

Trophoblast biology, responses to hypoxia and placental dysfunction in preeclampsia

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1. ABSTRACT

Preeclampsia is a hypertensive disorder unique to human pregnancy that can result in significant morbidity and mortality for mother and fetus. While the etiology of preeclampsia is unknown, the placenta in general and trophoblast in particular is a prerequisite for the disease. We overview normal development of the human placenta, describe the role of hypoxia and other insults in placental injury, and highlight how the dysregulation of villous trophoblast biology in the second half of pregnancy may incite the pathophysiology of preeclampsia in the mother.

2. INTRODUCTION

Preeclampsia is a pregnancy-specific syndrome of reduced organ perfusion secondary to vasospasm and activation of the coagulation cascade (1), commonly identified by new onset hypertension and proteinuria. Worldwide maternal deaths from preeclampsia are

estimated at 60,000 annually. The disorder is unique to human pregnancy, and the placenta is a prerequisite for development of the syndrome. The trophoblast is responsible for the development of preeclampsia and neither the fetus nor the extraembryonic membranes are necessary to develop the disease, as reflected by syndrome manifestation in women with hydatidiform mole or choriocarcinoma (2). The trophoblast need not be within the uterus, since preeclampsia occurs in rare cases of abdominal pregnancies (3). Importantly, preeclampsia occurs with increased frequency when trophoblast mass is excessive, such as in multi-fetal gestations, molar pregnancies, gestational diabetes, and hydrops fetalis (4). Taken together, the trophoblast derivatives of the products of conception are necessary, if not sufficient, to induce the preeclamptic syndrome

A popular model for the development of preeclampsia describes two stages (5). The first stage yields

mal-development of the basal plate of the placenta in early gestation, resulting from a genetic predisposition, an implantation defect, or a maternal medical condition. The consequences of this abnormal placental development predisposes to under-perfusion of the chorioallantoic villous tree of the placenta in the second half of pregnancy and ultimately, the maternal response to the placental dysfunction (5).

The variable phenotypes of patients assigned a diagnosis of preeclampsia underscore that the pathophysiology is a syndrome, not a single disease entity. The clinical phenotype exhibits multi-organ involvement unequally affected by vasospasm, endothelial dysfunction and a disproportionate inflammatory response above that of normal pregnancy. Onset in the mid- or early third trimester typically yields intrauterine growth restriction, predisposes to very preterm delivery, and predicts higher rates of recurrence in subsequent pregnancies(6). Key questions about preeclampsia are what injuries contribute to the placental dysregulation typical of the preeclamptic syndrome and how does the placenta cause the maternal syndrome? We overview normal human placental development, describe the role of hypoxia and other insults in placental injury, and highlight how the dysregulation of the biology of villous trophoblast in the second half of pregnancy may incite the maternal pathophysiology of preeclampsia.

3. HOW DOES THE HUMAN PLACENTA DEVELOP IN NORMAL PREGNANCY?

3.1. First trimester

The human blastocyst aggressively invades the uterine epithelial lining at day seven after fertilization yielding interstitial implantation within the endometrium of pregnancy or decidua. *In vivo* measurements in the first trimester show a $pO_2 < 20$ mm Hg in human implantation sites and no intervillous blood flow until beyond 11 weeks' gestation (7) and thus, early development of the conceptus and placenta normally occurs in a low oxygen environment. The trophoblast of the blastocyst differentiates into cytotrophoblasts that provide the stem cell populations for trophoblasts in three sites: the basal plate, chorioallantoic villi, and the chorion laeve. The basal plate extravillous cytotrophoblasts proliferate in the hypoxic environment as they emanate from distal components of anchoring villi to invade the decidua as interstitial trophoblasts or as they penetrate the lumen of spiral arterioles as endovascular trophoblasts. In the presence of these extravillous trophoblasts, the decidual spiral arterioles lose their smooth muscle coat and the vessel wall is replaced by fibrinoid. This anatomical change sets the stage for a many-fold increase in blood flow to the implantation site. The endothelium of these vessels is also replaced by endovascular trophoblasts that assume integrin expression typical of an endothelial phenotype (8). The chorioallantoic placenta develops during this same period as a tree of villi, some free floating and others anchored to the basal plate decidua. However, in the absence of maternal blood flow to the chorioallantoic villi, nutrition for the fetus and placenta derives from endometrial secretions called histiotroph (9).

3.2. Second and third trimesters

After 11 weeks' gestation, maternal blood from the decidual spiral arterioles circulates through the intervillous space to provide nutrition for the fetus for the remainder of the pregnancy via the placental villous tree. Free-floating villi are thereon exposed to an ambient pO_2 of 60 mm Hg that gradually declines to about 40 mm Hg by the third trimester of pregnancy (10). Importantly, oxygen and nutrient delivery to the fetus from this point forward is primarily the duty of the free floating villi, with their bi-layer covering of trophoblasts normally bathed in maternal blood.

The syncytiotrophoblast component of this bi-layer is a terminally differentiated, non-mitotic epithelium that also functions as an endothelium, as it lines the intervillous space where maternal blood circulates through the placenta. Syncytiotrophoblast is unique in human cell biology as a true syncytial epithelium, with multiple nuclei dispersed non-uniformly in a cytoplasmic mass without lateral cell membranes. The syncytium interfaces the maternal and fetal circulations and thereby is positioned to regulate maternal to fetal nutrient and oxygen transfer. The subjacent, mitotically active, mononucleated cytotrophoblasts provide a stem cell population, as some cells differentiate and fuse to replenish the overlying syncytiotrophoblast. Homeostasis of this trophoblast bi-layer in normal development, and in response to injury, requires modulation of the proliferation and differentiation of villous cytotrophoblasts. Turnover of syncytiotrophoblasts occurs in tandem, and this replenishment of the trophoblast components is reflected by shedding clusters of apoptotic nuclei, called syncytial knots, and by denudation of segments of syncytial cytoplasm that exhibit features of apoptosis, necrosis, or both cell death pathways (11). The villous trophoblast components share a basement membrane that de-limits the villous core connective tissue through which fetal vessels pass to the umbilical circulation of the fetus. The direct continuity of the intervillous space with the maternal circulation provides villous trophoblasts access to influence the endothelium within the maternal systemic vasculature.

The above overview indicates that a hypoxic environment is normal during early placental development until about eleven weeks. The subsequent role of placental villi is to supply oxygen and nutrients to the fetus. This responsibility requires an ongoing source of oxygenated maternal blood from the spiral arterioles to bathe the villi within the intervillous space.

4. HOW DOES PLACENTAL DEVELOPMENT DIFFER FROM NORMAL IN PREECLAMPSIA?

The histopathology of placentas complicated by preeclampsia is commonly characterized by incomplete invasion of maternal blood vessels by trophoblasts and inadequate modification of the spiral arterioles in the decidua and superficial myometrium (12). The decidual vessels in the basal plate, not to mention the chorion, may be affected by acute atherosclerosis, a lesion that mimics an arteriosclerotic plaque in an adult vessel lumen (13).

Logically, these histopathological lesions of the maternal spiral arterioles avail these vessels to an altered response to endogenous vasoactive agents, predisposing to reduced maternal blood flow to the intervillous space. Moreover, the histopathology of the chorioallantoic placental villi are abnormal and characterized by non-branching, slender villi, increased denudations of the syncytiotrophoblast layer on villi, enhanced fibrin deposition, altered syncytiotrophoblast cytodifferentiation with abnormal abundance of syncytial knots, and a prominence of villous cytotrophoblasts for placentas in the second half of pregnancy (4). The reduced villous surface area for transport and the enhanced injury to the syncytiotrophoblast, as this epithelium regulates nutrient transfer, predispose to the intrauterine fetal growth restriction associated with many cases of severe preeclampsia. However, there is an opposite end to the spectrum of histopathological findings in the placentas of women with the diagnosis of preeclampsia at or near term who deliver a larger than gestational age placenta with a histopathology characterized simply by prominent villous cytotrophoblasts (4).

The above extremes of placental pathology in preeclampsia complement the variability in phenotype that clouds the clinical categorization of the syndrome. We posit that the above variation in placental phenotypes is reconcilable if one considers that the placental capacity for repair to compensate for insults is what ultimately determines the presence or absence of placental dysfunction. Thus, placentas exposed to an insult but with an endogenous reserve or a robust repair response to exogenous stimuli will exhibit limited dysfunction. On the contrary, the placenta with a sub-optimal compensatory response to adverse implantation events, genetic endowment, or maternal constitutional factors may exhibit marked dysfunction and the extreme of histopathological injury.

5. DOES HYPOXIA RESULT IN VILLOUS INJURY AND PLACENTAL DYSFUNCTION IN PREECLAMPSIA?

5.1. Hypoxia and placental pathology

A debated question in preeclampsia is whether or not the placental dysfunction that typifies the preeclamptic syndrome is due to exposure to chronic hypoxia? Insight into this debate is gained through examination of the pathology of the placentas of pregnancies assigned the diagnosis of preeclampsia. Severe preeclampsia with onset preterm and often associated with intrauterine growth restriction commonly yields a placenta that is remarkably smaller in mass, compared to normotensive controls, with gross infarcts discernible by the untrained eye. In these cases, altered blood flow with hypoxia and ischemia is accepted by most to contribute to the placental damage (14-17). However, many placentas from pregnancy complications without preeclampsia also show a similar pathology that is characteristic, but not specific, for preeclampsia (15). Conversely, many women with features of the preeclamptic syndrome have placentas without gross lesions (18, 19), and the histopathology becomes an

important feature in distinguishing the sources of placental abnormality for the clinical disorder.

Can the above wide spectrum of placental pathology be related to underperfusion and chronic villous hypoxia? Some evidence supports this contention. Doppler studies of blood flow in uterine vessels in mid-trimester identifies a group of women with sub-optimal uteroplacental flow who have an increased risk for development of preeclampsia (20). This indirect evidence for placental hypoxia is buttressed by recent gene expression profiling of villous tissues (21). Villi from women with preeclampsia express a gene profile that is remarkably similar to villi of pregnancies at high altitude and to villous specimens from sea level exposed *in vitro* to low oxygen tensions. Interestingly, pregnant women at high altitude have maternal systemic blood pO₂ values in the 50-60 mm Hg range and a frequency of preeclampsia about four-fold higher compared to women at sea level (22). However, the pathology of the placentas from women at high altitude does not show generalized injury but remarkably, shows less fibrin deposition than placentas of either preeclamptic women or of pregnancies at sea level (23). These observations suggest that under-perfusion with hypoxia may contribute to placental injury and associate with preeclampsia. However, the histopathology of placentas of women exposed to high altitude hypoxia argues against chronic hypoxia as a primary source for all placental dysfunction in preeclampsia.

5.2. Increased placental apoptosis in preeclampsia

Placental villi of women with preeclampsia exhibit higher than normal levels of apoptosis (24, 25), and the increased apoptosis has been attributed to exogenous insults, including hypoxia and re-perfusion injury (24, 26). A splice variant of the pro-death molecule Matador/Bok (Mtd-P) in the Bcl-2 family of proteins is regulated by hypoxia and is assigned a role in the increased apoptosis in villi of women with early onset, severe preeclampsia compared to controls (27). Huppertz and colleagues (28) have estimated that several grams of trophoblast are normally shed into the maternal circulation each day, and the higher levels of apoptosis detected in placentas of preeclamptic women would be expected to yield more trophoblast trafficking to the maternal circulation. Compared to controls, villous trophoblasts from preeclampsia are more sensitive to exogenous stimuli, as they undergo higher levels of apoptosis *in vitro* in response to either TNF-alpha or hypoxia (29). This again illustrates that hypoxia is not the only insult that can induce enhanced cell death in trophoblasts. Moreover, thromboxane (30) and homocysteine (31) also induce increased levels of apoptosis in cultured term trophoblasts. In light of the higher than normal thromboxane levels (32) and hyperhomocysteinemia (33, 34) observed in some women with preeclampsia, these non-hypoxic insults may contribute to enhanced trophoblast death. Furthermore, Burton and colleagues (26, 35, 36) showed that an ischemia-re-perfusion model of injury creates a high level of oxidative stress and causes villous explants to undergo widespread trophoblast apoptosis. Importantly, hypoxia alone in the same model had little effect on apoptosis but

instead, yielded a higher frequency of necrotic cell death. Interestingly, the oxidative stress induced by the re-perfusion model may simulate what occurs repeatedly in the placenta at risk for varying maternal blood delivery through spiral arterioles. Collectively, these studies imply that hypoxia is one among several candidate stimuli to cause increased trophoblast cell death in preeclampsia. The studies also suggest that necrosis as well as apoptosis may be involved in the increased level of trophoblast demise.

5.3. Necrosis, trophoblast debris, and preeclampsia

What evidence is there for other forms of cell death? Trophoblasts on villous explants in culture undergo enhanced self-destruction when exposed to hypoxia but interestingly, with a mixed morphology of both apoptotic and necrotic cell death, so called aponecrosis (11). This has led to the hypothesis that the injury contributing to increased trophoblast turnover in preeclampsia may also be by aponecrosis. Packaging of cell remnants from necrotic cell death may not optimize phagocytic recognition of the corpses to clear them from the circulation, and may thereby allow prolonged systemic circulation of the cell debris shed into the maternal vasculature. Such debris may incite part, if not all, of the increased maternal inflammatory response associated with the preeclamptic syndrome. In support of this idea, women with preeclampsia exhibit increased shedding of not only trophoblast cells in the maternal circulation (37, 38), but also circulating syncytiotrophoblast membrane (STMB) fragments (39), cytokeratin 18 intermediate filaments typical of the trophoblast epithelium (40), and fetal DNA and RNA in syncytiotrophoblast micro particles (41-43). Again suggesting a role for hypoxia, fetal DNA derived from syncytiotrophoblast particles is increased in pregnancies exposed to hypoxia because of residence at high altitude, compared to women at sea level (44). Such micro-particles are identified in even higher numbers in pregnant women at high altitude who develop preeclampsia (44). These observations support the concept that the higher than normal level of circulating trophoblast debris derive from the trophoblast cell death response to hypoxia. The STMB and other syncytiotrophoblast micro-particles are candidates to activate the innate and adaptive immune system in the mother (45-47). Importantly, the source, and thereby packaging, of the micro-particles prepared from *in vitro* analyses determine the affect these membranes have on T lymphocyte activation, as perfusion derived particles activate T cells while villous explant and mechanically prepared membranes do not (48). Moreover, membrane fragments from trophoblasts cause endothelial cell dysfunction by suppression of cell proliferation in both cultured endothelial cells and in isolated vessels (49-51). Necrotic and apoptotic trophoblasts undergo phagocytosis by endothelial cells *in vitro* but phagocytosis of only necrotic trophoblasts induces enhanced expression of endothelial cell intercellular adhesion molecule 1 (ICAM-1) and facilitates monocyte adhesion to endothelial cells (52). These studies support the notion that the pathway to trophoblast cell death may influence the response of the maternal endothelium to higher than normal levels of trophoblast components. Indeed, hypoxia exposure of villous explants yields predominantly necrotic cell death while re-perfusion injury causes trophoblast apoptosis (26)

. This raises the possibility that re-perfusion injury *in vivo* may provide different levels and different sub-cellular compositions of trophoblast debris in the systemic circulation, compared to the trophoblast shedding in response to chronic placental hypoxia. Notably, placental dysfunction in other pregnancy complications shares some, but not all, of the features of preeclampsia. For example, cell free fetal nucleic acids are shed into the maternal circulation in higher than normal amounts in preterm labor, although fetal-maternal cell trafficking is not increased in this condition (53, 54). Despite sharing many of the histopathological placental features with preeclampsia, there is no increase in level of STBM in pregnancies complicated by intrauterine growth restriction without preeclampsia (51). These observations indicate that release of cellular components of syncytiotrophoblasts is not unique to preeclampsia and does not always correlate with the placental pathology.

5.4. Enhanced placental cell trafficking in preeclampsia

Trophoblasts and other fetal derived cells are normally shed into the maternal circulation in human pregnancies (17, 38, 55), with as many as 150,000 trophoblast fragments deported into the maternal circulation each day (56). The villous trophoblasts are a prime source for this fetal cell trafficking, but there are several other sources of fetal derived cells from the placenta. Ultrastructural evidence shows that there are breaks in both the fetal endothelium and syncytiotrophoblast barriers in placentas of preeclamptic women, and this injury correlates with development of a pathway for blood cells to pass outside the injured fetal vessels to gain access to the maternal circulation (57). Thus, a second source of fetal cells may be from loss of integrity of the villous structure.

Another non-villous alternative source of trophoblasts and debris in the maternal circulation is from the endovascular trophoblasts in the basal plate vessels. Apoptosis has been shown to occur in trophoblasts in the basal plate in early normal pregnancies, demonstrated by Fas-Fas ligand interaction during implantation (58). This ligand-receptor interaction is one of the pathways that invoke apoptosis, and the interaction is speculated to be responsible for the trophoblast invasion and transformation of the spiral arterioles (58). In later gestation, superficial vessel invasion and enhanced trophoblast apoptosis is apparent in the trophoblasts associated with the basal plate vessels in preeclampsia but not in normal pregnancy (59, 60). Conceivably, trophoblasts undergoing apoptosis in this vasculature may also gain entry into the maternal circulation. Importantly, women with preeclampsia have higher numbers of trophoblasts in the uterine vein effluent compared to normal, non-hypertensive pregnancy (37). Apoptotic fetal derived cells are even present in peripheral blood plasma of normal pregnant women (61). Most deported trophoblast cells likely derive from the process of trophoblast turnover that normally occurs on villi by apoptosis, especially at syncytial knots where clusters of nuclei with condensed chromatin reside (62, 63). The above studies clearly indicate that trafficking of trophoblast cells and fragments of syncytiotrophoblast occur with increased

frequency in preeclampsia. We therefore now focus on insults that contribute to the dysregulation of trophoblast cell death in response to injury and in preeclampsia. The steps involved in trophoblast apoptosis have been recently reviewed (60).

5.5. Insults that mediate or synergize with hypoxia to injure the placenta in preeclampsia

Although generalized hypoxia is a prime culprit to injure the placenta, there are other exogenous stimuli that are candidates to influence placental function. Local disruption of oxygen delivery to isolated villous trees, ischemia with re-perfusion of all or a segment of the placenta, or a response to toxic agents in maternal blood, such as autoantibodies, lipid peroxides, thromboxane, TNF- α , or higher than normal levels of homocysteine are all potential insults to the placenta. These stimuli may directly injure villi or indirectly affect the local expression of cytokines and growth factors to thereby adversely affect the placental response to the injurious agent. For example, thromboxane affects vessels by inducing vasoconstriction and platelet aggregation, findings typical of preeclampsia. This mediator is found in noticeably higher concentrations in women with preeclampsia compared to controls (32, 64-67). Moreover, cultured trophoblasts up regulate cyclooxygenase 2 to produce more thromboxane than controls in response to hypoxia (67, 68), and placentas of preeclamptic women produce not only more thromboxane than controls, but also more lipid peroxides (69, 70), reflecting enhanced oxidative stress. Consistent with the higher level of fibrin deposition in the placental histopathology in preeclampsia, cultured trophoblasts secrete increased amounts of thromboxane when exposed to a fibrin matrix (71). Thromboxane *in vitro* limits differentiation and induces apoptosis in term trophoblasts exposed to standard culture conditions (30). Notably, hypoxia does not uniformly enhance secretion of prostanoids from term trophoblasts, as prostaglandin E₂ release is not affected by low oxygen exposure (72). These results suggest that hypoxia induced changes in trophoblast secretion may yield a stimulus, such as increased thromboxane production, that limits the ability of villi to compensate for injury, by altering trophoblast turnover and limiting the differentiation of syncytiotrophoblasts during repair.

Another potential toxic agent in maternal blood independent of hypoxia is the autoantibody to the angiotensin II receptor, angiotensin receptor 1, as recently reviewed (73). This autoantibody is prevalent in women with preeclampsia but not in healthy controls or in women with chronic hypertension (74). The autoantibody interaction with the ligand receptor causes dysregulated G-protein signaling in cells expressing the receptor. Trophoblasts express angiotensin receptors and receptor stimulation by the autoantibody *in vitro* reduces trophoblast invasiveness (75), and activates transcription factors while generating reactive oxygen species (76). A role for the autoantibody in activating the NAD(P)H oxidases in the placenta has been suggested as one route for generation of injurious reactive oxygen species produced at higher levels in the placenta of preeclamptic women (77, 78). The

expression of a novel isoform of NADPH oxidase in villi of preeclamptic pregnancy has recently been identified to contribute to the increased oxidative stress found in these placentas (79). The specific role for the circulating autoantibody in preeclampsia awaits further studies, but the principle illustrated is that a "toxin" in maternal blood can adversely affect trophoblast function.

5.6. Hypoxia effects on trophoblast differentiation

What other responses does trophoblast exhibit to hypoxia? Third trimester villi exposed to hypoxia from residence at high altitude *in vivo* exhibit enhanced cytotrophoblast proliferation and reduced fusion to form syncytiotrophoblasts (23, 80). Moreover, low oxygen tensions limit differentiation of cultured trophoblasts from uncomplicated pregnancies, as reflected by reduced formation of differentiated syncytiotrophoblasts and lower secretion of human chorionic gonadotropin and human placental lactogen compared to trophoblasts exposed to standard conditions; (68, 81-83). Syncytin is a retroviral gene product expressed in villous trophoblasts that reflects differentiation as this protein is partly responsible for fusion of cytotrophoblasts with syncytiotrophoblasts (84, 85). Importantly, placental villi from preeclamptic pregnancies exhibit reduced expression of syncytin (86, 87) and *in vitro* hypoxia decreases syncytin gene expression (88-90). Collectively, these studies show that hypoxia hinders villous trophoblast differentiation and thereby, may contribute to the altered cytodifferentiation apparent in the trophoblast of villi from preeclampsia.

HIF-1 α is another potential agent that may play a role in the pathogenesis of preeclampsia. HIF-1 α is a known transcriptional factor activated by hypoxia in many tissues to adapt the cell to a lower oxygen level by decreasing metabolic activity and therefore, oxygen consumption. Expression of HIF-1 α is higher in villi in the first trimester ambient oxygen tension is low as described above. HIF 1 α expression diminishes in villi as the products of conception are exposed to oxygen tensions with the maternal blood perfusing the intervillous space. HIF-1 α affects the downstream regulation of several genes including the glucose transporter glut 1 (91) and TGF β 3, whose expression in the placenta is known to inhibit differentiation. Studies of placentas of pregnancies from high altitude residence demonstrate a reduction in the expression of the GLUT 1 glucose transporter on the trophoblast basal plasma membrane, and this may limit glucose transfer to yield smaller birthweight in preeclampsia (92). Expression of both HIF-1 α and TGF β 3 are higher than normal in preeclamptic placenta (93); (94); (95), and the biological effects of these mediators may limit differentiation of trophoblast on villi in the second half of pregnancy. Collectively, these observations suggest that up regulation of HIF-1 α in trophoblast exposed to hypoxia in preeclamptic pregnancy may adversely alter multiple placental functions.

Multiple cytokines are secreted differentially by placental villi from preeclamptic compared to normotensive pregnancies (96), and this secretion is altered in cultured

trophoblasts exposed to hypoxia. For example, hypoxia reduces IL-10 secretion by trophoblasts from preeclampsia, but hypoxia increases IL-6 and IL-8 secretion (97). Moreover, hypoxia induces cultured villi to secrete higher than control levels of TNF alpha, IL-1 alpha, and IL-1 beta (98). TNF alpha was one of the first cytokines identified to induce trophoblast apoptosis *in vitro*, and higher levels of this cytokine secreted in response to villous hypoxia may contribute to the enhanced apoptotic turnover of trophoblasts in preeclampsia as described below (29). Taken together, these studies suggest that the villous trophoblast response to hypoxia yields a cytokine secretion profile that is pro-inflammatory.

6. CAN DYSREGULATION OF TROPHOBLAST BE A MEDIATOR IN THE PATHOPHYSIOLOGY OF PREECLAMPSIA?

6.1. Villous trophoblast continuity with the maternal circulation

A key question is how might trophoblast have an affect on the systemic vasculature and the maternal inflammatory response to yield the pathophysiology characteristic of preeclampsia? There are two primary avenues to influence the maternal response: altered trophoblast secretion and increased trafficking of trophoblast components. Because the villous trophoblast bilayer lines the intervillous space, this epithelium is a specialized endothelium in direct continuity with the maternal systemic endovascular space. Secretory products such as those above from trophoblast, or other villous components, have direct access to the maternal blood stream. Altered secretion of vasoactive compounds, cytokines, and growth factors can thereby affect the endothelium, the coagulation cascade, and the inflammatory response of the mother. Similarly, abnormal turnover of the trophoblast layer can increase trophoblast cells and debris in the systemic circulation.

6.2. VEGF, PLGF, and sFLT

As introduced above, there are many secretory products of the trophoblast that are candidates to play a role in the pathophysiology of preeclampsia. Not yet discussed but high on the list are the angiogenic factors vascular endothelial growth factor (VEGF), and placental growth factor (PLGF), along with one of their receptors, the soluble fms-like tyrosine kinase (sFLT). The latter is an alternative splice variant of the *flt* gene surface membrane receptor that when injected into mice, induces hypertension and proteinuria simulating the clinical phenotype characteristic in humans (99). Women who develop preeclampsia commonly have a markedly higher level of circulating sFLT weeks prior to the onset of clinical signs of preeclampsia (99). Notably, sFLT is secreted in higher concentrations by trophoblasts exposed to hypoxic conditions, compared to standard culture conditions (100). Hypoxia *in vitro* also enhances trophoblast secretion of VEGF but suppresses PLGF secretion (100-103). Since VEGF facilitates syncytiotrophoblast differentiation, while PLGF has no effect, the trophoblast response to hypoxia may be one way that villi compensate for hypoxic injury (104). Collectively, the alterations in activity of the above

growth factors may play a role in the pathogenesis of preeclampsia by providing an antiangiogenic environment and dysregulated placental function during the weeks prior to manifestation of the syndrome.

6.3. Endothelin and eNOS

Endothelin 1 is another secretory product of villous trophoblast that is a potent vasoconstrictor proposed to play a role in the regulation of vascular tone in preeclampsia (105). Expression of this peptide is higher than normal in placental tissue from preeclamptic women (106) and secretion of endothelin 1 is notably higher in cultured trophoblasts derived from preeclampsia compared to normotensive controls (107). Moreover, endothelin 1 is attributed a role in the higher than normal oxidative stress associated with preeclampsia, by downregulation of antioxidants and enhanced production of oxidative stress molecules (105). In animal models, endothelin 1 mimics some of the effects seen in preeclampsia, including hypertension and proteinuria (108). Interaction of endothelin-1 with nitric oxide, another key vasoactive agent produced in the placenta, has been proposed to regulate vascular tone in the placenta (109, 110). Interestingly, hypoxia down regulates the expression of eNOS protein *in vitro* (111), and reduced L-arginine and eNOS expression constitute characteristic features of the placenta in preeclampsia (112). If hypoxia down regulates eNOS expression in trophoblast *in vivo*, this may adversely influence the balance of endothelin 1 and nitric oxide that regulates villous perfusion in later gestations.

7. SUMMARY AND PERSPECTIVE

The above discussion highlights the clinical variability of the end organ damage that is characteristic of preeclampsia. The mechanisms underlying the pathophysiology of this syndrome still remain largely unknown, but a mounting body of evidence points to dysfunction of the placenta in general, and of trophoblast in particular, as a major contributor to the pathophysiology of the maternal systemic disease. A predisposition to placental under-perfusion may derive from the effects of genetic constitution, implantation abnormalities, or co-existing maternal diseases that all can yield abnormal development of the maternal blood supply in early placental development. The differentiation and turnover of trophoblasts on villi plays a central role in growth and development of the fetus in normal pregnancy as the syncytiotrophoblast interfaces maternal-fetal exchange. The response of the chorioallantoic villous tree in later pregnancy to multiple and varied insults, including under-perfusion with chronic hypoxia, re-perfusion injury, or toxic mediators in the maternal circulation, determines the severity of the placental dysfunction and ultimately, the predisposition to systemic maternal disease. The trophoblast specific responses to exogenous insults influence the secretion of a wide variety of cytokines, growth factors, and hormones, and increase the maternal systemic burden of trophoblast debris. Do these events trigger the vasospasm, endothelial damage, and inflammatory reaction typical of the preeclamptic syndrome? The answer is that most likely some of the

responses derive from this dysregulation of trophoblast function, but it seems unlikely that trophoblast dysfunction is responsible for all of the varied pathophysiology associated with preeclampsia. What becomes clear is that new insights into the pathophysiology of preeclampsia, among other pregnancy disorders, will be derived from further study of the normal biology of villous trophoblasts and their response to exogenous stimuli.

8. ACKNOWLEDGEMENT

This work was supported by a grant from the NIH (HD29190).

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Key Words: Trophoblast, Hypoxia, Placenta, Preeclampsia, Review

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