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Beyond the threshold: an etiological bridge between hypoxia and immunity in preeclampsia

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Abstract

Taking a cue from the recent workshop 'Preeclampsia—A Pressing Problem' sponsored by the National Institutes of Child Health and Human Development, this review article takes a fresh look at hypoxia and a dysfunctional immune system as the key contributors to the etiology of preeclampsia and the mechanisms involved therein. In the context of epidemiological research on the intricate and multifactorial nature of preeclampsia, we focus on hypoxia as an upstream regulator of preeclampsia and its consequences in a model compromised by a deficiency in key pregnancy compatible immune modulators. It has been proposed that placental hypoxia releases cytotoxic factors produced at the maternal–fetal interface into the circulation to manifest the maternal symptoms associated with preeclampsia. However, it is not clear how this mechanism is empowered in pregnant women. Does systemic hypoxia exert preeclampsia-like effects on pregnancy? Are these effects further manifested by intrinsic inflammation in the absence of key immune modulators such as IL-10? Thus, it is of paramount importance that *in vivo* models be developed wherein the role of systemic hypoxia can be evaluated for preeclampsia-causing events. We present a discussion on whether prolonged exposure to hypoxia can lead to a perpetual cycle of compartmentalized uteroplacental tissue damage, release of anti-angiogenic and vasoconstrictive factors that impair trophoblast invasion and promote systemic vascular resistance resulting in the maternal syndrome.

Keywords

Preeclampsia; Hypoxia; IL-10 deficiency; Angiogenesis; Apoptosis

1. Introduction

Preeclampsia is a syndrome diagnosed by hypertension and proteinuria after 20 weeks of gestation with mild to severe microangiopathy of various target organs including kidney, liver, brain and placenta (ACOG Committee on Practice Bulletins—Obstetrics, 2002). The fact that placental tissue but not the fetus is necessary for the development of this disease and that the disease itself is always cured after delivery of the placenta underscores its indispensable role in the pathophysiology of preeclampsia (Moore-Maxwell and Robboy, 2004). The consensus reached at a recent NICHD/NIH sponsored workshop is that preeclampsia is a multifactorial disease whose pathogenesis is not solely vascular, genetic, immunologic, or environmental but a complex combination of factors (Ilekis et al., 2007). Moreover, the heterogeneity of the disease is suggested by diverse clinical manifestations

such as mild or severe preeclampsia, early onset (<34 weeks) or late onset (>34 weeks) of the disease and presence or absence of intra-uterine growth restriction (IUGR) with preeclampsia (Dekker and Sibai, 2001). One may wonder if these distinct clinical presentations have common underlying mechanistic underpinnings that simply manifest differentially based on contribution from predisposing maternal factors. Indeed, women with asthma, obesity, maternal infections, insulin resistance or adverse lipid metabolic profile all have an increased incidence of preeclampsia (Sibai et al., 2005).

Nevertheless, the consequence is that this multisystem disorder occurs in about 5–8% of all live birth pregnancies in the United States and is a leading cause of maternal and fetal mortality and morbidity. Moreover, a population-based cohort study suggests that women with early-onset preeclampsia are at greater risk of cardiovascular disease later in life (Irgens et al., 2001). Importantly, the hallmark pathological features of preeclampsia are shallow trophoblast invasion and poor spiral artery remodeling resulting in placental hypoperfusion, although not all women with reduced blood flow to the placenta experience preeclampsia (Roberts and Hubel, 1999; Burton et al., 2009). It is then possible that a second hit in terms of intrinsic or induced dysregulation of a pregnancy compatible factor(s) or inflammation at the maternal–fetal interface may culminate in full spectrum of symptoms as seen in preeclampsia.

2. Etiological factors associated with preeclampsia

Pregnancy is a physiological state that is characterized by an active dialog between maternal and extraembryonic tissues. The *in utero* milieu instructs developmental cues to the placenta which lead to the remodeling of delivery route for nutrients to the fetus. As a consequence, cytotrophoblasts efficiently invade the decidua and the maternal spiral arteries, a critical process that ensures supply of nutrients to the developing fetus. The outcome of this invasion is transformation of the resistant and small muscular blood vessels to large, vascular channels with high degree of plasticity and low resistance created by replacement of musculoelastic wall with fibrinoid material, thus greatly enhancing blood flow to the placenta (De Wolf et al., 1982; Pijnenborg et al., 2006). Although the precise period when the trophoblast invasion ceases is unclear, the remodeling is believed to occur during the first and early second trimester. On the contrary in preeclampsia, cytotrophoblasts fail to invade the spiral arteries deeply enough, resulting in poor transformation of these blood vessels and insufficient blood flow to the placenta (Brosens et al., 1977; Meekins et al., 1994). Normal trophoblast differentiation and invasion are associated with integral events in normal pregnancy (Zhou et al., 1997).

Why then do third trimester trophoblasts not serve this purpose in preeclampsia? We have recently demonstrated that unlike first trimester trophoblasts, term trophoblasts fail to interact with endothelial cells due to high expression of E-cadherin and poor expression of VEGF-C and VEGF receptor (Kalkunte et al., 2008). Impaired differentiation and transition of trophoblasts may contribute to defective trophoblast invasion and placentation (Zhou et al., 1993). In this regard, empowerment of uterine natural killer (uNK) cells that uniquely home to the pregnant uterus with angiogenic features not only promote tolerance through VEGF-C release, but also facilitate angiogenesis (Kalkunte et al., 2009). In both mouse and human models, uNK cells further promote trophoblast invasion and spiral artery remodeling by secreting IFN- γ , chemokines and modulators such as IP-10, IL-8 and CXCL12 (Ashkar et al., 2000; Hanna et al., 2006). Because of concurrent co-onset of maternal symptoms and placental pathology, it is now well appreciated that factors released from the placenta and found in the maternal circulation cause systemic endothelial dysfunction.

Similar to tumor growth, a balance of proangiogenic factors such as vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and anti-angiogenic factors including soluble *fms*-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) is established in the developing placenta. A number of studies have shown that increased production of sFlt-1 and sEng coupled with reduced VEGF and PlGF levels in preeclamptic women seem to correlate positively with the severity of the disease (Levine et al., 2004; Thadhani et al., 2004; Romero et al., 2008). It is important to point out that not all women with high sFlt-1 levels acquire preeclampsia and not all preeclamptic women have high sFlt-1 levels, again confirming the heterogeneity of the disease. Although the source of sFlt-1 seems to be the placenta, the symptoms of hypertension and proteinuria observed in non-pregnant mice imply that upstream factors are involved in the placental injury leading to multifold production and release of sFlt-1 and sEng into maternal circulation (Karumanchi and Stillman, 2006). These molecules would then sequester VEGF in target organs such as the kidney. The upstream factors that are likely to trigger production of anti-vascular factors at the maternal–fetal interface are poorly understood, but may include hypoxia/ischemia.

3. Is hypoxia an upstream regulator in the pathogenesis of preeclampsia?

Oxygen is a vital regulator of placentation. Embryonic and placental development during early pregnancy occurs in a predominantly low oxygen milieu, at least in part, choreographed by hypoxia signaling pathways (Fig. 1). Studies have shown that low oxygen tension can regulate differentiation of trophoblast stem cells dependent on hypoxia-induced factor-1 α (HIF1 α) signaling pathways (Maltepe et al., 2005). Recent studies have elegantly demonstrated *in vivo* that exposure of pregnant rats from gestation day 6.5 through day 13.5 to hypobaric hypoxia (11% oxygen) stimulated changes in the uterine mesometrial vasculature. This was associated with dramatic increase in vascularity and vessel diameter as well as increased endovascular trophoblast invasion and replacement of the endothelium. In these experiments, the animals were intermittently exposed to normoxia, which may create a hypoxia–reperfusion scenario. Under these conditions, hypoxia hastens the appearance of the invasive endovascular trophoblast (day 13.5) in the mesometrial regions as compared to normoxia (day 14.5), prompting the authors to suggest a possible window of “sensitivity and timing” to hypoxia that facilitates either early maturation of trophoblast or removal of maternal barrier (Rosario et al., 2008).

Nevertheless, systemic hypoxia is an effective stimulus eliciting adaptations at the maternal–fetal interface, additionally determining the depth of trophoblast cell invasion. It is then pertinent to contemplate if there is a window of “tolerance” to hypoxia and oxygen levels. Can prolonged exposure to severe systemic hypoxia beyond a threshold initiate placental pathology, placental ischemia, inhibition of trophoblast invasion, and release of factors that can initiate preeclampsia-like symptoms?

Preeclampsia is considered to be a two-stage disorder characterized by reduced placental perfusion, possibly due to abnormal development of placental vasculature in the first stage. The second stage is the maternal response to this condition characterized by widespread inflammation and maternal endothelial dysfunction (Roberts and Hubel, 1999). The pathophysiological link between the two stages of preeclampsia remains enigmatic, but placental ischemia/hypoxia is widely believed to be central to its cause. Although recent publications propose that placental pathology is due to deficient conversion of blood flow with rheological consequences rather than hypoxia (Burton et al., 2009), earlier studies suggest that hypoxia–reoxygenation caused by intermittent perfusion–reperfusion can cause placental oxidative stress (Hung and Burton, 2006). This is likely to trigger release of sFlt-1 and sEng and other pro-inflammatory cytokines such as IL-6 that are known to cause hypertension and proteinuria (Xiong et al., 2009a).

Current evidence linking hypoxia to placental pathology of preeclampsia stems mainly from *in vitro* studies using trophoblasts or *ex vivo* explants cultures. *In vitro* experiments also suggest that severe hypoxia (1%) can lead to increased apoptosis and oxidative stress, shedding of villous microparticles, and elevated expression of sFlt-1 (Nevo et al., 2006; Redman and Sargent, 2000). In the placental ischemic model of ligation of lower abdominal aorta and the ovarian arteries of pregnant rat, hypertension, IUGR, release of sEng, induction of HIF-1 α and decreased hemeoxygenase-1 expression in the placenta were observed, suggesting causal role of placental ischemia/hypoxia in preeclampsia (Gilbert et al., 2008). Moreover as suggested, reduced trophoblast invasion and associated changes in spiral arteries seen in early-onset preeclampsia would result in increased production of anti-angiogenic and inflammatory factors. Such an event will lead to the presence of these factors in the circulation and is likely to result in high systemic vascular resistance.

A scenario has been proposed for unlinking the systemic vascular resistance and delivery of blood to the intervillous space (Burton et al., 2009). However, it is widely accepted that factors that trigger this vascular resistance are derived from the placenta. sFlt-1 and sEng, whose levels increase in maternal circulation, are known to contribute to endothelial cell dysfunction and increased vascular resistance. In this context, trophoblast differentiation and functional properties may be affected by poor placental perfusion or vice versa. Recent studies have shown that unlike normal pregnancy, villous placental explants from preeclampsia exhibit increased sensitivity and susceptibility to apoptosis on exposure to pro-inflammatory cytokines, suggesting altered programming of apoptotic cascade pathway in preeclampsia (Crocker et al., 2004). A possible role of p53, Mdm2, p21, Bcl-2 family proteins has been proposed in placental apoptosis (Levy et al., 2002). These events may lead to increased apoptosis, caspase-3 activation, PARP inactivation and chromatin condensation as suggested by recent studies using placental explants subjected to intermittent placental hypoxia-reoxygenation injury (Hung et al., 2002). Additionally, recent studies have suggested the role of growth arrest and DNA damage-inducible 45 (Gadd45) family of protein as upstream regulators of sFlt-1 production via p38 MAPK pathway (Xiong et al., 2009a,b). It is then possible to envision that beyond the tolerogenic threshold of hypoxia, apoptosis is triggered via up-regulation of p53 expression that leads to apoptosis causing signaling cascade (Fig. 1). However, a direct *in vivo* evidence of the hypoxia-sFlt1-sEng-placental pathology-systemic vascular resistance axis is currently not available.

4. Hypoxia and immunity

NF- κ B is a crucial regulator of post-transcriptional stabilization and accumulation of HIF1 α protein in innate immune cells, particularly macrophages which encounter severe hypoxia at the site of injury or infection. Under these conditions, HIF promotes release of pro-inflammatory cytokines and VEGF, up-regulates TLR expression and induces iNOS expression (Nizet and Johnson, 2009). In this context, it is possible that intra-uterine cytokine milieu displays a vital regulatory role in hypoxia-driven consequences during placentation and preeclampsia (Fig. 1).

Among immuno-regulatory cytokines, IL-10 is thought to play a critical role at the maternal-fetal interface because of its potent anti-inflammatory activities. IL-10 is expressed in a gestational age-dependent manner and is down regulated in the placental-decidual microenvironment as part of “the normal mechanism” for the onset of labor (Hanna et al., 2000). Further, in unexplained spontaneous pregnancy loss compared to elective terminations, there is poor expression of IL-10 in placental and decidual tissues (Plevyak et al., 2002). Importantly, mice with a null mutation in the *Il10* gene are more sensitive to very low doses of toll-like receptor ligands such as lipopolysaccharide (LPS), poly I:C or CpG which cause fetal demise, premature delivery, and IUGR depending on the route and

window of administration. Pregnancy incompatible effects appear to be associated with cytotoxic activation of uterine immune cells (Murphy et al., 2005, 2009; Thaxton et al., 2009). More recently, it was shown that adenoviral gene transfer of recombinant human IL-10 ameliorated ischemia–reperfusion injury by decreasing hepatic necrosis and apoptosis by inhibiting caspase-3 activity and mitochondrial cytochrome c release, and up-regulating anti-apoptotic (Bcl-2) and antioxidant (HO-1) molecules (Li et al., 2009).

Preliminary studies from our laboratory suggest that pregnant mice exposed to hypoxia (9.5% O₂) for prolonged period (gd 7.5–15) experience preeclampsia-like symptoms with elevated sFlt-1 and sEng, providing direct evidence of systemic hypoxia in initiating preeclampsia. The effect of hypoxia was severe particularly in IL-10 null mutant mice as compared to their wild type counterparts. Moreover, placental pathology seems to imply hypoxia-driven apoptosis due to perturbed p53–Bax–Bcl-2–caspase-3 axis. Taken together, our studies seem to suggest that IL-10 may blunt the severe hypoxia-induced placental injury and apoptosis.

It is then possible that IL-10 deficiency in a subset of the population could be a predisposing factor to preeclampsia and may define its severity. As understanding of an intricate link between hypoxia and innate immunity emerges (Nizet and Johnson, 2009), future studies on the impact of hypoxia on uNK cell homeostasis and functions, response in the settings of infections and inflammation, and effect on endovascular activity at the maternal–fetal interface are highly warranted.

5. Conclusions

Preeclampsia is a heterogeneous disease. The mechanistic understanding of preeclampsia as understood today resembles an airline flight map converging on one destination, making it a multifactorial disorder. We posit that beyond the threshold hypoxic challenge during pregnancy is a crucial upstream trigger that leads to perpetual cycle of placental pathology, release of anti-angiogenic and vasoconstrictive factors that promote systemic vascular resistance leading to manifestation of the maternal syndrome.

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Fig. 1.

Hypoxia: beyond the threshold. Hypoxia regulates multiple pathways involving p53, nuclear factor- κ B (NF- κ B) and growth arrest and DNA damage-inducible 45 (Gadd45a). We posit that hypoxia beyond a threshold level triggers placental pathology associated with preeclampsia which is accentuated by IL-10 deficiency.