Pathogenesis of Preeclampsia

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Abstract

Preeclampsia is a systemic syndrome that occurs in 3 to 5% of pregnant women and classically manifests as new-onset hypertension and proteinuria after 20 weeks of gestation. Preeclampsia is a leading cause of maternal and neonatal morbidity and mortality. The only known cure is delivery of the placenta. Recent discoveries, however, have led to important advances in understanding the pathogenesis of the condition. Placental antiangiogenic factors are upregulated and disrupt the maternal endothelium. This change in the normal angiogenic balance toward an antiangiogenic state can result in hypertension, proteinuria, glomerular endotheliosis, HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, and cerebral edema—the clinical signs of preeclampsia and eclampsia. The regulation of these antiangiogenic factors in the placenta is unknown. The recent discoveries of upregulated antiangiogenic factors provide promise for future testing to predict and diagnose preeclampsia as well as therapeutic targets for amelioration of the clinical disease.
INTRODUCTION

Preeclampsia is a pregnancy-specific disorder that affects 3–5% (1, 2) of pregnant women worldwide and is one of the most frequently encountered medical complications of pregnancy. Classically, the condition presents with new-onset hypertension and proteinuria after 20 weeks of gestation (3). In developing countries where access to health care is limited, preeclampsia is a leading cause of maternal mortality, causing an estimated >60,000 maternal deaths worldwide per year (1). In developed countries, inducing premature delivery to protect the health of the mother results in significant morbidity and mortality for the neonate, due to the sequelae of prematurity and low birth weight (4). Preeclampsia is the third leading cause of maternal mortality in the United States and accounts for 20% of maternal deaths (5).

Delivery of the placenta remains the only known treatment for this clinical disease, suggesting that the placenta is the principal contributor to the pathogenesis of preeclampsia. High levels of antiangiogenic factors and low levels of proangiogenic factors released by the placenta contribute to the development of the maternal hypertensive syndrome of preeclampsia, which is thought to result from widespread endothelial dysfunction. In this article, we review recent discoveries that hold promise for the diagnosis and prediction of the disease and that suggest therapeutic modalities to be employed to ameliorate the condition.

Epidemiology and Risk Factors

Most preeclampsia occurs in healthy nulliparous women, in whom the incidence of preeclampsia may be as high as 7.5%. Although preeclampsia is classically a disorder of women in their first pregnancy, multiparous pregnant women with a new partner have an elevated risk of preeclampsia similar to that of nulliparous women (6). The increased risk may be due either to the change in paternity or to an increased interpregnancy interval. Additionally, women with a history of preeclampsia in a prior pregnancy are at increased risk of developing preeclampsia in future pregnancies, particularly if the preeclampsia had developed early in gestation (7, 8). Although most cases of preeclampsia occur without a known family history, the presence of preeclampsia in a first-degree relative increases a woman’s risk of severe preeclampsia two- to fourfold (9). A history of preeclampsia in the father’s mother also confers an increased risk (10).

Several medical conditions are associated with increased preeclampsia risk. These include chronic hypertension, diabetes mellitus, renal disease, metabolic syndrome, and hypercoagulable states (7, 8, 11). Very young maternal age and advanced maternal age are also independent risk factors for preeclampsia (8, 11). Obstetrical conditions with increased placental mass, such as multifetal gestation (8, 12) and hydatidiform mole (13), increase preeclampsia risk. Smoking protects against development of preeclampsia (14). These risk factors can be explained by mechanisms of preeclampsia that have already been deduced.

Clinical Features

Preeclampsia is a heterogeneous condition that can be challenging to diagnose, given the wide spectrum of presentation and the current lack of a robust diagnostic test. The cardinal features of preeclampsia are new-onset hypertension (defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) and proteinuria (300 mg or greater in a 24-h urine specimen) (3). With the classical presentation, women typically develop preeclampsia after 20 weeks gestation and prior to 48 h postpartum (15). A percentage of women present atypically without one of these cardinal signs, making the diagnosis difficult to confirm or exclude. Up to 20% of women with atypical preeclampsia have minimal or no proteinuria (16). The degree of proteinuria in preeclampsia may vary from minimal to nephrotic; however, the amount of proteinuria does not seem to affect maternal or fetal outcomes (15). Historically, edema was...
part of the diagnostic triad of preeclampsia (i.e., hypertension, proteinuria, and edema); however, edema is too nonspecific to be used for diagnostic purposes because a majority of pregnant women without preeclampsia develop edema toward the end of their pregnancies. The current criteria for the diagnosis of preeclampsia are based on clinical signs and symptoms (3) and are not always helpful in cases of atypical or superimposed preeclampsia (preeclampsia superimposed on chronic hypertension or chronic renal disease).

Preeclampsia has a wide spectrum with regard to presentation, time of onset, and severity. In severe disease, women may develop severe headaches or visual changes, right upper quadrant pain from acute liver injury, pulmonary edema, oliguria from acute renal failure, hemolysis and/or thrombocytopenia, and/or grand mal seizures or eclampsia. Current clinical guidelines support the differentiation of preeclampsia into mild and severe categories; these entities are treated differently, particularly at preterm gestations (15). Hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome is a severe variant of preeclampsia and may warrant expedient delivery to prevent development of life-threatening thrombocytopenia or hemolysis (16). Eclampsia complicates 2% of pregnancies with preeclampsia (18). Typically, eclampsia occurs after the onset of hypertension and proteinuria. A severe headache or visual blurring often heralds its onset. However, 20% of women who develop eclampsia do not have proteinuria (19). Eclamptic seizures can occur in the immediate puerperium and, infrequently, 48 h to one month postpartum, in which case the condition is described as late postpartum eclampsia. Interestingly, one-third or more of patients with postpartum eclampsia present without ever having manifested signs and symptoms of preeclampsia (20).

Although the maternal complications from preeclampsia are significant, the developing fetus can also be affected. Fetal and neonatal complications from preeclampsia include iatrogenic prematurity, fetal growth restriction, oligohydramnios, and increased risk of perinatal death (11). The exact pathogenesis of these fetal complications is unknown, yet impaired uteroplacental blood flow, placental abruption (which, when accompanied by gestational hypertension or preeclampsia, has been associated with a circulating antiangiogenic state), and infarction probably contribute.

Management of Preeclampsia

As mentioned above, despite research advances in understanding the pathogenesis of preeclampsia there remains no treatment except delivery of the placenta. Currently, there is no blood test to diagnose or exclude preeclampsia; diagnosis is confirmed by the clinical criteria outlined above. Once a diagnosis of preeclampsia is suspected, serial blood counts are conducted to monitor for development of thrombocytopenia, hemolysis, liver damage, or renal impairment. Blood pressure is controlled with medication if necessary. Close monitoring of the fetal status occurs with ultrasound surveillance and fetal heart rate testing. Mild preeclampsia can be managed expectantly until 37 weeks gestation (21). Women who develop severe preeclampsia can be managed expectantly with close monitoring of maternal and fetal status until (a) evidence of HELLP syndrome or eclampsia develops, (b) maternal blood pressure can no longer be controlled by medication, (c) the fetal status is nonreassuring, or (d) by the time 34 weeks of gestation has been attained, when the risks for the mother of continuing the pregnancy outweigh the risks for the baby (11). Patients with preeclampsia are often treated with magnesium for 24 h to decrease the likelihood of eclampsia (18).

Long-Term Morbidity and Mortality

The acute effects of preeclampsia resolve with delivery of the placenta; however, new research suggests that risks to the mother persist long after her reproductive years are completed. Approximately 20% of women with preeclampsia develop hypertension or
microalbuminuria within seven years of their pregnancy compared with only 2% among women with uncomplicated pregnancies. The risk of cardiovascular and cerebrovascular disease is doubled in women with preeclampsia and gestational hypertension compared with age-matched controls (22). This increase in subsequent cardiovascular disease is observed for both preeclampsia and gestational hypertension (2, 23), suggesting either common risk factors for these two syndromes or a common pathophysiology. Severe preeclampsia, recurrent preeclampsia, preeclampsia developing before 37 weeks of gestation, and preeclampsia with fetal growth restriction are most strongly associated with future adverse cardiovascular outcomes (24). Preeclampsia is also a marker for increased risk of subsequent end-stage renal disease, although the absolute risk is low (24).

Preeclampsia and cardiovascular disease share many risk factors, including chronic hypertension, diabetes, obesity, renal disease, and metabolic syndrome (2). However, the increased risk of cardiovascular events in women with a history of preeclampsia persists over the long term, even for previously healthy women with no known vascular risk factors. These studies suggest that preeclampsia itself may be a risk factor for future cardiovascular events, possibly through persistent subclinical systemic vascular damage or endothelial dysfunction occurring in women who were healthy prior to the onset of preeclampsia.

PATHOGENESIS

Preeclampsia is a systemic syndrome of pregnancy originating in the placenta. It is thought to be caused by inadequate placental cytotrophoblast invasion, followed by widespread maternal endothelial dysfunction. Research has demonstrated that excess quantities of the antiangiogenic factors soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) are released by the placenta into maternal blood, causing widespread endothelial dysfunction that results in hypertension, proteinuria, and other systemic manifestations of preeclampsia (25, 26). The molecular basis for placental dysregulation of these pathogenic factors remains unknown. The role of these antiangiogenic proteins in early placental vascular development and in trophoblast invasion is just beginning to be explored. Hypoxia is likely to be an important regulator. Additionally, perturbation of the renin–aldosterone–angiotensin II axis, excessive oxidative stress, inflammation, immune maladaptation, and genetic susceptibility may all contribute to the pathogenesis of preeclampsia.

Role of the Placenta

The placenta is essential to the development and remission of preeclampsia. Its importance is demonstrated in the case of hydatidiform moles. Women with hydatidiform moles, in which a fetus is absent, can still develop preeclampsia. This indicates that a placenta, but not a fetus, is required for the development of preeclampsia. The condition remits after curettage and removal of the mole (13). In a case of preeclampsia with an extraterine pregnancy, delivery of the fetus alone was not sufficient; symptoms persisted until the placenta was delivered (27). Cases of postpartum eclampsia have been associated with retained placental fragments, as the patients rapidly improved after uterine curettage (28).

Severe preeclampsia is associated with pathologic evidence of placental hypoperfusion and ischemia. Findings include acute atherosclerosis, a lesion of diffuse vascular obstruction that includes fibrin deposition, intimal thickening, necrosis, atherosclerosis, and endothelial damage. Placental infarcts, probably due to occlusion of spiral arteries (29), are also commonly observed in pathological analysis of the placenta. Abnormal uterine artery Doppler ultrasound, consistent with decreased uteroplacental perfusion, is frequently observed before the clinical onset of preeclampsia (30). The severity of the gross placental pathology appears to be correlated with the severity of the clinical disease, although these findings are not universal.
Placental Vascular Development

Because the placenta is central to the pathogenesis of preeclampsia, research has focused on the association between abnormal placental vascular development and the development of this disease. During early normal placental development, extravillous cytotrophoblasts of fetal origin invade the uterine spiral arteries of the decidua and myometrium. These invasive cytotrophoblasts replace the endothelial layer of the maternal spiral arteries, transforming them from small, high-resistance vessels into large-caliber capacitance vessels capable of providing adequate placental perfusion to nourish the fetus. In preeclampsia, this transformation is incomplete (31). Cytotrophoblast invasion of the spiral arteries is limited to the superficial decidua, and the myometrial segments remain narrow. One group of investigators revealed the importance of adhesion molecules for the cytotrophoblast invasion process by finding that cytotrophoblast expression of adhesion molecules was abnormal in preeclamptic placentas (32). During normal placental development, cytotrophoblasts undergo pseudovasculogenesis, or vascular mimicry, to assume an endothelial phenotype. Pseudovasculogenesis occurs through down-regulation of adhesion molecules and adoption of an endothelial cell-surface adhesion phenotype (33). In preeclampsia, cytotrophoblasts do not undergo this switching of cell-surface molecules and thus are unable to invade the myometrial spiral arterioles effectively (32, 34).

Angiogenic factors are thought to be important in the regulation of placental vascular development. Their receptors, Flt1 [also known as vascular endothelial growth factor receptor 1 (VEFGR-1)], VEGFR-2, Tie-1, and Tie-2, are essential for normal placental vascular development. Alterations in the regulation and signaling of angiogenic pathways in early gestation may also contribute to the inadequate cytotrophoblast invasion seen in preeclampsia. Mice engineered to have deletions in these genes have defective placental vasculogenesis and early embryonic mortality (35). In humans, VEGF ligands and receptors are highly expressed by the placental tissue in the first trimester. Invasive cytotrophoblasts express VEGF, placental growth factor (PIGF), and VEGFR-1; expression of these proteins, as elucidated by immunohistochemistry, is altered in preeclampsia (36).

sFlt1 has been shown to decrease cytotrophoblast invasiveness in vitro (36). Circulating sFlt1 levels stay relatively low early in pregnancy and begin to rise in the third trimester (37). This may reflect a physiologic antiangiogenic shift in the placental milieu toward the end of pregnancy, corresponding to the completion of the angiogenic phase of placental growth. Alterations in these angiogenic pathways during early gestation could contribute to the inadequate cytotrophoblast invasion observed in preeclampsia, thereby beginning a cycle of continued derangement in angiogenic balance. Indeed, gene-expression studies from chorionic villous biopsies at 11 weeks of gestation in women who subsequently developed preeclampsia showed marked alterations in angiogenic factors, including upregulation of sFlt1 message (38). By the third trimester, excess placental sFlt1, reflecting the degree of placental ischemia, accumulates in the maternal circulation and produces end-organ effects. However, in vivo evidence for the role of sFlt1 in placental pathology is still lacking. As with sFlt1, the presence of transforming growth factor beta (TGF-β) is inversely correlated with cytotrophoblast invasion. In normal pregnancy, TGF-β decreases at nine weeks gestation, promoting cytotrophoblast invasion. TGF-β is increased in preeclamptic placentas (39).

Inhibiting TGF-β activity with antibodies enhances the invasive properties of trophoblasts ex vivo. These data led Caniggia et al. (39) to hypothesize that failure to downregulate TGF-β results in shallow trophoblast invasion and preeclampsia. Similarly, endoglin, a receptor for TGF-β, has the same inverse relationship with cytotrophoblast invasion. Blockage of endoglin with specific antibodies also increases
trophoblast differentiation and invasion ex vivo. Therefore, it is possible that sEng produced by the placenta may be a compensatory mechanism to limit the effects of membrane-bound or surface endoglin. In preeclampsia, excessive production of surface endoglin leads to increased sEng in the maternal circulation. sEng together with sFlt1 may be responsible for maternal endothelial dysfunction and the clinical manifestations of preeclampsia (25, 26).

**Maternal Endothelial Dysfunction and Hemodynamic Changes**

Preeclampsia appears to begin in the placenta; however, the target organ is the maternal endothelium (40). Generalized damage to the endothelium of the maternal kidneys, liver, and brain at the cellular level probably occurs following the release of vasopressive factors from the diseased placenta (41). Many serum markers of endothelial activation and endothelial dysfunction are deranged in women with preeclampsia; these markers include von Willebrand antigen, cellular fibronectin, soluble tissue factor, soluble E-selectin, platelet-derived growth factor, and endothelin (42, 43). Incubation of serum taken from preeclamptic women with endothelial cells results in endothelial dysfunction (40, 44).

During normal pregnancy, there are physiologic decreases in arterial blood pressure and peripheral vascular resistance (43). Due to widespread vasoconstriction during the clinical manifestations of preeclampsia, systemic vascular resistance is high and cardiac output is low (45). Interestingly, it has been reported that, prior to the onset of clinical symptoms in some women destined to develop preeclampsia, cardiac output may be higher than in other women (45). There is also exaggerated sensitivity to the vasopressors angiotensin II and norepinephrine (46, 47). Women who develop preeclampsia have impaired endothelium-dependent vasorelaxation (48) and subtle increases in blood pressure and pulse pressure prior to the onset of overt hypertension and proteinuria (45).

**Pathological Changes: Liver, Renal, and Cerebral Changes**

Pathologic analysis of the organs of women suffering from preeclampsia and eclampsia show changes consistent with widespread hypoperfusion of organs. The liver and adrenals typically show infarction, necrosis, and intraparenchymal hemorrhage. The heart may reveal endocardial necrosis similar to that caused by hypoperfusion in hypovolemic shock (42). Injury to the maternal endothelium can be most clearly visualized in the kidney, which reveals the characteristic pathologic changes of preeclampsia. The term glomerular endotheliosis has been used to describe the ultrastructural changes in renal glomeruli, including generalized swelling and vacuolization of the endothelial cells and loss of the capillary space (Figure 1) (49). There are subendothelial deposits of fibrin that decrease the filtration surface area (50). Electron microscopy shows loss of glomerular endothelial fenestrae, which leads to a 40% decline in glomerular filtration rate (50). In contrast to other nephrotic diseases, in preeclampsia endothelial cells appear primarily to be injured; podocyte injury is usually restricted to the focal fusion of foot processes (43). Recently, podocyto-turia was noted in women with preeclampsia (51); whether this is a cause or an effect of proteinuria is unknown. Although glomerular endotheliosis was once considered pathognomonic for preeclampsia, recent studies have shown that trace to mild glomerular endotheliosis may also occur at term during normal pregnancy. This finding suggests that the endothelial dysfunction of preeclampsia may be an exaggeration of a normal physiological process that occurs near the end of pregnancy.

Cerebral edema and intracerebral parenchymal hemorrhage are common autopsy findings in women who died from eclampsia. However, cerebral edema in eclampsia does not correlate with the severity of hypertension, suggesting that edema is secondary to endothelial...
dysfunction rather than a direct result of blood pressure elevation. Findings from head computed tomography scans and magnetic resonance imaging (MRI) are similar to those seen in hypertensive encephalopathy, with vasogenic cerebral edema and infarctions in the subcortical white matter and adjacent gray matter, predominantly in the parietal and occipital lobes (Figure 2) (20). An eclampsia-like syndrome with these characteristic MRI findings has been associated with other clinical scenarios, specifically acute hypertensive encephalopathy in the setting of renal disease or immunosuppression (52) and following the use of antiangiogenic agents for cancer therapy (53). This syndrome is known as reversible posterior leukoencephalopathy or posterior reversible leukoencephalopathy syndrome (PRES). Its association with antiangiogenic therapy supports the involvement of innate antiangiogenic factors in the pathophysiology of preeclampsia and eclampsia.

MOLECULAR MECHANISMS

There are a number of mechanisms that contribute to the pathogenesis of preeclampsia. It is unclear whether the elucidated pathways are all interrelated, have synergistic effects, or act independently. However, endothelial damage induced by antiangiogenic factors, systemic inflammation, immunologic factors, and hypoxia all contribute to the development of this heterogeneous condition.
Figure 2
Cerebral edema in eclamptic subjects and in animal models of preeclampsia and eclampsia. (a) Serial magnetic resonance images obtained in the brain of a patient with eclampsia that developed 2 days after delivery. The left (from magnetic resonance imaging scan performed upon patient’s admission) and middle (obtained at the time of maximal signs) panels demonstrate cerebral edema in the posterior cerebral cortex. The right panel shows magnetic resonance images obtained from the same subject after IV gadolinium contrast demonstrating disruption of the blood-brain barrier. Figures reproduced with permission from Schwartz et al. (121). (b) Magnetic resonance images of brain from mouse overexpressing soluble fms-like tyrosine kinase 1 (sFlt1) or soluble endoglin (sEng) or both. Animals exposed to both sFlt1 and sEng demonstrate edema in the posterior cerebral cortex. Figures reproduced with permission from Maharaj et al. (60).

Altered Angiogenic Balance
Imbalance of innate angiogenic factors plays a key role in the pathogenesis of preeclampsia. Increased expression of sFlt1, associated with decreased PlGF and VEGF signaling, was the first abnormality described (25, 26, 37, 54). Compared to normotensive controls, in patients with severe preeclampsia, free PlGF and VEGF levels are significantly decreased (55–59), and sFlt1 levels are significantly elevated (37, 58, 59). VEGF stabilizes endothelial cells in mature blood vessels and is particularly important in maintaining the endothelium in the kidney, liver, and brain (25, 60). One of the major VEGF receptors is Flt1. sFlt1 is a truncated splice variant of the membrane-bound VEGF receptor Flt1. It consists of the extracellular ligand-binding domain without the transmembrane and intracellular signaling domains; it is primarily secreted by syncytiotrophoblasts into the maternal circulation (61). sFlt1 has also been found in monocytes (62). sFlt1 antagonizes both VEGF and PlGF by binding them in the circulation and preventing interaction with their endogenous receptors (63). Placental expression of sFlt1 is increased in preeclampsia and is associated with a marked increase in maternal circulating sFlt1 (25). Several investigators have confirmed that the increase in maternal circulating sFlt1 precedes the onset of clinical disease and is correlated with disease severity (37, 64–66). In vivo effects of sFlt1 administration include vasoconstriction and endothelial dysfunction. Maynard et al. (25) demonstrated that exogenous sFlt1 given systemically to pregnant or nonpregnant rats can produce a syndrome resembling preeclampsia that includes hypertension, proteinuria, and glomerular endotheliosis (Figure 1). New variants of sFlt1 have been discovered; these include a novel primate-specific variant sFlt1-14, which is also a potent VEGF inhibitor. sFlt1-14 (also referred
to as sFlt1-e15a) is the predominant VEGF inhibitor produced by human nonendothelial cells. It accumulates in circulation throughout pregnancy and may induce endothelial damage in distant organs affected by preeclampsia (67, 68).

VEGF is a central requirement for endothelial stability, and its blockade is an important part of the pathophysiology of preeclampsia. VEGF is necessary for glomerular capillary repair and may be particularly important in maintaining the health of the endothelium. VEGF is highly expressed by glomerular podocytes, and VEGF receptors are present on glomerular endothelial cells (69). Anti-VEGF therapies given to adult animals cause glomerular endothelial damage with proteinuria (70).

In a podocyte-specific VEGF knockout mouse, heterozygosity for VEGF-A resulted in renal disease characterized by proteinuria and glomerular endotheliosis (71). In humans, antiangiogenesis cancer trials with anti-VEGF therapies have led to proteinuria, hypertension, and glomerular endothelial damage (72, 73).

Fenestrated endothelium is found in the renal glomerulus, choroid plexus, and the hepatic sinusoids, organs that are disproportionately affected in preeclampsia (74). Thus, VEGF deficiency, whether induced by anti-VEGF antibodies, gene deletion, or excess sFlt1, is probably responsible for proteinuria and glomerular endotheliosis.

PlGF has structural homology to VEGF-A and is also a potent angiogenic growth factor that is thought to amplify VEGF signaling by displacing VEGF from the Flt1 receptor (75) and allowing it to bind to the more active kinase insert domain (KDR) receptor (or VEGFR-2) instead (76). PlGF appears to stimulate angiogenesis under conditions of ischemia, inflammation, and wound healing and may contribute to atherosclerosis (75, 77). During pregnancy, inhibition of both PlGF and VEGF is necessary to produce a preeclampsia-like syndrome in pregnant rats (25), indicating that PlGF blockade may also be important in the pathogenesis of sFlt1-induced endothelial dysfunction.

Importantly, changes in PlGF are noted quite early in women destined to develop preeclampsia, suggesting that an abnormally low level of PlGF is an important risk factor (37).

Derangements in other angiogenic factors have been observed. sEng is a truncated form of endoglin, a cell surface receptor for TGF-β. sEng is significantly upregulated in preeclampsia in a pattern similar to that of sFlt1 (78). sEng amplifies the vascular damage mediated by sFlt1 in pregnant rats, inducing a severe preeclampsia-like syndrome with features of HELLP syndrome (26). Overexpression of sFlt1 and sEng in rodents was also found to induce focal vasospasm, hypertension, choroid plexus endotheliosis, and increased vascular permeability with brain edema, producing MRI images reminiscent of PRES (Figure 2) (60).

This effect may be mediated by interference with nitric oxide (NO)-mediated vasodilation. As with sFlt1, circulating sEng levels are elevated weeks prior to preeclampsia onset (78). Interestingly, sEng is also elevated in small-for-gestational-age pregnancies that are not associated with preeclampsia (78, 79). The precise role of sEng in preeclampsia and its relationship with sFlt1 are currently being explored.

NO may be an important downstream mediator of both VEGF and TGF-β and has been suggested to be involved in the pathogenesis of preeclampsia. An initial animal model showed that inhibition of NO synthase via N-nitro-L-arginine methyl ester induced a preeclampsia-like syndrome characterized by hypertension, proteinuria, intrauterine growth restriction, and renal glomerular capillary endothelial lesions in pregnant rats (80, 81). NO has the vasodilator properties of endothelium and regulates the decrease in peripheral vascular resistance in pregnancy. Rats treated with L-arginine had lowered systolic blood pressure, increased mean birth weight, decreased proteinuria, and decreased injury of renal glomeruli (80). More recently, impaired production of NO metabolites was observed in patients with preeclampsia, and the impairment correlated with both circulating sFlt1 and sEng (82).

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**Placental Ischemia and Hypoxia**

Although incomplete remodeling of the uterine spiral arteries from partial cytotrophoblast invasion is a known precursor to preeclampsia development, it is unknown whether preeclampsia causes or results from placental hypoxia and ischemia. In pregnant primates and other mammals, constriction of uterine blood flow has been shown to induce hypertension and proteinuria (83, 84). However, in these animal models, uterine ischemia does not lead to seizures or HELLP syndrome. Conversely, fetal growth restriction secondary to placental insufficiency frequently occurs without preeclampsia. Placental ischemia and hypoxia are often interrelated. Defective trophoblast invasion and inadequate maternal spiral artery remodeling are common to both intrauterine growth restriction and preeclampsia. Paradoxically, cigarette smoking, an important risk factor for fetal growth restriction, is consistently associated with a reduced risk of preeclampsia (14, 85). Levels of circulating sFlt1 and sEng are significantly lower in women who smoke (86).

Women with preeclampsia also have alterations in placental hypoxia-inducible factor (HIF) and its targets (87). Women residing at high altitudes have similar alterations in HIF, and the rates of preeclampsia in populations at high altitudes are two- to fourfold greater (88). Many angiogenic proteins, including Flt-1, VEGFR-2, Tie-1, and Tie-2, are targets of HIF-1 regulation. These proteins are intimately linked to the regulation of normal placental vascular development. Invasive cytotrophoblasts express several other angiogenic factors regulated by HIF, including VEGF, PlGF, and VEGFR-1; expression of these proteins is altered in preeclampsia. TGF-β3, which has been shown to block cytotrophoblast invasion, is another HIF target (89). Hypoxia has been shown to upregulate expression and secretion of sFlt1 protein in primary trophoblast cultures from first-trimester placentas (90).

In vivo experiments in mice strongly suggest that placental hypoxia contributes to preeclampsia by upregulating soluble antiangiogenic factors affecting the vasculature (90). In recent work by Kanasaki et al. (91), pregnant mice deficient in catechol-O-methyltransferase (COMT) also showed a preeclampsia-like phenotype resulting from an absence of 2-methoxyoestradiol (2-ME), a natural metabolite of estradiol that is elevated during the third trimester of normal human pregnancy. The addition of 2-ME was shown to improve preeclampsia-like features without causing toxicity in the COMT knockout pregnant mice. Additionally, 2-ME was shown to suppress placental hypoxia, HIF-1α expression, and sFlt1 expression (91). Moreover, levels of COMT and 2-ME are significantly lower in patients with severe preeclampsia and may correlate with elevated sFlt1 levels. Whether the decreased COMT is the cause or the consequence of the abnormal placenta is still unclear.

In summary, the role of trophoblast invasion is clearly critical to successful support of a pregnancy. Placental ischemia and hypoxia resulting from impaired trophoblast invasion may be important secondary events.

**Renin-Aldosterone-Angiotensin Signaling**

The renin-angiotensin-aldosterone axis is suppressed in preeclampsia. Normally, during pregnancy aldosterone and angiotensin are increased. Women with preeclampsia have increased vascular sensitivity to angiotensin II and other vasoconstrictive agents (92), and plasma renin/aldosterone are suppressed in preeclamptic patients relative to women with normal pregnancies (93). Angiotensin II is a peptide mediator that increases blood pressure by signaling arterial vasoconstriction after binding to its receptor.

Angiotensin II hypersensitivity in preeclampsia may be secondary to formation of autoantibodies that bind and activate the angiotensin II receptor (94). These autoantibodies have been found to decrease the invasiveness of immortalized human trophoblasts in an in vitro assay (95). Studies have found that angiotensin
II receptor autoantibodies recovered from the circulation of women with preeclampsia induce key features of preeclampsia in pregnant mice, including placental damage, and stimulate the synthesis of sFlt1 (96). The effects of these antibodies can be blocked in vitro with losartan, a pharmacologic angiotensin II receptor blocker that also blocks TGF-β, or by an antibody-neutralizing peptide (95). Angiotensin II receptor autoantibodies are also increased in malignant renovascular hypertension (97) and renal vascular rejection (98). Angiotensin II receptor autoantibodies may be one of the insults or one of the precursors that contribute to the poor cytotorphoblast placental invasion, leading to the production of antiangiogenic factors and endothelial damage (Figure 3).

**Inflammation and Immunologic Alterations**

The gravid uterus is a site of immune privilege that permits a fetal-placental unit, a semi-allogeneic entity, to develop (99). Immune maladaptation is an important pathway that contributes to the inadequate invasion of cytotrophoblasts into the uterine decidua and may help explain why women with preeclampsia are typically nulliparous. An intact immune system is required for the development of preeclampsia, as women with untreated human immunodeficiency virus have a lower incidence of preeclampsia compared to the general population (100). The incidence reverts to that of the nonimmunocompromised population when these women are given antiretroviral therapy.

**Figure 3**

Summary of the pathogenesis of preeclampsia. Genetic factors, immune abnormalities [natural killer (NK) cell/human leukocyte antigen (HLA)-C axis], and other factors such as oxidative stress may cause placental dysfunction, which in turn leads to the release of antiangiogenic factors [such as soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng)] and other inflammatory mediators to induce hypertension, proteinuria, and other complications of preeclampsia. Abbreviations: AT1-AA, angiotensin type II receptor; COMT, catechol-O-methyltransferase; HELLP, hemolysis, elevated liver enzymes, and low platelet syndrome; PIGF, placental growth factor; VEGF, vascular endothelial growth factor.
If a woman has a pregnancy affected by preeclampsia, there is an increased risk that a subsequent pregnancy with a new partner will be affected by preeclampsia (6), supporting the theory that there is immune maladaptation at the fetal-maternal interface. Preeclampsia occurs more frequently in nulliparous women, after a change in paternity (6), or with long interpregnancy intervals (101). These observations may indicate that immune modulators such as dendritic cells may provide tolerance in future pregnancies to the insult that causes preeclampsia. Additionally, women who use barrier contraceptive methods that reduce exposure to sperm have an increased incidence of preeclampsia (102). Women who conceived via intracytoplasmic sperm injection in which the sperm had been obtained through testicular biopsy have a threefold-increased risk of preeclampsia compared to cases in which the sperm had been obtained through ejaculation (103). These observations support the theory that preeclampsia may involve an abnormal maternal immune response to fetal antigens.

Decidual cells are the major cell type of the pregnant endometrium. Natural killer (NK) cells, macrophages, and dendritic cells are mediators of innate immunity, and macrophages and dendritic cells are the major antigen-presenting cells in the uterus. The presence of macrophages and dendritic cells facilitates adaptation of the immune response to prevent rejection of the growing embryo (99). Macrophage infiltration is implicated in impaired trophoblast invasion, an underlying pathway for preeclampsia development (104). Studies have found a statistically significant increase in macrophages and dendritic cells in preeclamptic placentas compared to placentas from unaffected pregnancies (99, 104). An increase in the level of chemokines, molecules capable of recruiting macrophages and dendritic cells, has been found in preeclamptic placentas. The significantly increased presence of macrophages, chemokines, and dendritic cells in placentas affected by preeclampsia supports the notion that an inflammatory milieu present both in the first trimester and at the time of clinical presentation of preeclampsia may promote immune maladaptation, leading to impaired trophoblast invasion at the level of the spiral arteries. Shedding or release of syncytiotrophoblastic cell fragments and accompanying inflammation has also been proposed as a pathogenic mechanism to explain the maternal endothelial dysfunction; however, causal evidence for this hypothesis is still lacking (105). The syncytiotrophoblast debris may also serve as additional sources of sFlt1 and sEng in the circulation, as these antiangiogenic proteins are abundantly expressed in the syncytiotrophoblast.

It has recently been suggested that NK cells at the maternal-fetal interface may play a role in maternal vascular remodeling and thus may be involved in the pathogenesis of preeclampsia. Genetic studies of polymorphisms in the killer immunoglobulin receptors (KIRs) on maternal NK cells and the fetal human leukocyte antigen (HLA)-C haplotype suggest that patients with the KIR-AA genotype and the fetal HLA-C2 genotype may be at greatly increased risk of preeclampsia.

Oxidative stress in the placenta may be one mechanism for the impaired placentation of preeclampsia (106). Oxidative stress from the production of free radicals is known to contribute to vascular conditions such as atherosclerosis and therefore is thought to contribute to the pathogenesis of the placental atherosclerosis. Animal models and small studies in humans suggest a role for oxidative stress. However, large randomized placebo-controlled trials in pregnant women found that supplementation with vitamins C and E did not reduce the risk of preeclampsia, intrauterine growth restriction, or fetal death, nor did they improve maternal outcome (107, 108). In preeclampsia, markers of high oxidative stress are detectable through higher levels of lipid peroxidation, increased superoxide generation, and increased production of isoprostanes (106), although this finding has been disputed by some groups (109).

Small studies have also found that treatment with the antioxidant lycopene may reduce
the risk of preeclampsia (110). Very recently, it was reported that preeclamptic placentas have decreased expression of the antioxidant gene hemeoxygenase 1, which appears to be a proximal regulator of sFlt1 and sEng production (111). Whether this pathway is important in all patients or only in a subset of patients with preeclampsia remain unknowns. In summary, although oxidative stress is present in pregnancies affected by preeclampsia, the exact role of these free radicals has not been elucidated.

Genetics
As discussed in the previous section, most cases of preeclampsia occur in nulliparous women without a family history of the disease. However, the presence of preeclampsia in a first-degree relative increases a woman’s risk of severe preeclampsia two- to fourfold even after controlling for body mass index, smoking status, and age (9). Men who fathered one preeclamptic pregnancy had a significantly increased risk of fathering another preeclamptic pregnancy with a new partner (6, 112). These studies support a strong paternal component to the genetic predisposition. STOX1, a novel transcription factor gene, has shown inconsistent associations with preeclampsia (113–115). Studies using genome-wide association studies to pinpoint genes associated with preeclampsia are under way.

Speculations and Unanswered Questions
How placental dysfunction is related to placental sFlt1 and sEng production, and why placental perfusion is deranged in preeclampsia, remains unknown. Some of the early placental insults that have been suggested to contribute to sFlt1 production include reduced heme oxygenase expression, altered NK cell signaling, excess angiotensin II receptor signaling, and deficient COMT activity (Figure 3). However, it is also possible that subtle changes in the placental production of antiangiogenic factors during early pregnancy interfere with placental development, which then leads to further increases in sFlt1 and sEng production.

If antiangiogenic factors such as sFlt1 are an important cause of preeclampsia, there may be at least two kinds of predisposing factors. One may lead to the overproduction of sFlt-1, such as in multiple gestation, hydatidiform mole, trisomy 13, and possibly first pregnancy. A second set of predisposing factors may include disorders that sensitize the maternal vascular endothelium to the antiangiogenic effects of sFlt1; such factors may include obesity, preexisting hypertension or renal disease, diabetes, and preexisting vasculitis. Women who develop preeclampsia frequently have small elevations of blood pressure during the second trimester of pregnancy, well before the clinical onset of preeclampsia. These elevations may reflect preexisting endothelial damage, resulting in lower production of vasorelaxing factors and greater susceptibility to further endothelial damage by circulating antiangiogenic proteins (116). We do not yet know whether diabetes, hypertension, and preexisting renal disease predispose to preeclampsia by increasing the production of sFlt1 or by sensitizing the vascular endothelium to its presence. Hypoxia is known to increase the production of sFlt1 by placental trophoblasts (61), so placental ischemia may thereby trigger the preeclamptic syndrome. There is strong evidence for placental ischemia in many patients with preeclampsia, but not in others. Placental infarction unaccompanied by preeclampsia is a common finding in mothers with sickle cell anemia and in women whose fetuses are growth restricted. Placental overproduction of sFlt1, whatever its cause, may decrease angiogenesis locally and result in placental ischemia, thereby initiating a vicious circle leading to even greater sFlt1 production.

Three factors may conspire, in varying degrees, to produce the clinical syndrome of preeclampsia. These factors include (a) a change in the balance of circulating factors controlling angiogenesis/antiangiogenesis, attributable to placental overproduction of sFlt1.
and underproduction of PlGF, (b) increased vascular endothelial sensitivity to such factors (117), and (c) placental ischemia exaggerating the processes described in item a. It is not surprising that in human pregnancy, which is characterized initially by rapid angiogenesis localized to the placenta followed by regression of placental blood vessel growth close to the termination of pregnancy, there should occasionally occur systemic manifestations of a derangement of this remarkable process.

**CLINICAL APPLICATIONS OF RECENT RESEARCH**

Our understanding of the pathophysiology of preeclampsia within the past ten years has increased, leading to exciting new potential treatments, diagnostic tools, and screening tests for this heterogeneous disease. Preeclampsia remains a major cause of maternal and fetal morbidity and mortality and has implications for future pregnancies and future cardiovascular risk. A better understanding of this disease could lead to improved pregnancy outcomes for the women and their infants. Clinical experience suggests that early detection, monitoring, and supportive care are beneficial for both the woman and her fetus. Currently, there is no screening or diagnostic test approved for clinical use. Accurate diagnosis and early detection are the first steps in treating this disease. Preeclampsia is typically diagnosed using clinical criteria. However, it may present atypically or with features that resemble other conditions. In such cases, the clinical diagnosis may be wrong. No laboratory test to confirm or exclude preeclampsia exists; the diagnosis is based entirely on nonspecific markers and clinical presentation. Accurate diagnosis will help further medical research as well as lead to more appropriate therapy and better pregnancy outcomes.

At present, there is no reliable means of predicting the onset of preeclampsia; however, research has demonstrated alterations in serum levels of angiogenic factors weeks prior to the clinical onset of preeclampsia. Significant elevations in sFlt1 and sEng have been observed from midgestation onward, and the levels of these proteins rise many weeks prior to onset, particularly in those women with early-onset disease. The ratio of these antiangiogenic biomarkers to PlGF has been a reliable marker in large clinical studies (37, 78). A urine screening test for PlGF, in combination with a confirmatory blood test for circulating angiogenic proteins, may also help predict preeclampsia (118). Prediction of impending preeclampsia can assist clinicians in providing closer maternal and fetal monitoring, timely intervention with steroids to enhance fetal lung maturity, magnesium for seizure prophylaxis, treatment with antihypertensive medications, and expeditious delivery if necessary.

Delivery of the placenta and supportive care remain the only therapeutic options available for women with preeclampsia. Fortunately, after delivery acute symptoms and signs of preeclampsia resolve typically within 48 to 72 h (15). A novel therapeutic agent targeting the alteration in angiogenic balance such as VEGF-121 may permit clinical disease to be moderated so that delivery can be postponed and the fetus can continue to grow. Moreover, VEGF treatment may hasten the resolution of preeclampsia postpartum (119) and reduce risk of postpartum HELLP syndrome or eclampsia.

Although our knowledge of preeclampsia has advanced considerably, the initiating events in the placenta remain to be elucidated. Knowledge of the role of antiangiogenic factors and immunologic maladaptation may revolutionize the way preeclampsia is treated and managed. Prospective longitudinal studies monitoring alterations of urine and serum angiogenic factors are needed to determine the relevance of these markers to early identification of preeclampsia and prediction of its severity. Further work to determine the regulation of placental vascular development and expression of angiogenic factors in diseased pregnancies may lead to a better understanding of the disease and its heterogeneity.
DISCLOSURE STATEMENT

S.A.K. is named as a coinventor on multiple patents filed by the Beth Israel Deaconess Medical Center for the use of angiogenic proteins in the diagnosis and therapy of preeclampsia. S.A.K. is a consultant to Johnson & Johnson, Roche, Beckman Coulter, and Abbott Diagnostics. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

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