Preeclampsia: A renal perspective

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Preeclampsia: A renal perspective. Preeclampsia is a syndrome that affects 5% of all pregnancies, producing substantial maternal and perinatal morbidity and mortality. The aim of this review is to summarize our current understanding of the pathogenesis of preeclampsia with special emphasis on the recent discovery that circulating anti-angiogenic proteins of placental origin may play an important role in the pathogenesis of proteinuria and hypertension of preeclampsia.

Preeclampsia, the syndrome of hypertension and proteinuria that heralds the seizures of eclampsia, remains one of the great mysteries in the field of obstetrics. Although our understanding of the pathophysiology of preeclampsia has increased over the past 50 years, it is quite incomplete, and management remains supportive: close observation, treatment with antihypertensive agents and magnesium sulfate, and if progressive signs and symptoms occur, urgent delivery of the fetus. Preeclampsia is still one of the leading causes of maternal and neonatal mortality in the world.

Preeclampsia is characterized by a constellation of signs and symptoms, including the new onset of hypertension and proteinuria during the last trimester of pregnancy, usually associated with edema and hyperuricemia [1, 2]. It occurs only in the presence of the placenta, even when there is no fetus (as in hydatidiform mole) and remits dramatically postpartum [3]. The placenta in preeclampsia is usually abnormal, with evidence of hypoperfusion and ischemia. Although these placental changes are neither universal nor specific for preeclampsia, there appears to be a correlation between the severity of the disease and the extent of placental abnormalities. The clinical findings of severe preeclampsia are unified by the presence of systemic endothelial dysfunction and microangiopathy, in which the target organ may be the brain (seizures or eclampsia), the liver [the hemolysis, elevated liver function tests, and low platelet count (HELLP) syndrome], or the kidney (glomerular endotheliosis and proteinuria). Severe preeclampsia is also associated with small for gestational age (SGA) fetuses. Because of the strong clinical and experimental evidence of early placental involvement and dysfunction of the maternal endothelium, it is currently believed that preeclampsia has its origin in disordered vascular development of the placenta, which in turn leads to widespread maternal vascular endothelial effects [4, 5] (Fig. 1). This review will discuss mechanisms underlying the clinical manifestations of preeclampsia. In addition, we will describe evidence suggesting that placental secretion of an antiangiogenic protein may contribute to the endothelial dysfunction of preeclampsia.

RISK FACTORS FOR THE DEVELOPMENT OF PREECLAMPSIA

The risk factors for the development of preeclampsia are listed in Table 1 [1, 5–7]. Preeclampsia is more common not only in first pregnancies, but also in multigravidas who have a new partner, suggesting that prior exposure to paternal antigens may be protective [8, 9]. However, recent evidence from a large Norwegian birth registry suggests that prolonged interpregnancy interval, rather than primipaternity, accounts for this increase in risk [10], though why this occurs is unclear. Although preeclampsia is traditionally not considered to be a genetic disease, it is clear that genetic factors contribute to the susceptibility to preeclampsia [6]. A family history (mother, sister, or both) of preeclampsia is associated with a fourfold increased risk for preeclampsia [6]. Other epidemiologic studies suggest that paternal genetic contributions to the zygotic genotype in addition to

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maternal genes may contribute susceptibility to preeclampsia [11]. Polymorphisms of genes involved in the regulation of blood pressure or coagulation, such as renin, angiotensinogen (T235), endothelial nitric oxide synthase (eNOS), prothrombin, factor V Leiden, and methylenetetrahydrofolate (MTHFR), though promising in early studies [12–15], have not been confirmed in larger studies [16–20]. Genome-wide scanning of Icelandic families revealed a significant locus on chromosome 2p13 [logarithm of the odds (LOD) score 4.70] [21]. More recently, the 2p locus was confirmed in a study of patients from New Zealand and Australia [22]. Finally, a Dutch study reported linkage of HELLP syndrome, but not preeclampsia, with a locus on 12q, suggesting that genetic factors important in HELLP (hemolysis, elevated liver function tests, low platelets) syndrome may be distinct from those in preeclampsia [23]. Women with trisomy 13 fetuses have a higher incidence of preeclampsia, regardless of parity, suggesting that a gene on chromosome 13 may be important in preeclampsia [24]. An activating mineralocorticoid receptor mutation was described in a rare group of patients who have only pregnancy-induced hypertension without proteinuria [25]. The incidence of preeclampsia is also higher in women who live at high altitudes and in the third world, suggesting that hypoxia and/or hitherto unknown environmental factors may also contribute to the development of preeclampsia [26, 27].

**CLINICAL MANIFESTATIONS AND THEIR PATHOPHYSIOLOGIC UNDERPINNINGS**

**Hypertension**

While in normal pregnancy peripheral vascular resistance and blood pressure are decreased, in preeclampsia these changes are reversed. Increased peripheral vascular resistance, rather than increased cardiac output, is the chief cause of hypertension [28]. Sympathetic activation is noted in preeclampsia as it is in other forms of hypertension, a conclusion supported by electrical recordings of sympathetic nerve impulses [29] and by reports of increased concentrations of circulating catecholamines [30]. Sympathetic activation may be responsible for the increase in cardiac output noted by some in the early stages of preeclampsia [31]. Preeclampsia is also notable for an exaggerated response to angiotensin II, catecholamines, and other hypertensive stimuli when compared to normal pregnant controls [32, 33]. One group has reported that this response may precede the onset of overt hypertension by weeks to months [34] (a finding disputed by others [35]), reminiscent of the exaggerated response to vasoconstrictors described in normotensive relatives of patients with essential hypertension [36].

Although total plasma volume has been reported to be low in preeclampsia [37], there may be increased “effective circulating volume” as evidenced by suppressed renin and aldosterone [38, 39] and elevated brain natriuretic hormone [40] relative to normal pregnancy. These findings are reminiscent of the fall in plasma volume and rise in blood pressure produced by infusion of vasoconstrictors suggesting that peripheral vasoconstriction, especially of small venules, shifts blood to the arteries and central veins while elevating capillary pressure [41]. Blood pressure rises, and renin and aldosterone levels fall as a secondary phenomenon.

Generalized vascular constriction is universally present in preeclampsia, at least compared to the physiologic vasodilation of normal pregnancy [28]. There is substantial evidence that this may be due to endothelial dysfunction. A myriad of markers for endothelial activation and dysfunction, including endothelin, cellular fibronectin, plasminogen activator inhibitor-1 (PAI-1), and von Willebrand’s factor, are altered in preeclampsia. Women with preeclampsia have enhanced responsiveness to vasopressors as compared with normal pregnant women. Women with a history of preeclampsia exhibit evidence of impaired endothelial-dependent vasorelaxation as measured by brachial artery flow-mediated dilatation up to 3 years after delivery, implying these changes in the maternal endothelium may be more than transient [42, 43]. Alterations of endothelial function have been noted in preeclamptic vessels examined in vitro, supporting the hypothesis that endothelial dysfunction may underlie the hypertension of preeclampsia [4, 5]. The increased incidence of preeclampsia in women with chronic diseases such as diabetes and hypertension also suggests some factor in the maternal milieu may also lend susceptibility to preeclampsia. In addition to increased vascular reactivity, the vasoconstriction appears to be mediated at least in part by alterations in local concentrations of several vasoactive molecules, including the vasoconstrictors norepinephrine, endothelin, and perhaps thromboxane, and the vasodilators prostacyclin and perhaps nitric oxide.

Prostaglandin I₂ (PGI₂) (prostacyclin), a circulating vasodilator produced primarily by the endothelial and smooth muscle layers of blood vessels, is
Genetic factors

Environmental factors

Other

Placental dysfunction

Circulating factor(s)

Vascular endothelial dysfunction

Hypertension
Glomerular endotheliosis
proteinuria edema renal insufficiency
Headache
Cerebral edema
Seizures

↑LFTs HELLP syndrome

Fig. 1. Placental dysfunction and endothelial dysfunction in the pathogenesis of preeclampsia. Placental dysfunction, triggered by poorly understood mechanisms, which may include genetic, immunologic, and environmental factors, plays an early and primary role in the development of preeclampsia. The diseased placenta in turn secretes a factor(s) into the maternal circulation, causing systemic endothelial cell dysfunction. Most of the manifestations of preeclampsia, including hypertension, proteinuria (glomerular endotheliosis), seizures (cerebral edema and/or vasospasm), and the hemolysis, elevated liver function tests, and low platelet count (HELLP) syndrome can be attributed to vascular and endothelial effects.

Fig. 2. Glomerular endotheliosis. (A) Normal human glomerulus (hematoxylin and eosin stain). (B) Human preeclamptic glomerulus (hematoxylin and eosin stain). A 33-year-old woman with twin gestation and severe preeclampsia at 26 weeks’ gestation with urine protein/creatinine ratio of 26 at the time of biopsy. (C) Electron microscopy of glomeruli of the above patient described in (B). Note occlusion of capillary lumen cytoplasm and expansion of the subendothelial space with some electron-dense material. Podocyte cytoplasms show protein resorption droplets and relatively intact foot processes (original magnification 1500×). (D) Control rat glomerulus (hematoxylin and eosin stain). Note normal cellularity and open capillary loops. (E) Soluble Flt-1–treated rat (hematoxylin and eosin stain). Note occlusion of capillary loops by swollen cytoplasm with minimal increase in cellularity. (F) Electron microscopy of sFlt-1–treated rat. Note occlusion of capillary loops by swollen cytoplasm with relative preservation of podocyte foot processes (original magnification 2500×). All light micrographs taken at identical original magnification of 40×).
increased in normal pregnancy [44]. In preeclampsia, prostacyclin production is lower than in normal pregnancy well before the onset of hypertension and proteinuria [45, 46]. Endothelial cells incubated with serum from women with preeclampsia (vs. serum from normotensive pregnant women) produce less prostacyclin in vitro, suggesting the presence of a circulating factor suppressing prostacyclin synthesis [47]. Prostacyclin concentrations are normal in pregnant women with chronic hypertension but without preeclampsia [48], providing further circumstantial evidence that decreased prostacyclin is a contributing cause of preeclampsia rather than a consequence of hypertension.

Thromboxane A\(_2\) (TXA\(_2\)) is a potent vasoconstrictor synthesized by endothelial cells, activated platelets, macrophages, and other organs. Urinary excretion of TXA\(_2\) metabolites has been reported to be increased in preeclampsia by some [49] but not all investigators [46, 50]. TXA\(_2\) production appears to parallel the severity of preeclampsia, being higher in patients with coagulopathy and pronounced platelet activation [50]. Some attempts to prevent preeclampsia in high risk patients by inhibiting platelet thromboxane synthesis using aspirin were successful but others found no benefit, either in low risk or high risk populations [51, 52].

There is some evidence that nitric oxide, a vascular smooth muscle relaxant synthesized by vascular endothelium, may also mediate the generalized vasodilation of normal pregnancy [53, 54]. In rodent experiments, nitric oxide inhibition during pregnancy induces hypertension [55–57] and proteinuria [58] and reverses the norepinephrine and angiotensin II resistance characteristic of pregnancy [59, 60]. Thus, damage to vascular endothelium with decreased nitric oxide production might contribute to vascular constriction in preeclampsia. Although some human studies have reported decreased nitric oxide production in preeclampsia [61–63], others report nitric oxide production to be unchanged [64, 65] or even increased [66, 67]. Unfortunately, studies of nitric oxide production are difficult due to its extremely short circulating half-life and other challenges to its measurement.

Endothelin-1, a potent vasoconstrictor that can be released by vascular endothelial cells in response to injury [68], has also been implicated in the hypertension of preeclampsia. Reports of plasma endothelin concentrations in preeclampsia have been mixed, with most [69–71] but not all [50] investigators reporting a modest increase in circulating concentrations. The release of endothelin by cultured endothelial cells is enhanced by exposure to plasma from preeclamptic patients, as compared with plasma from women with normal pregnancies [72]. Limited animal data suggest that hypertension induced by endothelin-1 administration during pregnancy produces proteinuria [73].

**Renal dysfunction, proteinuria, and renal pathology**

In normal pregnancy, glomerular filtration rate (GFR) as measured by inulin clearance and renal plasma flow (para-aminohippurate clearance) increases by 40% to 60% during the first trimester [74, 75], resulting in a fall in serum markers of renal clearance, including blood urea nitrogen (BUN), creatinine, and uric acid. In preeclampsia, both GFR and renal plasma flow decrease by 30% to 40% compared with normal pregnancy of the same duration [76, 77]. Rarely, prolonged renal hypoperfusion with resulting “acute tubular necrosis” can occur in severe preeclampsia. It is important to note that in preeclampsia, BUN and creatinine often remain in the normal range for nonpregnant women despite a significant decrease in GFR from the high level of normal pregnancy.

Proteinuria may rarely precede hypertension but usually accompanies or follows it. After pregnancy is terminated, proteinuria commonly disappears within 3 to 8 weeks, but occasionally persists for months. The quantity of protein excreted in the urine varies widely from less than a gram to 8 to 10 g per day. The urinary sediment is usually bland; red blood cells and cellular casts are rare. Preeclampsia is the leading cause of nephrotic syndrome during pregnancy. Recent data suggest that a loss of both size and charge selectivity of the glomerular barrier contribute to the development of albuminuria [77].

Preeclampsia is associated with a characteristic glomerular lesion, “glomerular capillary endotheliosis” (Fig. 2A to C) [78–80]. By light microscopy, the glomeruli are enlarged and the glomerular capillary lumen is “bloodless” due to endothelial and mesangial cell swelling and hypertrophy. While these changes may be focally present in other conditions (such as abruptio placentae), only in preeclampsia is endotheliosis so prominent and widespread. Glomerular cellularity is at most slightly increased. Mesangial interposition may occur in severe cases or in the healing stages. Glomerular visceral epithelial cells (podocytes) usually appear swollen with periodic acid-Schiff (PAS)-positive hyaline droplets. Immunofluorescence may reveal deposition of fibrin or fibrinogen derivatives particularly in biopsies done within 2 weeks postpartum [81]. Electron microscopy is important to confirm endotheliosis and the loss of endothelial fenestrae. Remarkably, despite heavy proteinuria the podocyte foot processes are relatively preserved [82]. Glomerular subendothelial and occasional mesangial electron-dense deposits can be seen. These likely relate to fibrin or related breakdown products. Mild glomerular endotheliosis has been noted in up to 50% of patients with pregnancy-induced hypertension without proteinuria [83], suggesting that pregnancy-induced hypertension may in some cases reflect an earlier or milder form of the same syndrome. Glomerular enlargement and endothelial swelling usually disappear within 8 weeks of delivery, coinciding with resolution of the...
hypertension and proteinuria. Focal segmental glomerulosclerosis (FSGS) can accompany the generalized glomerular endotheliosis of preeclampsia in 50% or more of cases [84, 85].

**Edema**

Although the presence of edema is not necessary for the diagnosis, sudden weight gain, with edema of the feet, hands, and face, is a common presenting symptom in preeclampsia [32]. Salt loads are excreted more slowly than in normal pregnancy [86, 87]. The edema of preeclampsia is unlike that in congestive heart failure, hepatic cirrhosis, and nephrotic syndrome, where low effective circulating volume leads to high plasma renin and aldosterone concentrations and secondary renal sodium retention (“underfill” edema) [88]. Instead, renin and aldosterone concentrations are suppressed relative to normal pregnancy [39]. The edema of preeclampsia thus resembles the “overfill” edema of acute glomerulonephritis or of acute ischemic renal failure in which GFR is decreased out of proportion to the drop in renal plasma flow and in which glomerular-tubular imbalance has been invoked as a cause of salt retention.

Decreased GFR, increased capillary permeability, and hypoalbuminemia may contribute to the edema formation. Albumin-bound Evan’s blue dye disappears more quickly from the intravascular space in preeclamptic women compared with normal pregnant women, suggesting endothelial permeability is increased [89]. However, this phenomenon is also seen in non-pregnant patients with heart failure and the nephrotic syndrome [90, 91]. Hypoalbuminemia is common and may contribute to edema. However, correction of hypoalbuminemia with albumin infusions in preeclampsia patients usually does not produce a diuresis, suggesting that the hypoalbuminemia alone is not responsible for the edema [92].

**Hyperuricemia**

The association between preeclampsia and elevated serum uric acid was first noted more than 80 years ago [93]. Serum uric acid levels are usually elevated in preeclampsia, and the degree of uric acid elevation has been correlated with the severity of proteinuria [94], renal pathologic changes [83], maternal morbidity [95], and fetal demise [96]. Often, the elevation of uric acid precedes the onset of proteinuria and fall in GFR [97]. Although it has been proposed that uric acid generation may be increased in preeclampsia as a result of tissue ischemia [98], most evidence suggests that decreased renal clearance is the more important mechanism [99]. Similar decreases in uric acid clearance have been noted by infusions of vasoconstrictors in humans [100]. It has recently been suggested that hyperuricemia may also directly contribute to vascular damage and hypertension [101, 102].

**Coagulopathy and HELLP syndrome**

Preeclampsia is sometimes complicated by consumptive coagulopathy and thrombotic microangiopathy culminating in the HELLP syndrome. HELLP syndrome develops in approximately 10% to 20% of women with severe preeclampsia. The tendency of blood to coagulate is increased in normal pregnancy at least in part because of changes in the circulating concentrations of coagulant factors. In the indolent microangiopathy of preeclampsia, it is mainly the factors that are synthesized in the vascular endothelium that are abnormal. These include prostacyclins (PGI₂), von Willebrand factor, thrombomodulin, cellular fibronectin, and PAI-1 [103–106]. Even in the absence of overt coagulopathy, serum and urine fibrin degradation products are increased, implicating subclinical activation of the coagulation cascade.

These changes are not simply epiphenomena in response to hypertension. Plasma concentrations of cellular fibronectin may be increased weeks before the onset of hypertension [107]. Exposure of cultured endothelial cells to serum from women with preeclampsia triggers increased cellular fibronectin [108, 109] and thrombomodulin [110] release compared with serum from normotensive pregnant women, again suggesting that a factor in preeclamptic serum is directly “activating” endothelial cells. It is interesting to note that other endothelial disorders, including thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, vasculitis, and disseminated intravascular coagulation can produce similar alterations in markers of endothelial activation [111–113]. There is also evidence that platelet activation is present in preeclampsia as a consequence of endothelial damage. Markers of platelet activation such as beta thromboglobulin have been found to be elevated prior to the onset of clinical disease [114]. Furthermore, adhesion molecules such as soluble P-selectin, soluble E-selectin, and soluble vascular cell adhesion molecule-1 (VCAM-1) that reflect endothelial, platelet, and leukocyte activation have also been reported to be elevated in preeclampsia [115]. The severe thrombocytopenia that is occasionally seen is likely due to consumption during intravascular coagulation.

**Neurologic abnormalities**

Preeclampsia is occasionally complicated by the development of seizures ( eclampsia). Other neurologic symptoms include headache, blurred vision, and temporary loss of vision. The neurologic changes in eclampsia and preeclampsia have been attributed to cerebral edema and vasoconstriction. Brain edema by magnetic resonance
(MR) imaging and abnormalities in circulating endothelial markers correlate closely with the occurrence of eclampsia, suggesting that endothelial damage and disruption of the blood-brain barrier may be an underlying mechanism [116]. The cerebral edema of eclampsia involves predominantly the posterior portions of the white matter, and has been referred to as reversible posterior leukoencephalopathy syndrome (RPLS) [117]. Interestingly, patients with thrombotic thrombocytopenic purpura (TTP), another disorder with microangiopathy also have features of RPLS on MR imaging examinations [118], as do some patients with hypertensive encephalopathy.

ABNORMAL PLACENTATION IN PREECLAMPSIA

It was 1939, when E.W. Page first proposed that placenta plays a central role in preeclampsia [3]. Placentas from severe preeclamptic pregnancies classically have numerous infarcts, sclerotic narrowing of arteries and arterioles, and fibrin deposition and thrombosis. Physiologic studies have confirmed that uteroplacental blood flow is diminished and uterine vascular resistance is increased in preeclamptic women. In addition, placental ischemia induced by mechanical constriction of the uterine arteries or aorta produces hypertension, proteinuria, and, variably, glomerular endotheliosis, in a variety of species [119–121]. Placental ischemia may be contributed to by a decrease in placentation of nitric oxide [122]. However, placental ischemia alone, as in intravillus growth restriction, does not appear to be sufficient to produce preeclampsia. Thus, although uteroplacental ischemia is an important trigger of preeclampsia, the response to this ischemia—either at the placential or the maternal systemic level—must be variable.

Evidence suggests that placental ischemia in preeclampsia occurs as a result of defective placental vascular remodeling. Early in normal placental development, cytotrophoblast cells, fetal in origin, attach to the uterine deciduas by anchoring villi (see Fig. 3). Floating villi, containing fetal vessels which participate in nutrient exchange, are bathed in maternal blood which fills the intravillus space via uterine spiral arterioles. During placental vasculogenesis, a small percentage of cytotrophoblasts in the anchoring villi break through the syncytium and migrate into the endometrium. These extravillous trophoblasts invade the uterine spiral arteries of the myometrium, a process that peaks at about 12 weeks’ gestation. By 18 to 20 weeks, the endometrial and superficial myometrial segments of the spiral arteries are lined by cells of cytotrophoblast origin, with transformation of these vessels from small resistance vessels to flaccid, high-caliber capacitance vessels [123, 124]. This dramatic transformation allows the increase in placental blood flow needed to sustain the fetus through the pregnancy.

The characteristic pathologic placental lesion in severe preeclampsia is diminished endovascular invasion by cytotrophoblasts and failure of uterine spiral arteriolar remodeling [123, 125]. Cytotrophoblast invasion is limited to the proximal decidua, and the myometrial segments of the spiral arteries remain narrow and undilated [126], resulting in uterine hyperperfusion. Zhou et al [127, 128] have shown that invasive cytotrophoblasts down-regulate the expression of adhesion molecules characteristic of their epithelial cell origin and adopt a cell-surface adhesion phenotype typical of endothelial cells, a process referred to as pseudovascularogenesis. They hypothesized and confirmed that in preeclampsia, cytotrophoblast cells fail to undergo this switching of cell-surface integrins and adhesion molecules [129]. This work suggests that cytotrophoblast differentiation is abnormal in severe preeclampsia, and may be an early defect that eventually leads to placental ischemia. Others have demonstrated that hypoxia-inducible factor-1 (HIF-1) is up-regulated in preeclampsia and have suggested that HIF-1 target genes such as transforming growth factor-beta 3 (TGF-β3) may block the cytotrophoblast invasion [130–132]. More recently, heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) has been demonstrated to block the cytotrophoblast invasion [130–132]. More recently, heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) has been demonstrated to be decreased in preeclamptic cytotrophoblasts; however, its role in cytotrophoblast differentiation and invasion is unclear [133]. Interestingly, uteroplacental ischemia produced in monkeys by aortic constriction late in gestation does not produce the defective placental cytotrophoblast invasion seen in preeclampsia, though it is associated with proteinuria and hypertension [134].

THE PLACENTAL FACTOR IN PREECLAMPSIA

All of the clinical manifestations of preeclampsia can be attributed to endothelial cell dysfunction leading to end-organ damage and hypoperfusion. Based on this observation, it has been suggested that there may be a circulating factor, likely placental in origin, which affects systemic endothelial endothelial cell function and leads to the clinical syndrome of preeclampsia [4, 135]. Intense investigation has focused on the search for a placental factor which might induce the maternal syndrome. Although many candidate factors, including tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1α, IL-1β, Fas ligand, neurokinin B, and asymmetric dimethyl L-arginine (ADMA) have been suggested, none so far has been proven to be etiologic [1, 2, 5, 136, 137]. Recent data suggest that increased syncytiotrophoblast shedding as a consequence of placental apoptosis may contribute to endothelial dysfunction [138–140]; however, there is no in vivo evidence so far that circulating
Exchange of oxygen, nutrients, and waste products between the fetus and the mother depends on adequate placental perfusion by maternal vessels. In normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries, transforming them from small-caliber resistance vessels to high-caliber capacitance vessels capable of providing placental perfusion adequate to sustain the growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from an epithelial phenotype to an endothelial phenotype, a process referred to as “pseudovasculogenesis” (upper panel). In preeclampsia, cytotrophoblasts fail to adopt an invasive endothelial phenotype. Instead, invasion of the spiral arteries is shallow and they remain small caliber, resistance vessels (lower panel). This may result in the placental ischemia.

There is mounting evidence that VEGF, and possibly PlGF, are required to maintain endothelial health in several tissues including the kidney and perhaps the placenta. In normal pregnancy, the placenta produces modest concentrations of VEGF, PlGF, and soluble Flt-1. In preeclampsia, excess placental soluble Flt-1 binds circulating VEGF and PlGF and prevents their interaction with endothelial cell-surface receptors. This results in endothelial cell dysfunction, including decreased prostacyclin, nitric oxide production and release of procoagulant proteins such as von Willebrand factor, endothelin, cellular fibronectin, and thrombomodulin.
Recently, preeclampsia-like features were noted in p57kip2 (a cell-cycle inhibitor) knockout mice, however, the mechanisms responsible for this phenotype are unclear [141]. Some [142], but not all [143], investigators have found markers of oxidative stress to be elevated in women with preeclampsia, suggesting that generation of reactive oxygen-free radicals may contribute to endothelial dysfunction [2]. Decreased intake of the antioxidant vitamin C and low circulating ascorbic acid concentrations are associated with an increased risk of preeclampsia [144]. A small randomized controlled trial found that antioxidant therapy decreased the incidence of preeclampsia, suggesting that oxidative stress may be a cause, rather than a consequence, of the syndrome [145]. Additional studies are needed to test this hypothesis.

As noted earlier, increased sensitivity to the vasopressor effects of angiotensin is a well-established feature of preeclampsia. Two recent advances have illuminated possible mechanisms for this phenomenon. AbdAlla et al [146] reported increased angiotensin-1/bradykinin B2 receptor heterodimers in women with preeclampsia, and showed that such heterodimers can result in enhanced angiotensin II responsiveness [146]. In a similar vein, Wallukat et al [147] noted increased concentrations of agonistic antibodies to the angiotensin II type 1 (AT-1) receptor in women with preeclampsia. These autoantibodies may induce the production of reactive oxygen species and block cytotrophoblast invasion in vitro [148, 149], thus accounting for several of the clinical features of preeclampsia. Further work is needed to establish if these alterations are etiologic or epiphenomena that might be encountered in other examples of microangiopathy.

Recently, gene expression profiling has been used to search for candidate factors produced by the placenta in preeclampsia. Using this approach, we found that placental sFlt-1 mRNA (soluble fms-like tyrosine kinase-1) is up-regulated in preeclampsia [150]. sFlt-1 is a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1, lacking the transmembrane and cytoplasmic domains. sFlt is made in large amounts by the placenta and is released into the maternal circulation [151–153]. sFlt-1 acts as a potent VEGF and placental growth factor (PIGF) antagonist by binding these molecules in the circulation [154]. Circulating sFlt-1 concentrations are increased in women with established preeclampsia [150, 155–157]. Consistent with the antagonistic effect of sFlt-1, free (or unbound) VEGF and free (or unbound) PIGF concentrations are decreased in preeclamptic women at disease presentation and even before the onset of clinical symptoms [150, 158, 159] (see Fig. 4). When administered to pregnant and nonpregnant rats, sFlt-1 produces a syndrome of hypertension, proteinuria, and glomerular endotheliosis that mimics the human syndrome of preeclampsia (Fig. 2D to F) [150]. Recently, our laboratory as well as others have observed that there is a marked rise in circulating sFlt-1 concentration beginning about 5 to 6 weeks before the onset of clinical preeclampsia, accompanied by decreases in the circulating free PIGF and VEGF [160, 161]. Moreover, alterations in these circulating angiogenic proteins correlated with disease severity, earlier onset of preeclampsia, and the birth of an infant small for gestational age. Finally, we have also recently reported that decreased urinary concentrations of free PIGF during midgestation predict the subsequent development of clinical preeclampsia [162]. These data lend further support to the hypothesis that circulating sFlt-1 may have a pathogenic role in preeclampsia.

The possibility that antagonism of VEGF and PIGF might play a role in preeclampsia has sound physiologic underpinnings (Fig. 4). In addition to being a potent promoter of angiogenesis, VEGF is known to induce nitric oxide and vasodilatory prostacyclins in endothelial cells, decreasing vascular tone and blood pressure [163]. There is evidence from animal models that VEGF is important in maintaining glomerular endothelial cell health and healing [164, 165], and its absence induces proteinuria and glomerular endotheliosis [166, 167]. In antiangiogenic oncology trials, antagonism of VEGF using neutralizing antibodies and VEGF receptor inhibitors can produce headaches, hypertension, proteinuria, and coagulopathy in human subjects [168–170]. Furthermore, exogenous VEGF and PIGF can reverse the antiangiogenic properties of preeclamptic serum, as assessed by in vitro angiogenesis assays [150]. Thus, the antiangiogenic effects of sFlt-1 may account for many of the manifestations of preeclampsia, including the unique glomerular effects. Some tissues targeted in preeclampsia (i.e., renal glomeruli, hepatic sinusoids) have fenestrated endothelia, which are necessary for physiologic diffusion of water and solutes. It has been shown that VEGF, which is expressed constitutively in these organs, is necessary to induce and maintain the health of the fenestrated endothelium [167, 171]. Thus, it is intuitive that tissues with fenestrated endothelium, especially the renal glomerulus, might be particularly susceptible to damage by sFlt-1–mediated VEGF blockade.

How placental dysfunction is related to placental sFlt-1 production, and why placental perfusion is deranged in preeclampsia remains unknown. Recent data using in vitro primary cytotrophoblast cultures suggest that placental hypoxia may play an important role in up-regulating sFlt-1 production [172]. It remains to be shown if sFlt-1 can induce other features of preeclampsia such as the HELLP syndrome or if additional synergistic factors are needed. Additionally, the Flt-1 locus at 13q12 has not been localized within the genomic region identified by genetic linkage studies for preeclampsia. However, if the Flt-1 locus contributed to preeclampsia, one could...
hypothesize that patients who carry trisomy 13 fetuses should have a 50% increased risk of preeclampsia. Two case-control studies done several years ago did in fact demonstrate higher incidence of preeclampsia in mothers who carry trisomy 13 fetuses as compared to other trisomies or control pregnant patients [24, 173]. Although sFlt-1 levels were not measured in the patients with trisomy 13, these data are consistent with the hypothesis that elevated production of sFlt-1 may lead to preeclampsia.

**IMPACT OF PREECLAMPSIA ON LONG-TERM MATERNAL HEALTH**

Women with hypertensive disorders of pregnancy have an increased long-term incidence of dyslipidemias, insulin resistance, and cardiovascular diseases [174–176]. Sibai, el-Nazer, and Gonzalez-Ruiz [177] reported an 18-fold increase in the incidence of chronic salt-sensitive hypertension in women with a history of preeclampsia. It has been speculated that this late-onset hypertension may play a role in the eightfold increase in cardiovascular mortality that is also observed in these patients [176]. However, it is difficult to determine causality, because many of the risk factors for preeclampsia (such as diabetes mellitus, hypertension, and renal insufficiency) predispose to hypertension and cardiovascular disease as well. It has also been suggested that subtle renal injury caused by preeclampsia can eventually lead to the development of chronic hypertension [178, 179]. It is tempting to speculate that the long-term cardiovascular complications noted in some patients who have had preeclampsia may be due to a chronic antiangiogenic state resulting from polymorphisms in genes such as sFlt-1. Additionally, patients with preeclampsia are said to have a decreased long-term incidence of malignancy [180], a provocative observation disputed by some [181], that may suggest that the antiangiogenic state of preeclampsia may reflect a permanent state of the maternal milieu.

**CONCLUSION**

Preeclampsia is a systemic disorder characterized by widespread endothelial dysfunction. Substantial animal and human experimental evidence fulfilling Koch’s postulates [182] supports the hypothesis that placental sFlt1, an antiangiogenic protein, may be etiologic. Several important questions remain to be explored, and many of these can now be approached experimentally. Why do certain conditions (see Table 1) predispose to preeclampsia? Are there additional synergistic factors which might induce/predispose to HELLP syndrome? What is the relationship of sFlt-1 with other postulated mediators of the maternal syndrome such as hyperuricemia, ADMA, or antibodies to angiotensin II AT-1 receptor? What governs regulation of sFlt1 transcription and splicing, and why is it abnormal in preeclampsia? Is placental ischemia a cause or a consequence of excessive placental sFlt1? Large genetic trials such as GOPEC (Genetics of Preeclampsia) [6], ongoing proteomic/genomic studies of tissue and blood specimens from patients with preeclampsia, and further characterization of the sFlt-1 induced animal model of preeclampsia may help to answer these questions.

The implications of these advances on our clinical management of preeclampsia may be profound. Large, prospective studies are needed to describe in detail the changes in sFlt1, VEGF, and PlGF that precede preeclampsia onset; measurement of these factors in pregnant women may prove to be useful diagnostically. Pharmacologic intervention aimed at restoring sFlt1/PIGF/VEGF balance may prevent or modify the course of preeclampsia. Safely prolonging pregnancy by only a few weeks could substantially reduce maternal and neonatal morbidity and mortality resulting from preeclampsia, the world’s most common glomerular disease.

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