Placental particles in pregnancy and preeclampsia
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Chapter 8

English summary
Extracellular vesicles (EV) are small membrane-surrounded vesicles, which are secreted by a plethora of cells and are present in all body fluids during health and disease. During pregnancy, many types of EV are present in the maternal circulation originating from various cells, such as blood cells and endothelial cells, but interestingly also from the outer fetal layer of the placenta, the syncytiotrophoblast (STB). The syncytiotrophoblast extracellular vesicles (STB EV) enter the maternal circulation and increase in number during pregnancy. They can perform physiological functions, e.g. they may aid in the immunologic and hemostatic adaptations of the maternal body during pregnancy. Pregnancy has been described as a maternal systemic inflammatory state associated with changes in both the adaptive and innate immune response. Features of adjusted hemostasis, to avoid premature bleeding or excessive postpartum bleeding, have also been shown. In the present thesis we tested whether these changes in immunology and hemostasis during pregnancy may be influenced by STB EV.

STB EV are also believed to be involved in the pathophysiology of the pregnancy-complication preeclampsia (PE). PE is mainly characterized by gestational hypertension and proteinuria in the second half of pregnancy. PE features, amongst others, an exaggerated maternal inflammatory response and coagulopathies. The number of STB EV was even further increased in PE as compared to normal pregnancy. Furthermore, also the molecular load of STB EV has been shown to differ between normal pregnancy and PE, suggesting an altered functionality of STB EV between healthy and diseased pregnancy. Often, STB EV are subcategorized into syncytiotrophoblast microvesicles (STB MV) and exosomes, which differ by size and mode of formation. STB MV are relatively big particles, of 100 to 1000 nm in size and being formed by direct budding from the apical side of the STB plasma membrane. In contrast, exosomes are smaller particles of 30 to 100 nm in size and being formed in intracellular multivesicular bodies and released by fusion of the membrane of these intracellular multivesicular bodies with the STB plasma membrane. Based on their differing mode of formation, STB MV and exosomes are likely to posses, at least partially, different molecular loads and to perform different functions. From an immunological point of view, STB MV are believed to be activating, while exosomes are believed to be tolerance-inducing. Therefore, this thesis aimed to perform a comprehensive comparison of the function of the two main groups of STB EV, STB MV and exosomes, from placentae of normal and preeclamptic pregnancies, with respect to immunological and hemostatic functions.

In chapter 2 of this thesis, we described the development of an enzyme-linked sorbent assay to quantify STB EV in fluid samples. In that assay, phosphatidylserines on the STB EV surface are bound by immobilized annexin V and retained from the fluid sample. Next, placental alkaline phosphatase on the STB EV surface is used to catalyze the colorimetric detection reaction. Equipped with this tool, we performed a multi-center, prospective, blinded, prognostic marker
study exploring the usability of the peripheral plasma STB EV concentration as an accessory marker to mid-gestational uterine artery Doppler velocimetry in the prediction of the development of PE in a high risk population as described in chapter 3. We did not find a prognostic value of the peripheral plasma STB EV concentration in addition to mid-gestational uterine artery Doppler velocimetry. Concentrations of STB EV measured in this study in peripheral plasma of late pregnancy in the control group were used to determine the concentrations of STB EV used in the next chapters focusing on the immunologic function of STB EV.

In chapters 4 and 5, we stimulated whole blood samples of nulligravid women with physiologic concentrations of either STB MV or exosomes from either normal or preeclamptic placentae. In chapter 4, we studied the effects of STB EV on monocytes and granulocytes. STB MV and exosomes from both normal and preeclamptic placentae induced activation of monocytes and granulocytes as apparent by increased granularization of the cells and an increased expression of the activation marker CD11b. Additionally, both STB MV and exosomes from normal and preeclamptic placentae guided the maturation from CD16—classical monocytes towards CD16+ intermediate monocytes. Although STB MV and exosomes from normal and preeclamptic placentae induced similar effects, the exosomal effects were more pronounced. Interestingly, with respect to monocytes and granulocytes, the effects of STB EV from preeclamptic placentae were similar to the effects of the STB EV from normal placentae. In chapter 5, we studied the effects of STB EV on T cells, natural killer (NK) cells and natural killer T (NK T) cells. STB EV from normal placentae activated T cells and memory T cells, especially regulatory T (Treg) cell subgroups. They also stimulated cytotoxicity (expression of perforin and granzyme B) in NK (especially CD16+CD56++) and NKT cells. T helper (Th) cell subsets were not significantly affected by STB EV from normal placentae. Again, STB MV and exosomes from normal placentae featured similar effects, but the exosomal effects were more pronounced. STB EV from preeclamptic placentae failed to activate T cells, Treg cells and to induce cytotoxicity of NK and NKT cells.

The results from chapters 4 and 5 suggest that STB MV and exosomes from normal placentae may support the systemic inflammatory state of the maternal organism during normal pregnancy, e.g. by activation of monocytes and granulocytes. Additionally, STB EV from normal placentae affect predominantly regulatory lymphocytes (e.g. Treg and CD16+CD56++ NK cells), which may support the local uterine tolerance towards the fetus as well as ensure regulation of the controlled regulatory state during normal pregnancy. In contrast to earlier expectations, STB MV and exosomes were not solely activating (STB MV) or solely tolerance-inducing (exosomes), but showed similar effects. This suggests, that STB MV and exosomes from normal placentae do not necessarily perform opposite immunologic functions.

STB EV from preeclamptic placentae also activated both monocytes and
granulocytes, but did not induce an increased response in monocytes or granulocytes as compared with STB EV from normal placentae. However, they showed a loss of function in inducing regulatory T and NK lymphocytes. This suggests, that STB EV from preeclamptic placentae may not directly induce the exaggerated systemic inflammatory state of the maternal organisms during PE. Rather, STB EV from preeclamptic placentae seem to favor this state by not inducing regulatory mechanisms.

Additionally to the immunological effect of STB EV, in chapter 6 we also assessed their pro-coagulant effects. First, we studied the effect of suspensions from ex vivo placenta perfusion on thrombin formation and platelet aggregation. Perfusion suspensions from normal placentae induced only a very low thrombin formation and low platelet aggregation at a variable, individual aggregation rate. Next, we perfused normal placentae at low oxygen contents to mimic the preeclamptic placenta. The perfusion suspension of low oxygen perfused placentae led to an increased thrombin formation and highly deregulated platelet aggregation. Since the perfusion of placentae at low oxygen contents is regarded as a model for PE, this may suggest that a perfusion suspension from preeclamptic placentae may induce similar effects. A stepwise exclusion of cell debris, STB MV and exosomes from the perfusion suspension of low oxygen perfused placentae identified STB MV as the causing particles for the observed effects. Only the exclusion of STB MV reversed the effects on thrombin formation and platelet aggregation. In contrast to our immunologic studies, STB MV and exosomes do seem to feature different pro-coagulant functions, since only the STB MV influenced thrombin formation and platelet aggregation, but not exosomes. This may be related to the exposure of certain molecules, such as tissue factor and phosphatidylserines, on the STB MV surface but not on exosomes.

In conclusion, the present thesis showed that placental STB EV have immunological and coagulation properties. Immunological properties are observed with STB EV from both normal and preeclamptic placentae, while coagulation properties are only observed by STB EV from low oxygen perfused placentae (as a model for the preeclamptic placenta). Interestingly, the coagulation inducing properties were only observed with the STB MV and not with the exosomes. This finding is in line with the general suggestion that STB MV and exosomes have different (patho) physiological effects. Although it has also been suggested that STB MV and exosomes have different immunomodulating properties, we observed that STB MV and exosomes have similar immunomodulating properties, although the effects of the exosomes seemed to be stronger. This may be related to an, at least partially, different molecular load of STB MV and exosomes. Differences, however, were found between STB EV from healthy and preeclamptic placentae: although STB EV from healthy placentae and preeclamptic placentae activated monocytes and
granulocytes similarly, stimulation of lymphocytes, especially Treg and NK cells, was only observed by STB EV from healthy placentae. This suggests that STB EV may play an immunoregulatory role in normal pregnancy. In PE, however, the STB EV failed to induce this immunoregulation, which may favor the proinflammatory state of PE.