The Functions of Microparticles in Pre-Eclampsia

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ABSTRACT

Pre-eclampsia (P-EC), a heterogenic multisystem disorder characterized by hypertension and proteinuria, usually develops in the second half of pregnancy. The incidence is 2 to 5%, and P-EC is therefore a major cause of maternal and perinatal morbidity and mortality. Although the exact etiology is unknown, placental factors released into the maternal circulation lead to systemic maternal inflammation and endothelial dysfunction. Growing evidence indicates that placenta-derived microparticles, best known as syncytiotrophoblast microparticles (STBM), are important among these factors. This review provides an overview of the presence and function(s) of STBM and other cell-derived microparticles and exosomes.

KEYWORDS: Microparticles, pre-eclampsia, syncytiotrophoblast microparticles

Pre-eclampsia (P-EC) is a gestational heterogenic multisystem disorder characterized by hypertension and proteinuria, and in general it becomes clinically manifest in the second half of pregnancy. The incidence is 2 to 5% of all pregnancies, and P-EC is therefore a major cause of maternal and perinatal morbidity and mortality worldwide. P-EC can present as asymptomatic hypertension and proteinuria with little or no fetal compromise, but it may also be complicated by convulsions, cerebral hemorrhage, liver failure, acute renal failure, or respiratory insufficiency.[1] Because severe P-EC often presents with placental hypoperfusion, the child may also become compromised with subsequent growth retardation and fetal distress, sometimes resulting in intrauterine death. Prediction of P-EC is difficult at present, and preventive therapy is limited to low-dose aspirin to all pregnant women, offering a relative reduction of only 10%. The only definitive therapy is delivery, which is hazardous for the newborn in case of prematurity.

Although the exact etiology of P-EC is unknown, a general accepted hypothesis is a two stage model. In the first, clinically silent stage, placental hypoperfusion develops because of insufficient trophoblast invasion into spiral arteries of the uterus early in pregnancy (until 14 to 18 weeks of gestational age), which results in abnormal placentation (Fig. 1). In the second stage, placental factors are released increasingly into the maternal circulation, leading to a systemic inflammatory response and endothelial dysfunction and clinical manifest disease.[2] In addition, maternal susceptibility determines the chance of pregnant women developing P-EC in case of placental

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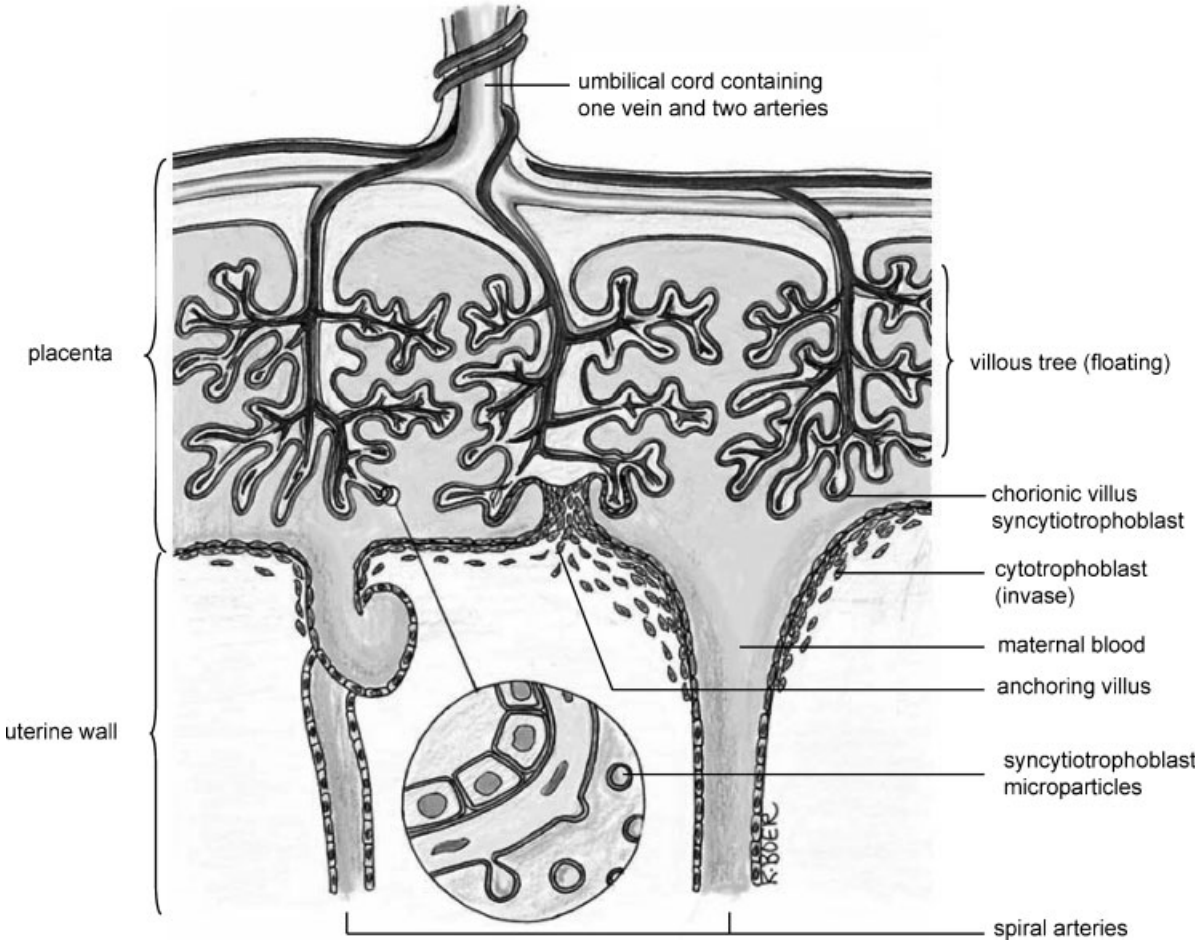

Figure 1 Placental hypoperfusion and syncytiotrophoblast microparticles (STBM) in pre-eclampsia. The figure on the left depicts a spiral artery whose endothelium is insufficiently replaced by invasive trophoblast cells and that consequently remains a narrow high-resistance vessel hypoperfusing the placenta. The insert shows the release of STBM. On the right a low resistance spiral artery is shown after successful invasion of the trophoblast cells.

hypoperfusion because patients with thrombophilia, vascular disease, or the metabolic syndrome have an increased risk of developing P-EC, and not all women with established placental hypoperfusion in pregnancy develop P-EC.[1]

MICROPARTICLES IN NORMAL PREGNANCY AND PRE-ECLAMPSIA

Microparticles, present in plasma samples collected from pregnant women and patients with P-EC, originate from blood cells, endothelial cells, and placenta syncytiotrophoblast microparticles (STBM). There is no consensus among investigators about the concentration of microparticles or subpopulations in P-EC.[3] Variations in concentration are due to patient selection, methodological differences, differences in gestational age, and intraindividual differences. P-EC is a heterogeneous disease, and clinical symptoms may vary from asymptomatic women to critically ill patients with signs of disseminated intravascular coagulation and hemolysis. Differences in patient selection may directly affect the numbers of microparticles and thus complicate comparison of data between studies. An additional complicating factor is the change in hemodynamics. Whereas uncomplicated pregnancy is characterized by hemodilution, P-EC is characterized by hemoconcentration due to fluid leakage into the extravascular space because of endothelial damage.

Upon analysis of blood samples of pregnant or P-EC women, by far the most abundant microparticles are those of platelet origin. The numbers of total and platelet-derived microparticles are decreased in P-EC compared with normal pregnancy, although the numbers of platelet-derived microparticles exposing P-selectin are increased.[4,5] These P-selectin–exposing microparticles reflect platelet activation, which is confirmed by increased numbers of platelets exposing P-selectin and increased numbers of platelet-monocyte complexes in P-EC patients.[6,7] Elevated concentrations of erythrocyte-derived microparticles have been reported in P-EC, which are due to hemolysis and/or hemoconcentration.[4]

In P-EC, increased levels of microparticles from T cells, monocytes, and granulocytes are present, and the numbers of granulocyte-derived microparticles correlate with elastase, a marker of granulocyte activation and secretion.[8–10] Also, some investigators, but not others, have reported elevated concentrations of endothelial cell–derived microparticles.[3,5,11,12]

The occurrence of placenta-derived STBM is unique for pregnancy because they originate from the placenta. The concentration of STBM increases during the course of pregnancy. This increase, which is most likely explained by the increasing placental volume, reaches the highest level in the third trimester.[4,13] Plasma samples from women with P-EC have increased levels of STBM compared with normal pregnancy also when corrected for gestational age, and this increase is thought to directly reflect placental hypoxia and apoptosis.[4,13–17]

FUNCTIONS OF MICROPARTICLES IN PRE-ECLAMPSIA

Functions of microparticles in P-EC have been studied mainly by isolating total fractions of microparticles from plasma samples or by preparing STBM in vitro. Before presenting an overview of these functions (Fig. 2), it must be taken into account that both approaches have several flaws. With regard to the isolation of microparticles from plasma samples, the most interesting (sub) populations may be absent due to binding in vivo (e.g., by binding to blood cells). Blood samples from pregnant women contain complexes of STBM and monocytes, thereby reducing the number of non–cell-bound STBM.[13] Furthermore, preanalytical and analytical conditions affect the structure and function of microparticles: Contaminants of plasma, medication, cells—in particular, small platelets—and other cell-derived vesicles such as exosomes can be present, and most experiments are

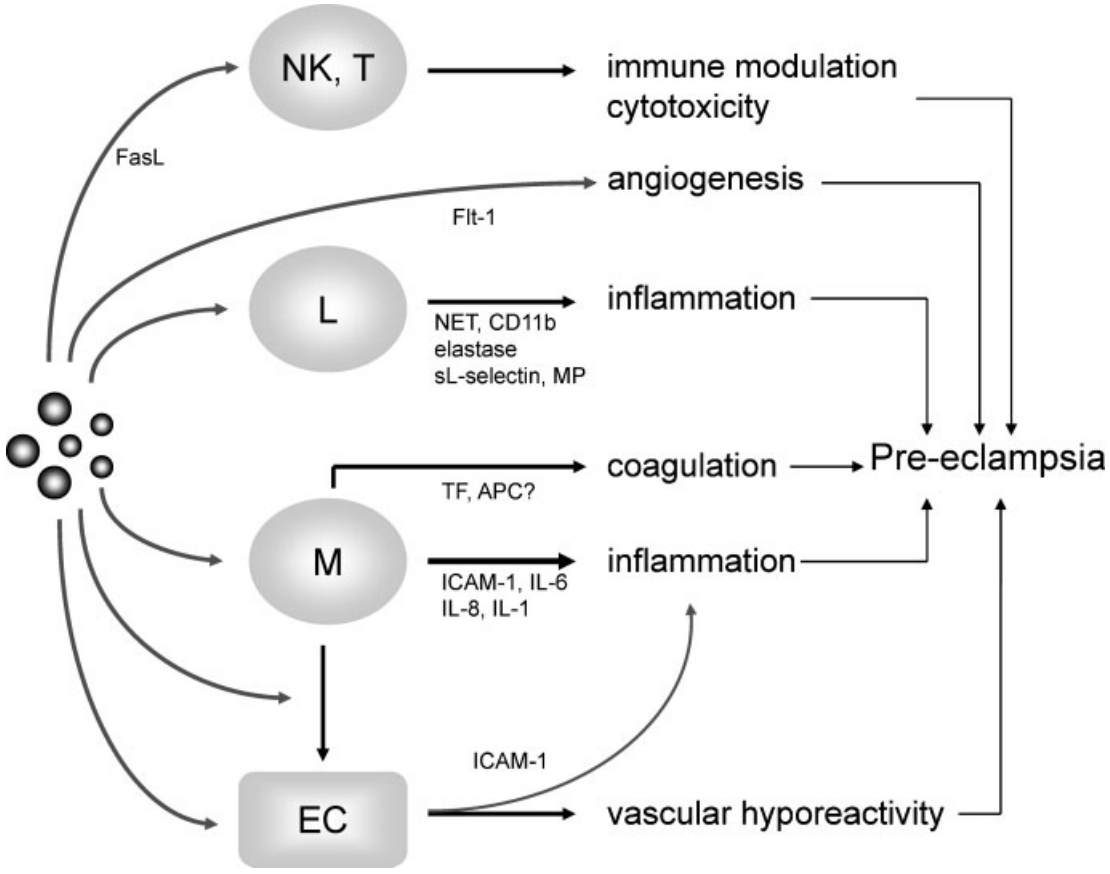

Figure 2 Overview of the functions of microparticles and exosomes in the development of pre-eclampsia (P-EC). Microparticles and exosomes (blue, left), present in the plasma of P-EC patients, originate from a variety of blood cells, endothelial cells, and the placenta syncytiotrophoblast microparticles. The microparticles interact with leukocytes (L), monocytes (M), and endothelial cells (EC), thus affecting inflammation, coagulation, and vascular reactivity. Microparticles expose Flt-1 in P-EC, which may affect angiogenesis. Exosomes expose Fas ligand (FasL) and bind to T cells (T) and natural killer (NK) cells, thereby modulating the immune response. The direct effects of microparticles on coagulation and activation of protein C are not shown. APC, activated protein C; Flt-1, vascular endothelial growth factor receptor 1; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; MP, microparticles; NET, neutrophil extracellular trap; sL, selectin: soluble leukocyte selectin; TF, tissue factor.

performed under static conditions. For instance, the effects of STBM on T-cell activation, proliferation, and cytokine release and on endothelial proliferation, detachment, and apoptosis depended on the methodology used to prepare STBM and whether the STBM originated from the placenta or from in vitro cell cultures.[18,19]

Vascular Reactivity

Microparticles from plasma samples of P-EC patients but not of normal pregnant women impair vascular dilation in vitro. Vanwijk et al showed an impaired endothelial-dependent dilation of myometrial arteries in response to microparticles from P-EC patients.[20] Similarly, Tesse and coworkers showed that microparticles from P-EC patients induced vascular hyporeactivity of human omental arteries and mouse aortas. This hyporeactivity was associated with increased nitric oxide (NO) production and was reversed by a NO synthase inhibitor.[8] Exposure of aortic rings from pregnant mice to microparticles from P-EC patients impaired the serotonin-induced constriction.[21] This vascular hyporeactivity was also observed in arteries isolated from mice that were pretreated with microparticles from P-EC patients, indicating the microparticle-induced vascular impairment occurs not only in vitro but also in vivo.[8] In contrast, discrepant results have been obtained when determining the effects of in vitro prepared STBM on vascular contractility. This may be due to differences in preparation or origin of STBM but can also be due to the copresence of microparticles from other cell types.[22,23]

The gene expression of endothelial cells is virtually unchanged when incubated with microparticles or STBM from P-EC patients.[24,25] In contrast, the proliferation of endothelial cells is inhibited in the presence of STBM, and various attempts have been made to purify compounds (from STBM) responsible for this inhibition and in fact even disruption of the endothelial cell monolayer.[26–28] Nevertheless, granulocytes, monocytes, and lymphocytes become activated, as measured by release of reactive oxygen species and changes in intracellular calcium and pH levels, when incubated with conditioned culture supernatants from STBM-pretreated endothelial cells.[29] Thus leukocyte activation can be achieved by factors released from STBM-treated endothelial cells or by factors released from endothelial cells in combination with STBM. In sum, the underlying mechanism suggests a complex role for cell-derived vesicles in the pathology of P-EC, in which the vesicles modify the sequence of several cellular responses that may contribute to the development of P-EC.

Coagulation

P-EC is characterized by a procoagulant and proinflammatory state. Microparticles contribute to coagulation by providing a membrane surface to which (activated) coagulation factors can bind, and by exposing tissue factor, the initiator of blood coagulation. Compared with nonpregnant women, pregnant women have an increased concentrations of plasma coagulation activation markers such as thrombin-antithrombin complexes and prothrombin fragment $1+2$ ($F1+2$), and these levels are further increased in P-EC patients.[30,31] Because the ability of microparticles to generate thrombin is not increased in P-EC but comparable with microparticles from nonpregnant as well as pregnant women, it seems unlikely that microparticles contribute to coagulation activation in P-EC directly.[30] In fact, there may be an alternative and more complex role for microparticles to modify coagulation in P-EC.

Activation of platelets increases not only their procoagulant phenotype but also their ability to inactivate factor Va by activated protein C, and ~25% of both procoagulant and anticoagulant activities are associated with platelet-derived microparticles.[32] In women with P-EC, the numbers of (phosphatidylserine-exposing) microparticles correlate inversely with the plasma concentration of $F1+2$,[31] and similar inverse correlations have been observed in healthy human subjects.[33] We speculate that under normal conditions and in P-EC, when circulating numbers of total (i.e., mostly platelet-derived), circulating microparticles are low, their ability to activate protein C may come in a critical zone. Insufficient activation of protein C results in reduced inhibition of (activated) coagulation factors Va and VIIIa, which may lead to a net increase in thrombin generation and coagulation activation. In both pregnancy and P-EC, acquired activated protein C resistance was reported.[30]

Inflammation

STBM from normal placental lobules stimulate the production of the proinflammatory cytokines tumor necrosis factor α, interleukin (IL) 12p70, and IL-18 by peripheral blood mononuclear cells from healthy nonpregnant women, indicating that STBM may contribute directly to the proinflammatory phenotype observed during pregnancy and P-EC.[13] In similar experiments, Messerli and coworkers showed that STBM, albeit strongly dependent on the preparation method they used, upregulated the cell surface exposure of intercellular adhesion molecule (ICAM)-1 (CD54) and the production of IL-6, IL-8, and IL-1β by monocytes.[34] Leukocyte activation in P-EC is confirmed by elevated plasma levels of soluble (s) L-selectin and elastase, elevated levels of various types of leukocyte-derived microparticles, and upregulation of nuclear factor (NF) κB-1A.[10] Syncytiotrophoblast microparticles activate neutrophils, as reflected by exposure of CD11b and formation of neutrophil extracellular traps (NETs).

NETs are fibrous extracellular lattices containing DNA, and large numbers of NETs are present in the intervillous space of P-EC placentae, suggesting that activation of neutrophils and formation of NETs may play an as yet unknown role in the development of P-EC.[35,36]

As mentioned before, isolated P-EC microparticles failed to induce major changes in RNA expression of inflammation-related genes in human umbilical endothelial cells.[24] Also the release of cytokines and gene expression of both human umbilical endothelial cells and human glomerular microvascular endothelial cells were unaffected upon incubation with microparticle-containing plasma from P-EC.[37] In co-culture with monocytes, however, increased exposure of CD54 (ICAM-1) was observed, suggesting a complex interaction between MP, monocytes, and endothelial cells (M. Faas and C. Lok, personal communication).

Complement activation is part of the systemic inflammatory response. With regard to P-EC, increased numbers of C-reactive protein-exposing microparticles were reported compared with pregnant controls, but no further evidence for their role in complement activation could be obtained.[38] Again, such CRP-exposing microparticles are likely to contribute to a proinflammatory phenotype, but their precise contribution remains to be established.

Angiogenesis

Plasma samples from P-EC patients contain platelet-derived and placenta-derived microparticles exposing full-length transmembrane vascular endothelial growth factor (VEGF) receptor Flt-1.[39] Whether or not the biological activity of microparticle-exposed Flt-1 differs from non-cell-bound (soluble) Flt-1 is unknown, but one may speculate that Flt-1, similar to other surface receptors, can be transported to target cells, thereby enabling these cells to bind VEGF and facilitate angiogenesis.

Exosomes and Immune Suppression

The role of exosomes in P-EC has not yet been thoroughly investigated yet. One of the main functions of exosomes is their ability to modulate the immune response.[40] Placenta-derived exosomes have emerged as immunosuppressive factors. Circulating levels of placental exosomes were almost twofold increased in pregnant women delivering at term compared with those delivering preterm, and these exosomes contained more Fas ligand (FasL) and were more efficient in suppressing genes involved in T lymphocyte apoptosis than exosomes from women delivering preterm.[41] Human placentae release exosomes bearing several different NKD2D ligands. Binding of exosomes exposing these ligands (i.e., the UL-16 binding proteins and MHC class I chain-related proteins A and B), to NKG2D receptors exposed on natural killer cells, CD8 lymphocytes, and a subset of T cells reduced their in vitro cytotoxicity, thus supporting fetal immune escape.[42] Exosomes of placental origin, isolated from maternal peripheral blood, suppressed T-cell signaling components in a partially dependent FasL-dependent mechanism.[43] To what extent immunologic maladaptation contributes to P-EC is unclear, and future studies are essential to further unravel the precise role exosomes play in the etiology of P-EC.

Vehicles of Genetic Information

Cell-derived vesicles contain genetic information that can be exchanged between cells. STBM vesicles, prepared in vitro, contain fetal DNA and RNA.[44] Already in 2003, Ng et al showed that maternal plasma contains mRNA transcripts from placenta-expressed genes, and these transcripts showed a "surprising stability."[45] This stability is explained by the fact that the mRNA transcripts are present *within* the vesicles and therefore are protected from degradation. The relative concentration of placental mRNA in maternal plasma may directly reflect the placental gene expression.[46] Microparticles containing DNA are known as "apoptotic bodies." Plasma samples from P-EC patients contain increased numbers of such DNA-containing microparticles compared with normal pregnancies, and they are thought to directly reflect the increased shedding of "placental debris" into the maternal circulation.[47] Labor increases the levels of STBM and placental mRNA in maternal blood but not the levels of fetal and maternal DNA, but the underlying mechanisms need to be elucidated.[48]

CONCLUSIONS AND CLINICAL APPLICATION

In pregnancy, a unique type of vesicles is present originating from the placenta. The numbers of the STBM are increased in P-EC compared with normal pregnancy, and these increased numbers are thought to reflect increased shedding and apoptosis of trophoblast cells due to placental hypoperfusion. The ability of STBM and total fractions of vesicles to modify vascular reactivity, coagulation and anticoagulation, inflammation, angiogenesis, and immune suppression is slowly becoming elucidated. The finding that STBM contain fetal DNA and RNA, which is efficiently protected against degradation, may offer novel diagnostic possibilities. It has already been shown that the prenatal diagnosis of Down syndrome may be possible by detecting a chromosome 21–encoded mRNA of placental origin in maternal plasma early in pregnancy.[49,50] We hypothesize that detailed analysis of mRNA and microRNA profiles of STBM, isolated from maternal plasma, may provide novel insights into placental development, not only in

normal pregnancy but also in pathological conditions such as P-EC. This might enable prediction of the development of P-EC in the preclinical phase and early therapeutic intervention for a gestational disease for which there is no good predictive test or real therapy available at present except for early delivery.

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