F, Greer IA: The neutrophil and preeclampsia. *Semin Reprod Endocrinol* 1998, 16:57–64
20. Goodyear AL, Walsh SW, Fisher BJ, Fowler AA: Oxidative stress and TNF $\alpha$  stimulate transendothelial migration of neutrophils. *J Soc Gynecol Invest* 1999, 6(Suppl):80A
21. Leik CE, Walsh SW: Neutrophils infiltrate resistance-sized vessels of subcutaneous fat in women with preeclampsia. *Hypertension* 2004, 44:72–77
22. Shah TJ, Walsh SW: Activation of NF- $\kappa$ B and expression of COX-2 in association with neutrophil infiltration in systemic vascular tissue of women with preeclampsia. *Am J Obstet Gynecol* 2006, (in press)