

Angiogenic Factors in the Pathogenesis of Preeclampsia

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Preeclampsia affects 5–10% of pregnancies and is responsible for substantial maternal and neonatal morbidity and mortality. It is believed to be a two-stage disease with an initial placental trigger with no maternal symptoms followed by a maternal syndrome characterized by hypertension, proteinuria, and endothelial dysfunction. The first stage is thought to be due to shallow cytotrophoblast invasion of maternal spiral arterioles leading to placental insufficiency. The diseased placenta in turn releases soluble angiogenic factors that induce systemic endothelial dysfunction and clinical preeclampsia during the second stage. This review will discuss the role of circulating angiogenic factors of placental origin as potential mediators of the systemic endothelial dysfunction and the clinical syndrome of preeclampsia and provide an evolutionary explanation for this phenomenon. © 2005, Elsevier Inc.

I. Introduction

Preeclampsia is characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation (Roberts, 2000; Roberts and Cooper, 2001; Walker, 2000). Preeclampsia is also frequently associated with edema and

hyperuricemia and it usually remits when the placenta is delivered. The placenta in preeclampsia is often abnormal, with evidence of hypoperfusion and ischemia. Vascular endothelial dysfunction and microangiopathy are present in the mother, but not in the fetus. Severe complications of preeclampsia can include acute renal failure, cerebral edema, cerebral hemorrhage, seizures (eclampsia), pulmonary edema, thrombocytopenia, hemolytic anemia, coagulopathy, and liver injury—including HELLP, the syndrome of *Hemolysis, Elevated Liver enzymes, and Low Platelets* (Sibai *et al.*, 2005). When preeclampsia threatens to lead to severe maternal complications, urgent delivery of the fetus and placenta is often undertaken to preserve maternal health.

Although preeclampsia has been traditionally thought of as a pregnancy-specific syndrome, data suggests that women with a history of preeclampsia have an eightfold increased risk of cardiovascular death when followed over their lifetime (Irgens *et al.*, 2001). Risk factors for preeclampsia include nulliparity, preexisting hypertension, obesity, diabetes mellitus, thrombophilias, and a family history of preeclampsia (Dekker, 1999).

Observations to date suggest that the earliest pathological change in preeclampsia occurs in the uteroplacental circulation resulting in placental insufficiency or ischemia, which may be considered stage 1 of the disease (Roberts, 2000). In stage 2, the diseased placental tissue (ischemic placenta) in turn secretes circulating factors that cause generalized endothelial cell injury in the mother, resulting in the clinical syndrome of preeclampsia (Roberts, 2000; Roberts *et al.*, 1989). Current understanding of the pathogenesis of preeclampsia will be reviewed with an emphasis on evidence that an imbalance in circulating angiogenic factors (Bdolah *et al.*, 2004) and their interaction with the maternal vasculature may be responsible for the clinical phenotype of preeclampsia.

II. Abnormal Placentation and Placental Ischemia (Stage 1)

It is widely accepted that the placenta plays a central role in the pathogenesis of preeclampsia as it occurs only in the presence of the placenta and the clinical symptoms remit dramatically postpartum after the delivery of the placenta (Page, 1939). In a case of preeclampsia with extrauterine pregnancy, removal of the fetus alone was not sufficient; symptoms persisted until the placenta was delivered (Shembrey and Noble, 1995). One report suggests that in cases of preeclampsia with discordant twins, selective feticide reverses preeclampsia, with the attenuation of symptoms occurring in a timeframe consistent with placental involution (Heyborne and Porreco, 2004).

Several lines of evidence suggest that placental insufficiency is central to the pathogenesis of preeclampsia (Karumanchi *et al.*, 2004): (1) Pathological

examination of placentas from preeclamptic pregnancies reveals numerous placental infarcts and sclerotic narrowing of arterioles (De Wolf *et al.*, 1975; Khong *et al.*, 1986); (2) placental bed biopsies in preeclamptics have been noted to have inadequate trophoblastic invasion of maternal decidual arterioles leading to tight and constricted vessels (Gerretsen *et al.*, 1981; Robertson *et al.*, 1967); (3) maternal risk factors for preeclampsia include medical conditions that predispose a patient to underlying vascular insufficiency such as chronic hypertension, diabetes, systemic lupus erythematosus, as well as acquired and inherited thrombophilias (Dekker, 1999); (4) obstetrical conditions such as multiple gestations or hydatiform moles that increase placental mass but with a relative decrease of placental blood flow increase the risk of preeclampsia (Dekker, 1999); and (5) animal models of preeclampsia involve creating placental insufficiency by disrupting uterine blood flow (Casper and Seufert, 1995; Kumar, 1962).

During normal placental development, cytotrophoblasts invade the maternal spiral arterioles and completely remodel the maternal spiral arterioles into large capacitance vessels with low resistance (Gerretsen *et al.*, 1981; Robertson *et al.*, 1967). This endovascular cytotrophoblast invasion involves replacement of not only the endothelium but also the highly muscular tunica media. In preeclampsia, there is shallow placental cytotrophoblast invasion of uterine spiral arterioles, leading to reduced placental perfusion and consequently placental insufficiency (Brosens *et al.*, 1972). Paradoxically, even in the normal placenta, there is little or no invasion of the uterine venules. The primary event that contributes to the failed trophoblast invasion/differentiation in preeclampsia is unknown, but genetic, immunological, and environmental factors (such as hypoxia and nutritional deficiencies) are thought to play a role. Extensive studies (by Fisher *et al.*, 1981) suggest that differences in O₂ tension may be the governing factor that regulates the invasive behavior of the cytotrophoblasts (Genbacev *et al.*, 1996, 1997). The remodeling of the spiral arterioles is thought to begin in late first trimester and is complete by 18–20 weeks. Although the exact gestational age at which the trophoblast invasion of these arterioles ceases is unclear, histological studies show that fewer invasive trophoblasts are seen in the decidua with increasing gestational age.

Differentiation of trophoblasts along the invasive pathway involves alteration in expression of a number of different classes of molecules, including cytokines, adhesion molecules and extracellular matrix molecules, metalloproteinases, and class Ib major histocompatibility complex molecule, HLA-G (Damsky *et al.*, 1992, 1994; Fisher and Damsky, 1993). During normal differentiation, invasive trophoblasts alter their adhesion molecule expression from those that are characteristic of epithelial cells (integrin α_6/β_4 , α_v/β_5 , and E-cadherin) to those of endothelial cells (integrin α_1/β_1 , α_v/β_3 , PECAM, and VE-cadherin), a process referred to as pseudo-vasculogenesis (Zhou

et al., 1993, 1997b). Both *in vitro* and *in vivo* studies show that trophoblasts obtained from patients with preeclampsia fail to undergo these alterations of adhesion molecules and pseudo-vasculogenesis (Lim *et al.*, 1997; Zhou *et al.*, 1997a), a finding that is disputed by some groups (Kaufmann *et al.*, 2003). The molecular pathways that regulate pseudo-vasculogenesis may involve a vast array of transcription factors, growth factors, and cytokines (Zhou *et al.*, 2003). Considerable attention has been given to angiogenesis-related gene products such as VEGF, angiopoietin/tie, and ephrin family proteins and their role in regulating pseudo-vasculogenesis and invasiveness. Interestingly, invasive trophoblasts have been found to express VEGF, PlGF, VEGF-C, and their receptors. Furthermore, blocking their signaling pathways decreases the expression of integrin $\alpha 1$ (a marker of pseudo-vasculogenesis) *in vitro* (Zhou *et al.*, 2002). However, *in vivo* evidence directly linking abnormalities of VEGF signaling to impaired pseudo-vasculogenesis is lacking at the present time. More recently, the invasive trophoblasts were also found to express L-selectin, an adhesion molecule that mediates leukocyte migration from blood to tissues (Genbacev *et al.*, 2003). It has been hypothesized that abnormalities of the selectin system at the fetal-maternal interface may account for implantation failures and preeclampsia. Finally, trophoblast expression of HLA-G, a nonclassical class I molecule that has shown to be decreased in preeclampsia, has been hypothesized to protect trophoblasts from NK cell attack at the implantation site (Moffett-King, 2002).

Long-standing and severe preeclampsia is associated with placental changes such as atherosclerosis, fibrinoid necrosis, thrombosis, and placental infarction (De Wolf *et al.*, 1975). Although not all these lesions are uniformly found in patients with preeclampsia, there appears to be a correlation between the severity of the disease and the extent of the lesions. Furthermore, in about one third of preeclamptic women (especially in those with term preeclampsia), these placental changes are not present. Abnormal remodeling of the spiral arterioles results in placental ischemia, which in turn is thought to lead to the secretion of soluble factors into the maternal bloodstream. However, evidence establishing a causal relationship between abnormal placentation and the maternal syndrome is lacking.

III. Systemic Endothelial Dysfunction (Stage 2)

Generalized endothelial dysfunction can account for most of the clinical aspects of preeclampsia (Roberts, 1998): hypertension through disturbed endothelial control of vascular tone, proteinuria from increased glomerular vascular permeability, coagulopathy as a result of abnormal endothelial expression of procoagulants, and liver dysfunction from hepatic ischemia.

Data from several studies support the theory that the maternal response in preeclampsia is secondary to generalized endothelial dysfunction. Studies have reported increased circulating fibronectin, factor VIII antigen, and thrombomodulin, all markers of endothelial cell injury in patients with preeclampsia (Friedman *et al.*, 1995; Hsu *et al.*, 1993; Taylor *et al.*, 1991). Flow-mediated vasodilation has also been found to be impaired in preeclamptic vessels, further suggesting abnormal endothelial function (Cockell and Poston, 1997; McCarthy *et al.*, 1993). Decreased production of endothelial-derived vasodilators such as prostacyclins, increased production of endothelins and enhanced vascular reactivity to angiotensin II also suggest abnormal endothelial function (Clark *et al.*, 1992; Gant *et al.*, 1973; Mills *et al.*, 1999). Renal biopsies from patients with preeclampsia reveal diffuse glomerular endothelial swelling referred to as glomerular endotheliosis (Fisher *et al.*, 1981). Finally, serum from preeclamptic women causes endothelial activation in human umbilical vein endothelial cells *in vitro* (Roberts *et al.*, 1992).

The identification of circulating factors mediating endothelial dysfunction has been the source of great research interest for decades. Several groups have reported alterations of cytokines/growth factors/chemicals such as TNF- α , IL-6, IL-1 α , IL-1 β , Fas ligand, neurokinin-B, oxidized lipid products, and ADMA (asymmetric dimethyl arginine) that are released by the placenta and/or other maternal sources in preeclampsia (Benyo *et al.*, 2001; Conrad *et al.*, 1998; Page *et al.*, 2000; Roberts and Cooper, 2001; Savvidou *et al.*, 2003). However, there is no evidence that any of these molecules are etiological. Increased sensitivity to angiotensin II, a consistent feature of preeclampsia, has been found to be secondary to increased bradykinin (B2) receptor upregulation in preeclamptic patients (AbdAlla *et al.*, 2001). This, in turn, was found to lead to heterodimerization of B2 receptors with angiotensin II type I receptors (AT1), and this AT1/B2 heterodimer was shown to increase responsiveness to angiotensin II *in vitro*. It is unclear, however, whether these alterations are pathophysiological or epiphenomena. Along similar lines, increased circulating concentrations of agonistic antibodies to the angiotensin-1 (AT-1) receptor have been reported in women with preeclampsia (Wallukat *et al.*, 1999). Stimulation of the AT-1 receptor by these autoantibodies might contribute to the vascular damage and the enhanced angiotensin II sensitivity noted in preeclampsia (Dechend *et al.*, 2003; Xia *et al.*, 2003). These antibodies have also been encountered in other examples of vascular injury such as vascular rejection (Dragun *et al.*, 2005), suggesting that they may be secondary to the generalized microangiopathy of preeclampsia.

Studies from several laboratories have demonstrated an increased placental expression and secretion of sFlt1 (soluble fms-like tyrosine kinase 1; see following), a naturally occurring circulating vascular endothelial growth

factor (VEGF) antagonist in patients with preeclampsia (Koga *et al.*, 2003; Maynard *et al.*, 2003; Zhou *et al.*, 2002). Importantly, when administered exogenously to rats, sFlt1 alone has been shown to be sufficient to induce a preeclampsia-like phenotype (Maynard *et al.*, 2003).

A. Excess Circulating sFlt1, Impaired VEGF Signaling, and Antiangiogenic State

VEGF is an endothelial-specific mitogen that plays a key role in promoting angiogenesis. VEGF's activities are mediated primarily by its interaction with two high-affinity receptor tyrosine kinases: kinase-insert domain region (KDR) and fms-like tyrosine kinase-1 (Flt-1) that are selectively expressed on vascular endothelial cell surface (Dvorak, 2002). Alternative splicing of Flt-1 results in the production of an endogenously secreted protein referred to as sFlt1, which lacks the cytoplasmic and transmembrane domain but retains the ligand-binding domain (He *et al.*, 1999b; Kendall and Thomas, 1993). Thus, sFlt1 can antagonize circulating VEGF by binding to it and preventing VEGF's interaction with its endogenous receptors located in the vasculature (Fig. 1). sFlt1 also binds and antagonizes placental growth factor (PlGF), another member of the VEGF family that is made in the placenta predominantly (Fig. 1).

In vitro studies confirm that excess placental sFlt1 production induces an antiangiogenic state in the serum of preeclamptic women that can be rescued by exogenous VEGF and PlGF (Maynard *et al.*, 2003). sFlt1 alone, when administered to pregnant rats, induced albuminuria, hypertension, and renal pathological changes of glomerular endotheliosis by antagonizing circulating VEGF and PlGF and inducing endothelial dysfunction. In addition, circulating levels of free VEGF and free PlGF were found to be decreased in conjunction with elevated sFlt1 in the bloodstream at the time of disease presentation (Chaiworapongsa *et al.*, 2004; Koga *et al.*, 2003; Maynard *et al.*, 2003; Tsatsaris *et al.*, 2003). Although sFlt1 is made in small amounts by other tissues (endothelial cells and monocytes), the placenta seems to be the major source of circulating sFlt1 during pregnancy as evidenced by a dramatic fall in circulating concentrations of sFlt1 after delivery of the placenta (Maynard *et al.*, 2003). The increase in sFlt1 precedes the onset of clinical disease by at least 5 weeks (Hertig *et al.*, 2004; Levine *et al.*, 2004) and appears to be more pronounced in severe and early-onset preeclampsia (Levine *et al.*, 2004). When free PlGF and free VEGF were measured throughout pregnancy, it was found to be decreased in preeclamptics well before the onset of clinical disease (Levine *et al.*, 2004; Polliotti *et al.*, 2003; Taylor *et al.*, 2003). Finally, decreased urinary PlGF has also been reported to precede clinical preeclampsia (Levine *et al.*, 2005).

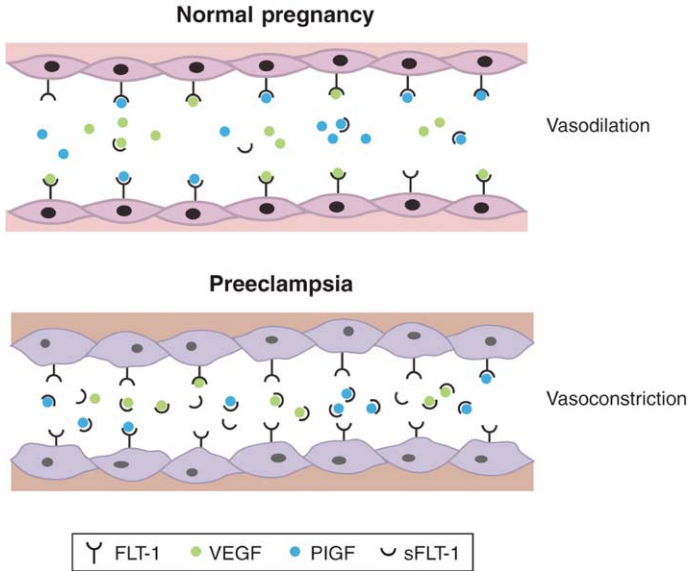


Figure 1 Mechanism of action of sFlt-1. sFlt-1 protein, derived from alternative splicing of Flt-1, lacks the transmembrane and cytoplasmic domains but still has the intact VEGF- and PlGF-binding extracellular domain. During normal pregnancy, VEGF and PlGF signal through the VEGF receptors (Flt-1) and maintain endothelial health. In preeclampsia, excess sFlt-1 binds to circulating VEGF and PlGF, thus impairing normal signaling of both VEGF and PlGF through their cell-surface receptors. Thus, excess sFlt-1 leads to maternal endothelial dysfunction. Reproduced with permission from [Bdolah et al., 2004](#).

VEGF is known to stimulate angiogenesis and promote vasodilation by stimulating NO and prostacyclin formation (signaling molecules that are decreased in preeclampsia) ([He et al., 1999a](#)). Furthermore, a significant percentage of cancer patients receiving VEGF-signaling antagonists develop hypertension and proteinuria ([Kabbinavar et al., 2003](#); [Yang et al., 2003](#)). Even a 50% reduction of renal VEGF production in genetically modified mice resulted in glomerular endotheliosis and proteinuria ([Eremina et al., 2003](#)). These data suggest that excess sFlt1, by neutralizing VEGF and PlGF, may play a causal role in the pathogenesis of the maternal syndrome in preeclampsia. Data suggests that VEGF may be particularly important in maintaining the health of fenestrated endothelium ([Risau, 1998](#)), which is found in the renal glomerulus, choroid plexus, and the hepatic sinusoids—organs disproportionately affected in preeclampsia. It has been shown that VEGF induces endothelial fenestrae *in vitro* ([Esser et al., 1998](#)) and even a 50% decrease in VEGF production in the glomerulus in mice leads to not only glomerular endotheliosis but also loss of glomerular endothelial fenestrae ([Eremina et al., 2003](#)).

B. sFlt1 and Maternal–Fetal Conflict

Substantial evidence suggests that sFlt1 is, at least partially, responsible for the endothelial dysfunction of preeclampsia. Why then does the placenta release a factor that damages the maternal endothelium? A conventional interpretation would be that sFlt1 performs some other function and that endothelial damage is an occasional maladaptive side effect of its release into the maternal circulation, perhaps in women who are particularly “susceptible.” Another possibility should also be considered. The placenta may release sFlt1 into the maternal circulation to cause endothelial dysfunction because the associated vasoconstriction benefits the fetus by directing a greater share of maternal cardiac output to the intervillous space of the placenta. In this view, stage II of preeclampsia is an adaptive response of the conceptus to the placental insufficiency arising from stage I of preeclampsia (Haig, 1993, 1996).

During pregnancy, the maternal systemic circulation can be conceptualized as consisting of two subcirculations, placental and nonplacental, arranged in parallel: the placental subcirculation consists of all the vessels that supply maternal blood to the intervillous space of the placenta; the nonplacental subcirculation consists of all vessels that supply other tissues of the maternal body (Fig. 2). Increases in the nonplacental resistance (R_n), as occurs in preeclampsia, would result in increased blood flow through the placental subcirculation, other things being equal (i.e., for unchanged cardiac output and placental resistance, R_p). More generally, any increase in the ratio of nonplacental to placental resistance (R_n/R_p) will result in a larger fractional share of maternal cardiac output flowing through the intervillous space (Haig, 1999).

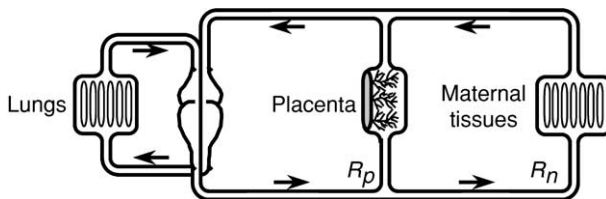


Figure 2 A simple model of the maternal circulation during pregnancy. Maternal systemic cardiac output is shared between placental and nonplacental subcirculations. In stage I of preeclampsia, inadequate modification of maternal spiral arterioles results in increased placental resistance (R_p). In stage II of preeclampsia, placental release of sFlt1 causes a disproportionate increase in the nonplacental resistance (R_n) relative to the placental resistance (R_p). As a result, an increased share of maternal cardiac output is directed to the placental subcirculation. Modified from Haig, 1999.

The hypothesis that preeclampsia is an adaptation of malnourished fetuses to increase their supply of nutrients posits that the endothelial dysfunction of preeclampsia disproportionately increases nonplacental resistance relative to placental resistance. This seems plausible—given the remodeling of spiral arterioles that occurs during the first half of pregnancy—but is yet to be experimentally demonstrated. The hypothesis is based on the evolutionary theory of parent–offspring conflict (Trivers, 1974) that what is “best” for a parent is not always “best” for an offspring, and vice versa. This conflict is illustrated in the clinical dilemmas of treating preterm preeclampsia: the longer that induction of delivery is delayed, the greater the risk to a mother’s health but the greater the benefits to the fetus.

If the induction of preeclampsia is an adaptation to enhance fetal nutrition, then the adaptation need not be simple and could involve the release of multiple placental factors that target different physiological systems of the mother. That is, sFlt1 may be just one component, albeit an important component, of a cocktail of substances that are released into maternal blood in nutritionally compromised pregnancies.

C. Speculations About the Mechanisms of Preeclampsia

If sFlt-1 is an important cause of preeclampsia, there might be at least two kinds of predisposing factors. One might involve the overproduction of sFlt-1. Conditions falling in this category might include multiple gestation, hydatiform mole, trisomy 13, and, possibly, first pregnancy. Another set of predisposing factors would include disorders that sensitize the maternal vascular endothelium to the antiangiogenic effects of sFlt-1. Such factors might include obesity, preexisting hypertension or renal disease, diabetes, and preexisting vasculitis. It is interesting that the alteration in the angiogenic factors in the serum of obese patients with preeclampsia was somewhat lower than that in lower-weight preeclamptic patients (Levine *et al.*, 2004; Thadhani *et al.*, 2004a,b). It is not yet known whether diabetes, hypertension, and preexisting renal disease predispose to preeclampsia by increasing the production of sFlt-1 or by sensitizing the vascular endothelium to its presence.

D. Unanswered Questions

There are limitations and several unanswered questions to the sFlt1 story. The precise mechanisms of excess sFlt1 production by the placenta are not known, and importantly, the role of sFlt1 in normal placental development and in placental pseudo-vasculogenesis is not clear. No coagulation or liver

function abnormalities or brain abnormalities (eclampsia) were reported in sFlt1-treated animals. Moreover, genetic studies provide little support for a role for sFlt1. For example, both an Australian/New Zealand cohort (Moses *et al.*, 2000) and an Icelandic cohort (Arngrimsson *et al.*, 1999) have suggested a maternal susceptibility locus on chromosome 2, bearing no known relationship to sFlt1. One possible interpretation is that these studies detect a locus responsible for susceptibility to stage I of preeclampsia, whereas placental production of sFlt1 is responsible for stage II of the disease. Although, it is also possible that such loci are associated with transcription factors or splicing factors affecting sFlt1 production, it seems more likely that there are other yet unidentified genetic factors that contribute to this multifactorial disease.

On the other hand, the hypothesis that excessive production of sFlt1 may play a causal role in preeclampsia is supported by studies of the occurrence of this syndrome in mothers of infants with trisomy 13. The genes for sFlt1 and Flt-1 are carried on chromosome 13. Fetuses with an extra copy of this chromosome should theoretically produce more of these gene products than their normal counterparts. The incidence of preeclampsia in mothers who carry fetuses with trisomy 13 is in fact greatly increased, as compared with all other trisomies or with control pregnant patients (Tuohy and James, 1992). It has been noted the women carrying trisomy 13 fetuses have a greater concentration of circulating sFlt1 as compared to normal karyotype controls, thus providing a molecular explanation for the increased risk of preeclampsia observed in these patients (Bdolah and Karumanchi, unpublished observations).

Serum concentrations of sFlt1 have been found to be modestly elevated in patients with IUGR without preeclampsia (Tsatsaris *et al.*, 2003), a finding that has not been confirmed by others (Shibita *et al.*, 2004). Finally, although sFlt1 was elevated in most patients with preeclampsia, it was not elevated in some patients with mild preeclampsia (Levine *et al.*, 2004). Thus, it is likely that additional synergistic factors that are elaborated by the placenta may yet be identified that play a role in the pathogenesis of the generalized endothelial dysfunction and vascular damage noted in preeclampsia.

IV. Conclusions

In summary, preeclampsia is believed to be a two-stage disease with an initial placental syndrome that is followed by the maternal syndrome (Fig. 3). The maternal syndrome in preeclampsia is a state of generalized endothelial dysfunction secondary to excessive amounts of circulating antiangiogenic factors (such as sFlt1) that are released by the diseased placenta (Fig. 3). The excess sFlt1 theory in the pathogenesis of preeclampsia fits very well with

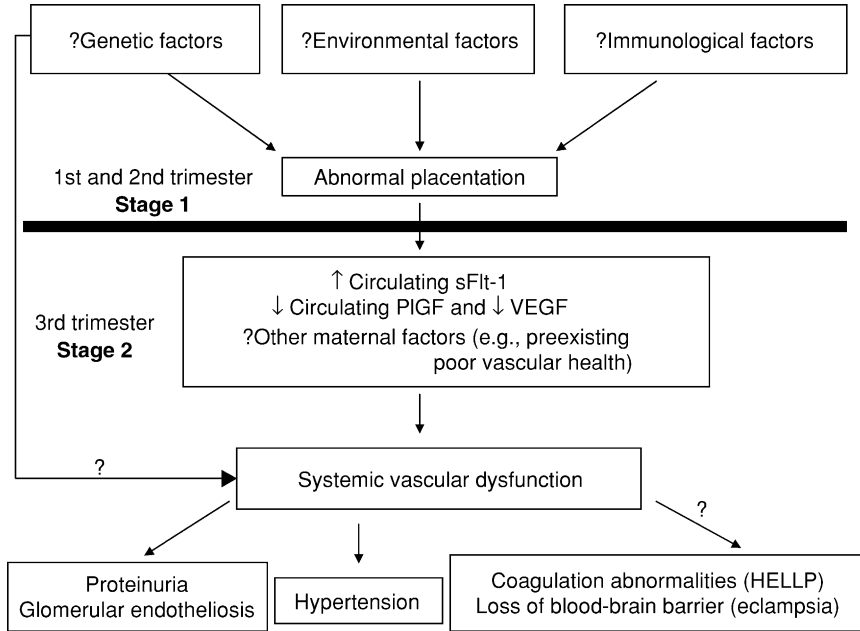


Figure 3 Summary of the pathogenesis of preeclampsia.

the maternal–fetal conflict that has been previously proposed as the basis of the development of preeclampsia. Understanding the mechanisms of placental dysfunction in preeclampsia should further clarify the etiology of preeclampsia. Future studies specifically characterizing the various circulating proteins elaborated by the preeclamptic placenta and understanding their relationship with already-identified mediators of endothelial dysfunction such as AT1-AA trophoblast debris and sFlt1 should help in clarifying the pathogenesis of the maternal syndrome. Although improvements in obstetrical and perinatal care have dramatically reduced morbidity and mortality from preeclampsia (especially in the developed world), there have been no significant breakthroughs in the treatment of preeclampsia over the last 40 years. The promising early results of agents such as aspirin and calcium supplementation have not been borne out in large randomized, controlled trials (Caritis *et al.*, 1998; Levine *et al.*, 1997). Therapeutic strategies aimed at rescuing the endothelial dysfunction with agents such as VEGF, PIGF, and prostacyclins should be tested in patients with severe disease and hence might allow the delivery to be safely postponed. As understanding continues to advance based on molecular and genetic techniques, hopefully new interventions may improve the management of this important syndrome in the near future.

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