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Placental hemostasis and sterile inflammation: New insights into gestational vascular disease

Shrey Kohli, Berend Isermann*

Institute of Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke University, Magdeburg, Germany

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ABSTRACT

Activation of the coagulation and inflammatory systems are physiologically occurring during pregnancy. However, excess activation of either system is well documented in gestational vascular diseases (GVD). GVD are placenta-mediated pregnancy complications and a major cause of feto-maternal morbidity and mortality. The causal relevance of excess coagulation and inflammatory responses for GVD remains largely unknown. Deciphering the causal relationship of excess coagulation and inflammation in GVD may allow conceptualizing new therapeutic approaches to combat GVD. Platelet activation and procoagulant extracellular vesicles (EVs) provide a link between coagulation and inflammation and their activation or generation in GVD is well established. As recently shown EVs cause sterile placental inflammation by activating maternal platelets that release ATP and activate purinergic receptor signaling and NLRP3 inflammasome in the embryonic trophoblast. This thrombo-inflammatory mechanism suggests a novel link between coagulation activation and sterile inflammation in GVD. These findings highlight a role of anti-platelet therapies in GVD. In addition, targeting the inflammasome alone or in combination with platelet inhibition may provide a new therapeutic strategy in GVD.

1. Introduction

Pregnancy is physiologically associated with hypercoagulability and low-grade inflammation [1,2]. Somewhat paradoxically, immune-tolerance of the allogenic fetus and the placenta parallels the low-grade inflammation and the slow- to no-flow vascular bed within the placenta is largely devoid of occlusive thrombi. These apparent paradoxes reflect the profound changes of coagulation and inflammation during pregnancy. The importance of the immune-system and coagulation system for successful placentation are underscored by the catastrophic consequence (potential fetal demise) in pregnancy associated diseases such as preeclampsia or HELLP syndrome, which are associated with a further increase of coagulation activation and inflammation [3,4]. Why and under which circumstances the inflammatory and coagulation system reach a state of pathophysiological activation and whether the excessive activation of these systems is related to the disease onset or propagation remains largely unknown. Addressing these questions may provide not only new mechanistic insights, but may also unravel new therapeutic approaches. Recent insights start to unravel these questions.

2. Platelets in placental vascular complications

Gestational vascular diseases (GVD) such as preeclampsia and HELLP syndrome affect approximately 5–7% of pregnancies and are a major cause of maternal, fetal and neonatal morbidity and mortality [5]. These pregnancy-associated diseases are associated with endothelial dysfunction and a low platelet count. One of the key diagnostic criteria of HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) is a drop of platelet numbers in the peripheral blood (below 100,000/μl) [4]. The drop of platelet numbers frequently precedes the onset of placental and renal changes, suggesting a causative role of platelet activation [6]. Intriguingly, platelet properties and reactivity change in the context of GVDs, as they display an increase in membrane fluidity, cholesterol concentration (independent of plasma levels), an increase ratio between unsaturated and saturated fatty acids, and increased propensity to secrete ATP in GVDs [7–9]. Increased platelet activation, as reflected by markers such as β-thromboglobulin, thromboxane β2, or platelet factor-4, are associated with GVD, suggesting that platelet activation may aggravate the disease course. Collectively, these studies imply a function of platelet activation and potentially platelet derived mediators for pregnancy associated vascular dysfunction and fetal loss. It is conceivable that platelets either directly cause or propagate the disease process. However causality and potential mechanisms remain to be established.

While these clinical data suggest a function of platelets in pregnancy-associated complications, successful pregnancy is possible in mice with severe qualitative or quantitative platelets defects

* Corresponding author: Berend Isermann, MD, Institute of Clinical Chemistry and Pathobiochemistry Otto-von-Guericke-University Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany. Tel.: +49 (0)391-13900; Fax: +49 (0)391-13902.
E-mail address: berend.isermann@med.ovgu.de (B. Isermann).

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[10,11]. Unlike in mice, severe platelets defects are associated with an increased risk of hemorrhage in both the mother and the fetus, but except for these serious and potentially life-threatening complications development proceeds normal. The observation that platelets – in particular in mice – are dispensable for successful reproduction should not be misinterpreted as an indication that platelets have no function at all during pregnancy. As noted above, GVDs such as preeclampsia or HELLP syndrome are primarily characterized by excess platelet activation, not a loss of platelets. Taken together, while platelets appear to be dispensable for normal physiological pregnancy, they can acquire pathophysiological importance in GVDs such as preeclampsia or HELLP.

A causative role of platelet activation for pregnancy impairment was initially demonstrated in mice with increased coagulation activation. Placental hypercoagulability due to the presence of maternal factor V Leiden mutation causes embryonic lethality if the trophoblast expresses a hypomorphic thrombomodulin variant ($TM^{Pro/Pro}$) [12]. However, occluding thrombi are not increased in the placenta of these embryos, arguing against a causative role of occluding placental blood clots. Rather, placentae of these embryos are smaller and lack a well-formed labyrinth layer, reflecting a failure of vascular remodeling and potential invasion of embryonic blood vessels into the developing placenta. Similar observations have been made in embryos lacking thrombomodulin or its co-receptor endothelial protein C receptor in placental tissues [13–15]. At least the embryonic loss of thrombomodulin deficient embryos can be partially rescued by concomitant platelet deficiency. Likewise, embryonic demise of factor V Leiden females carrying embryos expressing the hypomorphic $TM^{Pro/Pro}$ variant can be rescued by concomitant platelet deficiency. These results demonstrated that platelets can cause placental failure, and suggested that the detrimental function of platelets is independent of occluding blood clots. The latter conclusion is supported by the differential effect of anticoagulants in this model. While low molecular weight heparin protects from the platelet induced fetal loss, other anticoagulants like the direct thrombin inhibitor lepidurin failed to do so. These observations argue against a predominant role of thrombin mediated platelet activation and support a function of platelets independent of hemostasis. Intriguingly, platelets have been linked with sterile inflammation in recent years.

3. Placental sterile inflammation

As outline above low-grade inflammation is a characteristic finding in pregnancy. Inflammation in the absence of an infectious agent, as observed during pregnancy, is referred to as sterile inflammation. One of the key systems controlling sterile inflammation is the inflammasome. Several inflammasome have been identified [16]. Among these, the NLRP3 inflammasome is the best studied. Upon sensing PAMPs (pathogen associated molecular patterns) or DAMPs (danger associated molecular patterns) *via* pattern recognition receptors (PRR), including toll-like receptors, a multiprotein complex is formed intracellular, comprising NRLP3 and the adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) [17,18]. This complex interacts with procaspase-1, generating active caspase-1, which cleaves the pro-forms of IL-1β and IL-18, generating the mature cytokines [19]. While this system has been initially described in myeloid innate immune cells, with monocytes being the main source of IL-1β, a functional relevance of the inflammasome in non-myeloid cells such as microglia, endothelial cells, retinal pigment epithelial cells, or renal podocytes, has been increasingly recognized [20–22]. We and others recently demonstrated expression of inflammasome regulators in human and mouse trophoblast cells [23–25]. During pregnancy, there are several host-derived molecules potentially acting as DAMPs, which may contribute to pregnancy associated inflammasome activation [26]. Furthermore, the inflammasome is activated in women with preeclampsia, as reflected by increased plasma levels of IL-1β as well as increased expression of TLR, IL-1β, and NLRP3 in neutrophils [27,28]. However, the pathophysiological relevance of inflammasome activation in GVDs remained unknown. Interestingly, inflammasome activation in platelets has been recently demonstrated, suggesting a mechanistic link between platelets and inflammation via the inflammasome [29].

4. Extracellular vesicles provide a mechanistic link between and inflammasome, platelets, and placental vascular complications

Extracellular vesicles (EVs) are membrane bound vesicles that range from micrometer to nanometer in size. EVs carry specific surface markers representing their cellular origin and they are "loaded" with cargo such as proteins, lipids, RNA species (mRNA, miRNA), or DNA derived from the original cell [30]. As such, they can affect the function of distant cells. In healthy pregnancy the frequency of EVs already increases during gestation when compared to non-pregnant healthy women. EVs during pregnancy can be differentiated into three main categories – macrovesicles, microvesicles and exosomes – depending upon their size and mode of release from their cell of origin [31]. Pregnancy associated macrovesicles typically comprise large multi-nucleate syncytial aggregates originating from the placental syncytiotrophoblast. Their diameter ranges from 20 μm to several hundred micrometer and they carry fetal protein, RNA and other factors. Microvesicles (MVs) are derived from budding of plasma membrane of stressed or apoptotic cells and range from 0.1–1 μm in diameter. Exosomes range from 20 to 100 nm in diameter. The physiological role of exosomes, during pregnancy remains ill defined, but they may contribute to the feto-maternal cross talk [32]. MVs remain the most studied type of EVs during pregnancy and GVDs.

In women with GVDs the frequency of EVs increases even compared to healthy pregnant women, and this quantitative change is associated with qualitative alterations [33]. MVs derived from different cell sources such has platelets, endothelial cells, monocytes or placenta have been shown to play important roles in regulating processes such as invasion, migration, proliferation, angiogenesis or apoptosis [33–35]. MVs typically bear the coagulation pathway initiation factor, tissue factor (TF), and negatively charged phospholipids [36]. Accordingly, these MVs are procoagulant and capable to alter the hemostatic system. Healthy pregnant women carry MVs with high procoagulant activity compared to non-pregnant women. Several studies have suggested an association of pro-coagulant MVs with GVD and fetal loss [37,38] and *in vitro* MVs isolated from women with pregnancy complications cause endothelial dysfunction in isolated myometrial arteries from healthy pregnant women [39]. Of note, not only platelet derived MVs, but also MVs of other cellular origin are associated with impaired pregnancy outcome [40]. Indeed, syncytiotrophoblast derived MVs interact with thrombin activated platelets, and this interaction increases when using syncytiotrophoblast derived MVs from pregnant women with preeclampsia compared to those from women without preeclampsia [41]. The occurrence of MVs may hence reflect general cell-activation and may be part of a self-propagating disease process. Accordingly, endothelial cell activation is well established in GVDs, which likely contributes to MV formation and itself may cause platelet activation [42]. Other potential causes of increased platelet activation in GVDs are activation of the renin-angiotensin-aldosterone system, increased levels of cytokines (TNFα, IL1β), or changed prostacyclin and thromboxane synthesis [43]. However, whether quantitative and qualitative changes of EVs in women with pregnancy associated vascular complications are of disease relevance remained unknown

We recently demonstrated a pathophysiological function of procoagulant EVs in mice [23]. Injection of procoagulant EVs into