

Endothelial barrier function in preeclampsia

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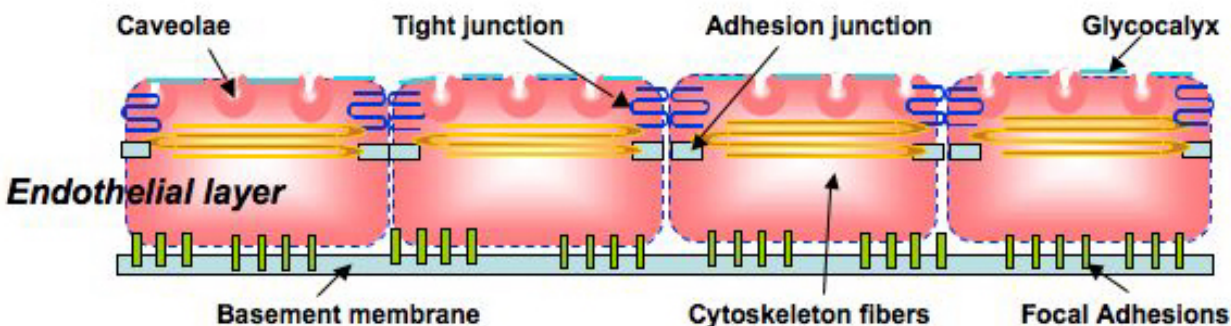
1. ABSTRACT

Impaired endothelial barrier function resulting in increased vascular permeability is a characteristic vascular response in preeclampsia, a hypertensive and multiple systemic disorder of human pregnancy. During the last two decades, endothelial function in preeclampsia has been intensively studied and significant progress has been made in understanding the cellular and molecular bases of the altered endothelial cell response. In this review, we address the nature and mechanisms that are proposed to underlie the disturbed endothelial barrier function in preeclampsia and discuss the potential relevance of the endothelial cell responses to the initiation and/or progression of this vascular syndrome. Insights gained from the characterization of the endothelial cell phenotype assumed by the preeclamptic microvasculature may lead to novel therapeutic strategies for the management of this syndrome.

2. INTRODUCTION

Increased vascular permeability and vasoconstriction are characteristic features of the endothelial dysfunction that accompanies preeclampsia. Increased vascular permeability in the kidneys results in proteinuria, while interstitial edema is the result of the impaired endothelial barrier function in the systemic circulation. These consequences of preeclampsia are highly correlated with disease severity, as reflected by the quantity of urinary proteins and the intensity of interstitial edema. Proteinuria and edema with expanded extracellular volume are clinical indicators of disease severity and progression, while resolved proteinuria and edema after parturition are positive clinical signs of disease resolution. Functional integrity of vascular endothelium is a requirement for proper control of transcapillary fluid and solute exchange, and for maintenance of vasomotor tone during a normal

Intravascular luminal surface



Extravascular compartment

Figure 1. Key endothelial structures and proteins that maintain endothelial integrity and barrier function ensuring that the intravascular solutes do not leak into the extravascular compartment. Tight junction proteins and adhesion junction proteins seal the intercellular junction between endothelial cells. Both tight junction and adhesion junction proteins bind to membrane proteins to form junctional complex and interact with actin cytoskeleton to increase the strength of cell-cell adhesion. Focal adhesions of endothelial cells with the underlying matrix provide an important structural basis for normal endothelial function and barrier properties. Caveolae, as vesicle carriers responsible for transcellular transport of albumin and other plasma proteins across endothelium, may help to maintain the transcapillary protein oncotic pressure gradient, which is critical for normal fluid balance homeostasis. The glycocalyx is a negatively charged surface coat of proteoglycans and absorbed plasma proteins lining the luminal surface of vascular endothelium. The net negative charge of the glycocalyx repels red blood cells and protects the vascular endothelium from platelet and leukocyte adhesion.

pregnancy. Understanding how and why endothelial cells become dysfunctional during preeclampsia is essential for the development of effective therapeutic strategies for this serious and prevalent disorder of human pregnancy. Figure 1 illustrates key endothelial structures and molecules that maintain endothelial integrity and barrier function ensuring that the intravascular solutes do not leak into the extravascular compartment. Figure 2 presents potential stressors in the maternal circulation that disturb endothelial barrier function and increase endothelial permeability in preeclampsia which are discussed in this review.

3. INCREASED VASCULAR PERMEABILITY IN PREECLAMPSIA

3.1. Endothelial function during pregnancy

The vascular endothelium is a functionally complex tissue that forms an active boundary between the bloodstream and underlying tissues. The endothelial cell is an exquisite “sensor” that responds to diverse signals present in the blood circulation, generated by the sub-endothelial matrix, and produced as a result of their interaction with circulating blood cells (1). The integrity of the vascular endothelium is an essential requirement for controlling vascular permeability and for preventing the vessel wall from platelet deposition and thrombus formation. Vascular endothelium plays an important role in the regulation of vascular tone, coagulation and fibrinolysis, and inflammatory responses to internal and external stimuli. In normal pregnancy, physiological adaptations in the maternal vascular system include increased cardiac output and circulating blood volume, and decreased vascular resistance, all of which serve to provide

adequate placental perfusion for fetal development. Plasma volume increases approximately 40-45% during pregnancy, compared to the non-pregnant state. With this remarkable increase in blood volume, maintenance of normal endothelial integrity is necessary to ensure that the increased intravascular volume does not leak into the extravascular compartment.

3.2. Imbalance of plasma and interstitial colloid osmotic pressure and capillary hydrostatic pressure in preeclampsia

It is well known that extracellular fluid volume is markedly expanded in most women with severe preeclampsia, in which vascular endothelial damage with subsequent capillary leakage into the extra-cellular space plays a major role. Qian and his colleagues studied fluid transport between the plasma and interstitial fluid compartment in normal and preeclamptic pregnancies (2, 3). They collected interstitial fluid from subcutaneous tissue by implanted wicks and then determined interstitial fluid colloid osmotic pressure and hydrostatic pressure. In their study, capillary hydrostatic pressure was calculated in 10 normal pregnant women during the first trimester and the third trimester, in 15 patients with “mild” preeclampsia, and in 13 women with “severe” preeclampsia. Their results showed that in normal pregnancies, capillary hydrostatic pressure measured on thorax was 8.3 ± 1.9 mmHg (mean \pm SD) in the first trimester and 11.5 ± 2.3 mmHg in the third trimester, which increased by about 30% between the first and third trimesters. The capillary hydrostatic pressure values were not significantly different between those in the third trimester of normal pregnancy and in mild preeclampsia. However, in severe preeclampsia capillary

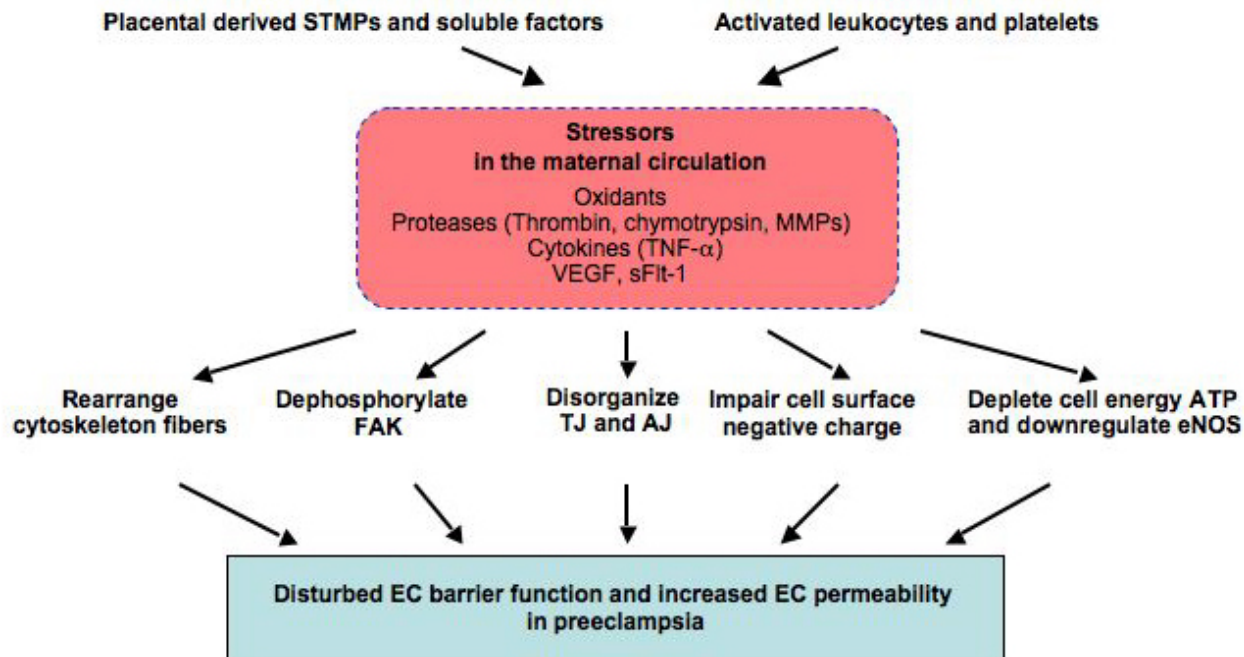


Figure 2. Proposed mechanisms of stressors in the maternal circulation that disturb endothelial barrier function and increase endothelial permeability in preeclampsia. Proposed stressors (toxic agents) in the maternal circulation may derive from placental trophoblasts (syncytiotrophoblast microvillus membrane particles (STMPs)), and activated leukocytes and platelets, which include oxidants (reactive oxygen species), proteases (thrombin, chymotrypsin, and MMPs), cytokines, angiogenic factor VEGF and its antagonist sFlt-1, etc. These stressors are able to affect on endothelial cells in multiple ways by disorganization of junctional proteins (tight junction – TJ; adhesion junction – AJ), rearrangement of cytoskeleton fibers, dephosphorylation of focal adhesion kinase (FAK), impairment of cell surface negative charge glycocalyx, depletion of cell energy ATP and downregulation of eNOS.

hydrostatic pressure was significantly lower (40%) than that in mild preeclampsia (6.7 ± 1.8 mmHg vs. 12.2 ± 1.6 mmHg, respectively) (3). They further noticed that plasma colloid osmotic pressure is reduced in severe preeclampsia. In contrast, interstitial colloid osmotic pressure is increased in severe preeclampsia compared to that in mild preeclampsia (2). These data indicate that increased microvascular permeability may lead to increased pooling of plasma proteins into the subcutaneous interstitium, which contributes to the reduction of plasma protein concentration and decreased plasma colloid osmotic pressure in preeclampsia.

3.3. Evidence of increased vascular permeability during preeclampsia

Evan's blue is a low molecular weight dye that binds to plasma protein to form Evan's blue-plasma protein complex. Normally, the bound Evan's blue dye retain within circulation. However, when increased vascular permeability occurs the Evan's blue-plasma complex extravasate rapidly, generating a clearly defined blue spot in the hyper-permeabilized area of the vasculature (4). Schwartz and colleagues established a methodology to monitor transendothelial passage of Evan's blue dye (5, 6). When dye was injected intravenously, a characteristic and consistent pattern of dye uptake by vascular endothelium was obtained. They showed that the areas of increased vascular permeability with dye uptake might represent

areas of spontaneous injury (5). Using albumin-bound Evan's blue dye to study endothelial barrier function and vessel injury *in vivo*, Brown *et al.* observed that there was a higher rate of disappearance of the albumin-bound Evan's blue dye from the intravascular space of in women complicated by preeclampsia [-0.472 (slope $\times 10^{-3}$)] compared to women with normal pregnancies [-0.183 (slope $\times 10^{-3}$)] (7). Their data revealed a more rapid Evan's blue disappearance rate and a lower plasma volume in preeclamptic than in normal pregnant women, suggesting that increased microvascular permeability in the systemic circulation account for the interstitial edema in preeclampsia. Campbell and Campbell reported similar observations from their study (8) and a recent *ex vivo* study by Svedas *et al.* also revealed enhanced Evan's blue dye staining in subcutaneous arteries from women with preeclampsia, compared to those from normal pregnancies (9).

Electron microscopic studies by Svedas *et al.* provided convincing evidence that the increased vascular permeability in preeclampsia may be directly related to morphological changes in the endothelial barrier (9, 10). In their study, maternal myometrial and subcutaneous vessel samples from normal pregnancies and from pregnancies complicated by preeclampsia were examined by scanning electron microscopy. Their results showed an augmentation of intercellular gaps in the endothelial lining in arterial

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samples from women with preeclampsia, which was correlated with enhanced Evan's blue dye staining of the same vessel samples (9). In a separate study, the same authors also noted significant differences in endothelial morphology between arteries from normal pregnant women and those from women with preeclampsia (10). In normal myometrial arteries, a continuous sheath of elongated endothelial cells with thick intact membranes covered the luminal surface of the intima with tightly connected endothelial cells. However, in vessel samples from preeclampsia, numerous morphological changes were observed: 1) shrunken endothelial cells with blebbing and thin plasma membranes detaching from the basal lamina; 2) platelets and non-specific protein aggregates adhering on the endothelial surface; and 3) endothelial cell layers with disrupted intercellular junctions and enlarged intercellular spaces. These morphological findings strongly support the idea that the enhanced vascular permeability in preeclampsia results from enlarged intercellular junctions. These observations are also consistent with activation of the coagulation system in preeclampsia as evidence by platelets adhering on the endothelial surface of vessel samples from preeclampsia (10).

4. HUVEC as a model to study endothelial barrier function *in vitro*

4.1. Electron microscopic findings

An electron microscopy study of umbilical arteries of babies born to preeclamptic mothers by Dadak *et al.* showed dilatation of the endoplasmic reticulum, increased cell organelles and loss of intercellular junctions (11). Sulbaran *et al.*'s work also revealed comparable cellular and structural changes in endothelium of umbilical cord vessels from mothers with preeclampsia. They observed a marked enlargement of the subendothelial space with distended extracellular spaces (12). Their findings demonstrated that disruption of and injury to the endothelial layer also occur in umbilical vessels in babies born to mothers with preeclampsia. The similar morphological changes (enlarged and distended intercellular space) in endothelial cells of umbilical cord vessels with systemic maternal vessels demonstrated by electron microscopy, and their ease of collection of human umbilical cord vein endothelial cells (HUVECs) and maintenance in culture, have provided a strong rationale for using HUVECs as a model for studies of endothelial barrier function in preeclampsia.

4.2. Altered endothelial junction distribution and expression of VE-cadherin and occludin: molecular basis of the altered endothelial barrier function in preeclampsia

Although HUVECs are endothelial cells derived from the fetal source, these cells, like their maternal counterparts, are also exposed to circulating substances that pass into fetal blood from the maternal circulation. Evidence from HUVECs derived from preeclamptic patients manifest both the morphological and functional changes that occur in the maternal circulation, supporting the view that both fetal and maternal endothelial cells assume a similar altered phenotype during preeclampsia (7, 9, 13-17).

We have used HUVECs as a model to study the changes and underlying mechanisms of endothelial barrier function in preeclampsia. Using measurement of horseradish peroxidase (HRP) leakage through HUVEC monolayers grown on inserts, we have demonstrated that HRP leakage is significantly increased in HUVECs derived from preeclamptic mothers, compared to HUVECs from uncomplicated pregnancies (14). We also examined the expression and distribution of the endothelial junction protein, Vascular endothelial cadherin (VE-cadherin), and the tight junction protein, occludin. We found that HUVECs from pregnancies complicated by preeclampsia exhibit multiple morphological changes (when primary isolated and/or first passage cells were compared to their counterparts from uncomplicated pregnancies) including irregular cell shape, disorganized VE-cadherin and occludin distribution at cell junctions, contracted junctional fibers, and the presence of intercellular gaps/pores at cell contact regions (14). We also noted the disappearance of the reduced and disorganized expression of VE-cadherin and occludin that were observed in the primary isolated preeclamptic HUVEC cells when the passage 2 and passage 3 cells were studied. These observations suggest that cultured primary isolates and early passage HUVECs maintain the phenotypic changes that are manifested *in vivo* during preeclampsia (14). These findings also indicate that the isolation and culture procedures do not appear to immediately eliminate functional differences in cells from uncomplicated and preeclamptic pregnant women at least in the primary isolated and first passage cells. The fact that these cellular alterations are only present among the early passage cells also supports the concept that endothelial junction disorganization is a central cellular and molecular basis for the altered endothelial barrier function and increased vascular permeability in preeclampsia. Furthermore, the restoration of a normal cell junction pattern in late-passaged HUVECs from women with preeclampsia is also in line with clinical observations, i.e., the abnormal clinical signs in the maternal vascular system (increased blood pressure, proteinuria, and edema) observed before delivery, are restored after parturition in women with preeclampsia (14, 18).

Both adhesion and tight junctions are important cellular structures that regulate the barrier function of endothelial cells. VE-cadherin is selectively expressed in endothelial cells of all types of vessels and is the major structural protein that mediates Ca^{++} -dependent homophilic binding and adhesion of adjacent cells. It also acts as both a receptor and ligand. The VE-cadherin cytoplasmic tail binds to membrane proteins (β -catenin, p120-catenin, or plakoglobin) and forms a junctional complex with actin cytoskeleton to increase the strength of cell-cell adhesion. Therefore, it is believed that VE-cadherin is an important regulator of endothelial barrier "sealing" and is required for the maintenance of normal barrier function and antithrombotic properties (19-21). The tight junction is a transmembrane structure, which forms a continuous impermeable barrier between adjacent cells that regulate the flux of molecules through the paracellular space. Occludin is the first identified tight junction protein and has been demonstrated to be a critical structural and functional

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component of the tight junction (22-25). Occludin distribution at cell junctions is also an indicator of endothelial integrity. Lack of VE-cadherin and occludin expression at cell contact regions and disruption of VE-cadherin and/or tight junction complexes have been used as an indicator of disturbed endothelial barrier function. Several excellent reviews have intensively analyzed the cellular and molecular structure and functions of VE-cadherin and occludin as guardians of endothelial barrier function (1, 26-29). Because VE-cadherin and occludin are required for proper assembly of endothelial adhesion and/or tight junctions, our findings of altered distribution and expression of VE-cadherin and occludin at cell contacts in preeclamptic HUVECs provide an important cellular and molecular basis for altered endothelial barrier function in this pregnancy disorder.

5. ENDOTHELIAL COMPONENTS THAT MAY CONTRIBUTE TO THE DISRUPTED ENDOTHELIAL BARRIER FUNCTION IN PREECLAMPSIA

5.1. Caveolae and endothelial NO synthase (eNOS)

Caveolae are a subset of lipid rafts that are prevalent on the plasma membrane of endothelial cells. They make up approximately 15% of the total endothelial cell volume (approximately 10,000 – 30,000 caveolae/cell) (1). Caveolae are invaginations in the plasma membrane, which compartmentalize many signal transduction systems such as the production of nitric oxide (NO) by the caveolae resident enzyme endothelial NO synthase (eNOS). Several recent reviews address the importance of caveolae as vesicle carriers responsible for transcellular transport of albumin in endothelial cells (1, 30). Caveolae, by transporting albumin and other plasma proteins across endothelium, may help to maintain the transcapillary protein oncotic pressure gradient, which is critical for normal fluid balance homeostasis (1). Interstitial edema and reduced circulating albumin levels are characteristic features of the maternal syndrome in preeclampsia. Although the renal glomerular injury that results in proteinuria is considered the main reason for albumin loss and low circulating albumin levels during preeclampsia, the possibility of endothelial damage related to a disturbed transcapillary oncotic pressure gradient across capillaries as a result of altered caveolae function cannot be excluded.

Although there is no information available about caveolae function related to the altered vascular permeability in preeclampsia, we have observed a reduction of eNOS expression in HUVECs from preeclamptic compared to those from normal pregnancies (31). Although baseline monolayer permeability levels are not dependent on eNOS (32), inhibition of eNOS by N^G monomethyl-L-arginine (L-NMMA), a non-selective eNOS inhibitor, does result in increased monolayer permeability, suggesting that decreased eNOS activity or reduced NO availability could contribute to the altered endothelial barrier function in preeclampsia (31). Whether decreased eNOS activity is associated with altered caveolae function in the plasma membrane of endothelial cells in preeclampsia warrants further attention.

5.2. Focal adhesion kinase (FAK)

Focal adhesion of endothelial cells with the underlying matrix provides an important structural basis for normal endothelial function and barrier properties. Focal adhesion assembly and activation serve as a vital signaling event in the modulation of vascular permeability under various physiological and pathophysiological conditions. FAK is an important tyrosine kinase that contributes to the regulation of the cell-matrix interactions. Several groups of investigators suggest that activation of FAK enhances endothelial barrier function. For example, phosphorylation of FAK397, which is coupled to the assembly of focal adhesion, is responsible for maintaining integrity and barrier function in endothelial cells (33). Upon activation, FAK autophosphorylates tyrosine 397 and a number of downstream events ensue, leading to focal adhesion assembly or reorganization. These events include integration of scaffold or signaling molecules, stabilizing cells against lateral contractile force, and providing anchorage support for endothelial cells binding to matrix proteins (34). Furthermore, spatial and temporal changes in FAK activation and cell morphology are also closely correlated with changes in barrier function (34). It was found that inhibition of FAK tyrosine phosphorylation prevents the formation of focal adhesion (35) and association with cell contraction, whereas tyrosine phosphatase inhibitors promote the formation of focal adhesion (36). This is supported by a reciprocal relationship between FAK activity and monolayer permeability (37, 38). Recent studies have also demonstrated that site-specific tyrosine phosphorylation exerts differential effects on endothelial monolayer permeability. For example, both the endothelial-protective molecule sphingosine-1 phosphate (S1P) and endothelial – disruptive agent thrombin could induce FAK576 phosphorylation in cultured human pulmonary artery endothelial cells (39).

Endothelial cells experience an oxidative stress during preeclampsia and it has been proposed that oxidative stress is a major underlying factor in the pathogenesis of preeclampsia (40). We recently reported increased superoxide generation in HUVECs derived from preeclampsia, providing direct evidence for increased oxidative stress in fetal endothelial cells in preeclampsia (41). Although direct evidence of increased oxidative stress in the maternal vasculature is still scarce, enhanced nitrotyrosine staining in subcutaneous vascular tissue from women with preeclampsia has been reported (42), suggesting that vascular system experiences a nitrosative stress in preeclampsia. Nitrotyrosine is formed most commonly when tyrosine in proteins is modified by peroxynitrite, which are reactive oxygen and nitrogen species. We have reported that the disruption of VE-cadherin and occludin at inter-endothelial junctions induced by peroxynitrite is accompanied by the dephosphorylation of FAK397 (43), which links oxidative stress to FAK involvement in the regulation of endothelial barrier function.

5.3. Adenosine triphosphate (ATP)

The endothelium is a local source of adenosine triphosphate (ATP) within vascular beds as evidenced by

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the basal release of ATP across the apical membrane of endothelial cells. Several lines of evidence suggest that ATP and other purines may exert physiologically relevant barrier-protective effects by enhancing the restrictive properties of the barrier through remodeling of cell-cell junctions (44-47). In *in vitro* experiments on cultured cells, Kolosova *et al.* found that ATP and ADP increased transendothelial electrical resistance in a concentration-dependent manner (45). ATP also increased cell-cell interface surface area as measured by VE-cadherin-labeled cell-cell junctions. A more regular and continuous tight junction protein ZO-1 distribution was observed after ATP treatment (45). In contrast, energy depletion may increase endothelial monolayer permeability and intercellular gap formation. Energy depletion of endothelial cells leads to cell-cell separation and enhanced macromolecule permeability of the endothelial cell barrier due to rapid, but reversible disintegration and destruction of F-actin cytoskeletal structures (48, 49). Endothelial dysfunction stemming from structural/phenotypic changes is a common theme in preeclampsia. Although evidence of endothelial cell energy depletion during preeclampsia is lacking, ATP depletion may well be a consequence of endothelial activation/dysfunction that accompanies preeclampsia. Lack of energy generation in dysfunctional endothelial cells may lead to disintegration of junctional and cytoskeletal organization in endothelial cells and result in increased vascular permeability in preeclampsia.

5.4. Glycocalyx

The glycocalyx is a negative charged surface coating proteoglycan and absorbed plasma proteins lining the luminal surface of vascular endothelium. The net negative charge of the glycocalyx repels red blood cells and protects the vascular endothelium from spontaneous leukocyte adhesion. Proteins of the glycocalyx can be shed from endothelial cells in response to formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation (50). It is believed that the glycocalyx serves as a barrier to adhesion and that its shedding during natural activation of endothelial cells may be an essential part of the inflammatory response. Studies have shown that neutralizing of the apical negative charge on endothelium using cationic ferritin increases the transendothelial permeability of albumin, suggesting that glycocalyx plays a unique role in maintaining endothelial barrier function by charge-selective exclusion of plasma proteins (1). Jeansson and Haraldsson studied the endothelial cell glycocalyx and glomerular barrier function in a C57BL/6 mice model (51). They found that decreasing the thickness of the glomerular endothelial cell glycocalyx increased the fractional clearance of albumin, demonstrating that morphological alterations in the endothelial cell glycocalyx have functional consequences for glomerular permeability (51).

Renal glomerular injury is a unique end-organ injury response to preeclampsia. Positive proteinuria is the key diagnostic criterion for preeclampsia, and the quantity of protein in urine is one indicator of syndrome severity. Although the glomerular podocyte is considered to play a major role in maintaining an intact glomerular barrier, the diminished glomerular endothelial cell glycocalyx and the

resultant change in charge-selective properties of the glomerular barrier may make an equally important contribution to the proteinuria in preeclampsia. Although it remains unclear whether the glycocalyx on glomerular endothelial cells is altered in preeclampsia, an analysis of the glomerular glycocalyx would provide valuable insight into the role of charge-selective filtration in the kidney injury and the mechanisms of the negative charged surface coating proteoglycan in modulating proteinuria associated with preeclampsia.

6. CIRCULATING MEDIATORS OF ENDOTHELIAL BARRIER DYSFUNCTION IN PREECLAMPSIA.

6.1. Oxidants

Oxidative stress is suspected to be an important factor that contributes to the maternal and placental vascular dysfunction in preeclampsia (40, 52, 53). Both *in vivo* models and *in vitro* cell culture systems have revealed a role for reactive oxygen species (ROS) as important mediators of the increased vascular permeability that accompanies a variety of pathological conditions (54-57). Oxidant species such as lipid peroxides, nitrosothiols, S-nitrosoalbumin, and antibodies to oxidized low-density lipoprotein (LDL) are higher (18, 58-60), whereas antioxidant agents including α -tocopherol levels, superoxide dismutase activity, and antioxidant capacity are lower (58, 61) in the maternal circulation in women with preeclampsia. Although the exact causes of endothelial barrier dysfunction in preeclampsia are yet to be determined, increased oxidative stress in the environment of the maternal circulation, and intrinsic endothelial responses to these circulating oxidants and oxidant-dependent processes that disturb endothelial barrier function are perhaps the most likely events to provoke the many differing stresses in preeclampsia. One potential source of ROS in the maternal circulation is activated leukocytes. Enhanced leukocyte activation occurs during preeclampsia. Activated leukocytes not only generate more ROS but also release proteases such as elastase and matrix metalloproteinases (MMPs). Both ROS and proteases act on endothelial cells to increase vascular permeability (see below). This exogenous source of ROS, coupled to an increased flux of ROS generated by endothelial cells *per se*, are likely to modify endothelial barrier structure and function, providing a basis for the vascular permeability responses in preeclampsia.

6.2. Proteases

Evidence has shown that the proteolytic properties of proteases can both directly and indirectly affect the endothelial barrier. Protease levels and activities in maternal blood of women with preeclampsia are elevated relative to normal pregnancies (16, 62-64). These proteases include thrombin, chymotrypsin (chymase), MMPs, etc. Proteases such as thrombin can increase endothelial monolayer several fold through a variety of mechanisms. First, the proteolytic activity can directly disrupt the homophilic binding of the adhesion junction protein VE-cadherin as well as the tight junction protein occludin. Second, these proteolytic properties could dissociate the intercellular junction complex, such as the VE-cadherin

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intracellular tail with catenins. Thrombin interferes with the linkage between catenins and VE-cadherin (65). Third, proteases stimulate platelet aggregation and neutrophil activation and consequently elicit the interaction of platelets and neutrophils with the endothelium, which can ultimately cause junction protein redistribution and actin-mediated cell contraction. Finally, proteases also regulate the induction, release and activation of MMPs 1, 2, and 3, which can degrade the underlying basement membranes and influence vascular solute permeability.

Proteases regulate many endothelial functions via a specific group of membrane receptors called protease-activated receptors (PARs). PARs are G-protein-coupled receptors of which four have been identified (PAR 1-4). PAR-1 is cleaved by thrombin and PAR-2 is cleaved by trypsin and chymotrypsin (chymase). We recently reported the upregulation of mRNAs for PAR-1 and PAR-2 in HUVECs derived from women with pregnancies complicated by preeclampsia (15). Strikingly, PAR-2 is only expressed in HUVECs from preeclampsia, suggesting that upregulation of PAR-2 could be a novel inflammatory endothelial phenotype changes in preeclampsia. Thrombin induced increases in endothelial monolayer permeability appear to involve PAR-1 receptor activation (65, 66). It has also been reported that PAR-2 activation in the lung induces airway constriction, lung inflammation, and provoke a protein-rich pulmonary edema (67). Most recently, we noticed that preeclamptic placenta-conditioned medium disorganized the spatial distribution of endothelial junction proteins, an effect that can be blocked by transfection of PAR-2 siRNA in endothelial cells (unpublished data), suggesting that interference with PAR-2 activation might directly affect endothelial monolayer permeability via modification of cell junction proteins. Hence, there is evidence suggesting that increased protease levels/activities in the maternal circulation contributes to the increased vascular endothelial permeability in preeclampsia.

6.3. Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF) was first described as vascular permeability factor (VPF) by Senger *et al.* (68). They found that tumor ascite fluids from guinea pigs, hamsters, and mice contain activity that rapidly increases microvascular permeability. This tumor-derived VPF was later found to be an endothelial cell mitogen and therefore later re-named as VEGF (69). VPF/VEGF acts selectively on vascular endothelial cells to diminish their restriction to circulating macromolecules and to stimulate their replication. Subsequent studies show that VPF/VEGF is not only necessary for endothelial survival but also prevents endothelial cell senescence (70, 71).

Several studies have shown that total maternal VEGF levels are elevated in pregnancies complicated by preeclampsia, compared to normal pregnancies (72-75), and it has been proposed that the increased VEGF levels may be directly related to the maternal endothelial dysfunction that accompanies preeclampsia. Although the source of VEGF in the maternal circulation during preeclampsia is not defined, many believe *placenta*-derived

VEGF is the major source of maternal VEGF during pregnancy. Placental trophoblast cells produce VEGF and an *in vitro* study has demonstrated that trophoblast cells release increased amounts of VEGF when the cells are cultured under hypoxic conditions (76). Studies have also demonstrated dysregulated VEGF ligands and receptors in cytotrophoblast cells from severe preeclampsia (77). The exact mechanism underlying the higher total VEGF in preeclampsia is not known, however due to the nature of VEGF function, higher VEGF production by placental trophoblasts (if valid) could be compensatory processes involved in tissue defense, renewal, and acting to restore normal vascular endothelial functions during preeclampsia. This concept is based on the significantly higher soluble VEGF receptor (sFlt-1) levels in the preeclampsia patients (78-80). The early detection of elevated sFlt-1 levels and lower free placental growth factor (PlGF) and VEGF levels (81) in those patients before clinical symptoms become evident further support this idea. It is proposed that excess sFlt-1 released from the placenta into the maternal circulation contributes to the preeclampsia syndrome, endothelial dysfunction and kidney injury (82). An animal model study has demonstrated that administration of sFlt-1/Fc chimera could induce hypertension, proteinuria and glomerular endotheliosis in studied animals (83). Therefore, higher sFlt-1 levels may result in depletion of the protective effects of free VEGF/PlGF on the renewal and repair of vascular endothelial cells and this leads to endothelial damage during preeclampsia.

6.4. Cytokines

An imbalance in Th1 and Th2 cytokines may be another important component of the exaggerated inflammatory response in preeclampsia (84). Numerous reports have described higher maternal blood levels of tumor necrosis factor-alpha (TNF α) and interferon-gamma (INF γ) in women with preeclampsia compared to uncomplicated pregnancies (85-88). These pro-inflammatory Th1 cytokines produced by activated leukocytes (see below) exert both direct and indirect effects on vascular endothelium mostly dysregulating endothelial function (see below). For example, cultured endothelial cells became elongated and intercellular gaps are formed when exposed to TNF α and INF γ (89). TNF α also destabilizes endothelial barrier and increase vascular permeability by up-regulating endothelial phosphodiesterase-2 (90). Elevated interleukin-8 (IL-8) levels may also play a role in mediating the increased vascular permeability of preeclampsia (91).

6.5. Leukocytes

Leukocyte activation, especially neutrophil activation, has been demonstrated in women with preeclampsia (92-95). Activated neutrophils act on vascular endothelium directly by adhering to the endothelial surface and releasing ROS, proteases, and/or lipid mediators, each of which has the potential to affect endothelial function and increase microvessel permeability (27). Although the ability of activated neutrophil-mediated microvascular leakage has not been directly demonstrated in preeclampsia, it is well known that leukocytes may directly signal endothelial cells at sites of substrate attachment. It

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has been demonstrated that leukocyte-endothelial interactions induce a transient increase in endothelial intracellular $[Ca^{2+}]_i$ with a corresponding increase in monolayer permeability (96). Neutrophil-induced increases in coronary microvascular permeability is an important pathophysiological event in heart disease. Guo *et al* reported that activated neutrophils caused a concentration-dependent increase in FAK tyrosine phosphorylation with a time course matching that of venular hyperpermeability (97). Leik and Walsh reported increased neutrophil infiltration by examining vessels from subcutaneous fat obtained from women with preeclampsia (98). They found more vessels with neutrophils infiltrated into the intima and more neutrophils per vessel in tissue sections derived from women with preeclampsia compared to the vessels from control pregnant women (98), suggesting that elevated neutrophil extravasation occurs during preeclampsia.

6.6. Platelets

Platelet activation is another pathophysiological phenomenon in preeclampsia (99-101). Activated platelets release the vasoconstrictors thromboxane and serotonin, the protease thrombin, as well as ATP, platelet-activating factor (PAF), and other alpha granule and dense granule contents such as von Willebrand's factor, calcium, and serotonin etc. These platelet-derived molecules (especially thrombin) play significant roles in promoting platelet aggregation and stimulating chemotaxis of neutrophils and monocytes. The proteolytic role of thrombin may also possibly act directly on endothelial cell junction molecules. Thrombin also regulates the induction and release of MMPs. PAF is an oxidized phospholipids and the effect of PAF to induce changes in endothelial barrier function have been demonstrated in both *in vivo* and *in vitro* studies (102-104). Although platelet activation may not be an early pathophysiological event during preeclampsia, there is no doubt that consequence of platelet activation due to the release of its granular contents contributes to the vascular endothelial pathology in preeclampsia.

7. POTENTIAL ROLE OF PLACENTAL FACTORS IN REGULATION OF ENDOTHELIAL BARRIER FUNCTION

It is clear that the circulating agents mentioned above all have the potential to act upon on vascular endothelium and affect endothelial barrier function either directly or indirectly. Compelling evidence also indicate that trophoblast products, either solid particles (apoptotic cells/cell fragments) or soluble molecules (e.g. lipid peroxides, cytokines, proteases, sFlt-1/VEGF, etc.), can be released into the inter-villous space and enter the maternal circulation to affect maternal endothelial cell function. It has been proposed that deported (shed) trophoblasts from the placenta could cause endothelial dysfunction and the concentration of shed trophoblasts is higher in the blood of women with preeclampsia compared to women with uncomplicated pregnancies (105). Placental syncytiotrophoblast microvillous membrane particles (STMPs) may influence endothelial cell growth by suppressing proliferation and disrupting the cell monolayer (106). Furthermore, *in vitro* studies have also shown that

placental STMPs impair maternal vascular endothelial function, in which subcutaneous fat tissue arteries perfused with STMPs showed a significantly reduced relaxation to acetylcholine (107). Endothelial disruption was also observed in these arteries perfused with STMPs (107). These findings suggest that deported trophoblast fragments or apoptotic trophoblasts exert harmful effects on maternal vascular endothelium.

To study whether soluble factors released from the preeclamptic placenta could influence endothelial functions, we developed an *in vitro* cell co-culture model, i.e. trophoblast cells co-cultured with endothelial cells (108, 109). Using this model, we found that endothelial cells co-cultured with trophoblast cells derived from preeclamptic placentas showed significant upregulation of endothelial adhesion molecules (ICAM, VCAM, P-selectin, and E-selectin). We noted significant elevations in the expression of P-selectin and E-selectin between the endothelial cells co-cultured with preeclamptic trophoblast cells and the endothelial cells co-cultured with normal trophoblast cells (108). Furthermore, we observed that endothelial junctions were disrupted in cells that were co-cultured with trophoblast cells from preeclamptic placentas as evidenced by altered junction protein distribution and expression of VE-cadherin and occludin (109). The disturbed junction protein distribution was correlated with reduced trans-endothelial electrical resistance and increased horseradish peroxidase leakage (109). Although the disrupting agents produced by the placenta need to be defined, proteases, cytokines, ROS, etc. derived from the placenta likely play an integral role in altering endothelial barrier in preeclampsia.

8. SUMMARY AND PERSPECTIVE

In this review, we have summarized evidence that the increased vascular permeability in preeclampsia reflects structural and functional altered junctional complexes in vascular endothelium. Both intrinsic and extrinsic stimuli likely mediate these junctional abnormalities and the resultant increase in vascular permeability. However, it appears that several mechanisms underlie the endothelial barrier dysfunction, and the relative contribution of each remains to be defined. In addition, further studies to define the identity and cellular source of the initiating agents that disrupt the endothelial junctional complex in preeclampsia are warranted. Fruitful areas for future investigations also include an assessment of the contribution of endogenous endothelial barrier protective agents such as sphingosine-1-phosphate, pregnancy hormones, and endothelial progenitor cells in preeclampsia. These investigations may help to confirm the promise of endothelial junctions as a target for therapeutic intervention in this unique disorder of human pregnancy.

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