

Macrophages: Are They Involved in Endometriosis, Abortion and Preeclampsia and How?

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Abstract

Macrophages hold a key role in both regulating and executing the body's own immune response under various conditions. Hence, although endometriosis, preeclampsia and abortions are clinically different, all three are regarded to involve highly complex immunological processes. The aim of our current work was to evaluate the role of macrophages within these gynaecological disorders. Macrophages have been shown to invade endometriosis lesions and to mediate propagation of endometriotic cyst growth. However this is the first time that significant GPER up-regulation in macrophages is demonstrated. This highlights a potential alternative way through which estrogen may modulate immune response of macrophages in endometriosis. In addition, during spontaneous miscarriages the macrophage population increases significantly. This deregulation may possibly support an inflammatory scheme further triggering abortive procedures. Macrophage-mediated apoptosis of extravillous trophoblasts (EVT) has been associated with preeclampsia. Larger numbers of apoptotic EVT were detected in preeclamptic placentas compared with normal. In preeclamptic placentas, decidual macrophages were found to be Fas ligand (FasL)-positive. Our results highlight a new aspect of macrophage biology in endometriosis and pregnancy physiology and pathophysiology. Further studies with larger samples are needed to verify the current results and evaluate their clinical impact. Our data strongly indicate that macrophages hold key roles in various gynaecological disorders and might be crucial to further elucidate their pathophysiology.

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Introduction

Endometriosis, abortion and preeclampsia form three important yet different gynaecologic entities that are linked to complex immunologic processes. Each of them implies macrophage activation and differentiation. Macrophages play a key role in immune response and their activation and differentiation are altered in these cases of immune reaction. These cells of the monocyte-macrophage line are characterized by diversity and plasticity and therefore can respond to environmental stimuli by acquiring specific phenotypes. In response to external cues they will undergo classical M1 activation with high levels of inflammation and microbicidal and tumoricidal activity. Alternatively the M2 pathway contains mostly parasite containment, tissue remodelling and most importantly in this case immunomodulatory functions¹.

Endometriosis as defined by extrauterine endometrial cells reacting to the cyclic estrogen stimulus affects 5–10% of women in reproductive age. It causes mostly pelvic pain, dysmenorrhea, dyspareunia, infertility and menstrual irregularities². As a hormone dependent process it is mostly associated with estrogen but also genetic and environmental aspects play a role in development of endometriosis³. The available evidence stresses the role of inflammatory factors and especially the role of macrophages in the process of endometrial tissue adhesion and growth⁴. The G-protein coupled estrogen receptor (GPER) is considered an estrogen receptor transducing estrogenic signals in a rapid non-genomic mode, however the evidence in the literature regarding a possible role of GPER in ovarian physiology and pathophysiology is rather restricted. In endometriotic tissue the GPER had been shown to be up-regulated. In analogy to this finding an alternative way of influencing macrophages and therefore influencing immunologic environment of endometriosis can be postulated⁵. Peritoneal macrophages retain receptors encoding for estrogen and progesterone and ovarian steroids also participate in producing an inflammatory

response in pelvic cavity and are involved in the growth of endometriosis⁶. Endometriosis associated macrophages undergo specific changes in estrogen receptor profile and maturation in dendritic cells.

Macrophages also play an important role at the feto-maternal interface during pregnancy. Besides many other causes like chromosomal abnormalities and infections that are blamed in case of early miscarriage, immunological tolerance at the feto-maternal interface is crucial for early pregnancy maintenance. Aiming at a well regulated trophoblast invasion and balancing maternal immunotolerance this process involves several types of cells with immunologic origin⁷. Among those T cells and natural killer (NK) cells have been described essential for early pregnancy development and implantation. However, decidual macrophages (DM) are the second most predominant cell population at the site of trophoblast invasion and are described as antigen presenting cells thus producing a variety of cytokines and regulating T cell activation⁸. Their role in maintaining early pregnancy or miscarriages has not been cleared yet. Both implantation and abortion are linked to the Fas/Fas ligand (FasL) system⁹. FasL is a transmembrane protein that by binding to Fas receptor, triggers apoptosis to the Fas-expressing cells, like trophoblast and decidual cells do to the Fas bearing T cells of the feto-maternal interface¹⁰. Therefore trophoblast and decidual cells can influence the risk of rejection of the semi-allogenic embryo. Abortion has already been described as a state characterized by a high expression of FasL in decidual leukocytes¹¹. As DM are expressing FasL¹² it is of interest whether FasL might be deregulated in DM in cases of spontaneous miscarriages.

Correct trophoblast invasion and its consequences reach as far as to the third trimester of pregnancy and placenta function. At that time of pregnancy a considerably high number of women (3–14% depending on the population studied¹³) suffer from elevated blood pressure and proteinuria. Preeclampsia (PE) is defined by a newly elevated blood pressure of above 140 mmHg systolic and 90 mmHg diastolic combined with urinary excretion of more than 300 mg protein in 24 hr urine collection

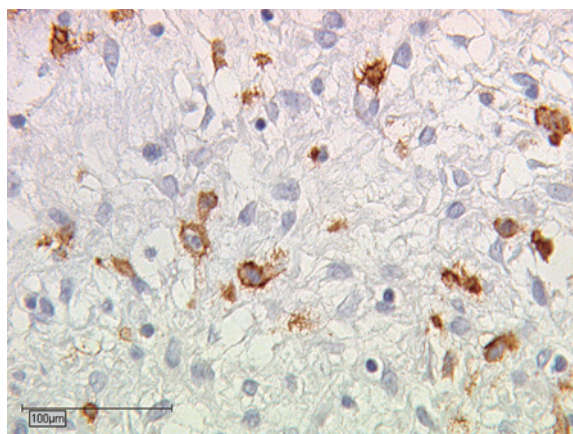


Fig. 1. Visualization of the DC sign in macrophages in ovarian endometriosis. Macrophage plasticity could be shown by an elevated IR score concerning DC sign in endometriotic tissue compared with physiological ovarian tissue.

and is often accompanied by headache, blurred vision or abdominal pain^{14,15}. Placental malfunction in sense of improper cyto-trophoblast invasion of spiral arteries in the endometrium is considered to be one of the reasons for the development of PE. Alternatively large placental mass as in twin pregnancies or deterioration of placenta as in late term pregnancies are supposed to induce PE by a vast amount of soluble factors leading to the characteristic systemic vascular reaction of PE¹⁶. The inflammatory character of PE as pregnancy associated disease has been focused as pathophysiological pathway in the last several years¹⁷. Especially DM that are able to confer and interact with trophoblast cells are likely to play an important role in balancing and regulating trophoblast function. In pregnancies complicated by PE activated macrophages have been shown to secrete tumor necrosis factor α (TNF α at levels that lead to the death of the invading extravillous trophoblast (EVT) cells and therefore prevent them from invading and repairing damaged vessels¹⁸. However, their exact function and timeline in this interaction is largely unexplored and remains unknown. Obviously macrophages as part of the innate immune system have a major part of endometriosis as state of chronic inflammation and take also an important role in interaction between

trophoblast and immune system as seen in miscarriages and PE. This work is intended to show our results on macrophage function, characteristics and special plasticity and differentiation in these partly immunological diseases.

Endometriosis

Fifty-one women at reproductive age were included in this study to get insight in macrophage presence, activation and differentiation at endometriosis manifestations. Normal ovaries (n=18) were from patients who underwent bilateral adnexectomy at the Department of Obstetrics and Gynecology of the Ludwigs-Maximilians-University (LMU, Munich, Germany). Ovaries with ovarian endometriosis (n=33) were randomly selected from the archives of the Department of Obstetrics and Gynecology of the LMU (Munich, Germany). The ovarian tissue showed according to previous studies¹⁹ an elevated concentration of CD68 positive cells by immuno-histochemical staining. Comparing tissue from control ovaries with endometriotic ovarian tissue there was a 1.5 fold increase in concentration of CD68 positive macrophages. Besides of an elevation in macrophage concentration at the stained tissue there is evidence for macrophage plasticity concerning dendritic cell maturation and other lymphatic cell lines²⁰. The DC sign implementing the maturation of dendritic cells also appeared to be elevated in ovarian tissue affected by endometriosis compared to the control group (**Fig. 1**). Finally we were able to identify the co-expression of GPER in about 90% of CD68 positive cells at endometriotic tissue. Regular ovarian tissue showed this co-expression of CD68 and GPER in less than 20% of the CD68 positive cells. GPER which had been shown to be distributed widely in the human body including brain, liver, lung, heart, bones, placenta and others, is clearly identified in breast glands, endometrium cells and ovarian tissue²¹. Although GPER function in the human ovary has not been clarified yet⁵ it is now found not only in ectopic endometrium but also in macrophages at the manifestation site of endometriosis. Macrophages react to hormonal

stimuli like estrogen by the classical nuclear steroid hormone receptor²² which is expressed in macrophages thus implying that in endometriosis inflammation and activation of immune reaction could be estrogen dependent. Now for the first time GPER has been demonstrated to be up-regulated in macrophages involved in endometriosis, which allows the hypothesis of a different endocrine activation of macrophages promoting endometriosis via GPER.

Abortion

To analyze macrophage activation and the role of FasL in DM a total of 20 cases were included in a comparative study with 9 cases of elective termination of pregnancy (ETP) and 11 cases of spontaneous miscarriages (SM). Samples from ETP were provided from private practice clinics in Munich, Germany. Maternal and gestational age-matched tissues samples from spontaneous miscarriages were additionally collected from the archives of the Department of Obstetrics and Gynaecology, Innenstadt, Munich, Germany. Thrombophilia and autoimmune diseases were ruled out and cryotypic analysis was performed in all samples to exclude chromosomal abnormalities. Microbiologic analysis ruled out infectious causes for miscarriage. The samples of ETP and SM were compared after double immunofluorescence and SM samples showed an extensive up regulation of FasL. As had been shown earlier DM express FasL¹² and therefore further analysis to detect macrophages as the carrier of elevated concentrations of FasL in abortion samples was needed. Apoptosis was found to be significantly more present in SM than in ETP samples by using immunohistochemistry and confirming these results by the TUNEL assay. Using dual immunofluorescence with HLA-G (an extravillous trophoblast marker) and anti-M30 cytodeath trophoblast apoptosis could be revealed markedly increased in spontaneous abortions. Furthermore macrophages were identified by immunohistochemistry against CD68 near the trophoblast in normal pregnancies as well as in spontaneous abortions. However, in the latter the

number of macrophages is increased. Finally dual immunofluorescence against CD69 and FasL showed a significant increase of FasL in macrophages, that are involved in spontaneous abortions⁷.

Summing up today's knowledge about DM two classical phenotypes -M1 as promotor of inflammation and M2 as immuno-modulatory phenotype- are known. Therefore M2 phenotype would be expected during regular developing pregnancy keeping balance at the feto-maternal interface. The M1 phenotype could be linked to pregnancy failure and miscarriage. As macrophages have been demonstrated to present dynamic plasticity this rather simplified model of thought is abandoned. Obviously macrophages can react to stimuli according to the surrounding tissue and conditions, which leads to the consideration of a more complex function of DM in the context of early pregnancy and spontaneous abortion. So far only few reports on macrophages in terms of population and polarization in regard of miscarriages exist. Though in some studies an increase in number has been described, the differences in number between SM and ETP and recurrent abortions and ETP respectively were not considered significant^{23,24}. We could demonstrate an increase in DM population in cases of SM, which might support a process of inflammation and trigger further abortion. The role of the Fas/FasL system in the conditions of spontaneous abortion and pregnancy had been described for T cell apoptosis by decidual and trophoblast cells earlier¹². Our group was able to show an increased expression of FasL in DM in cases of spontaneous miscarriages. Baseline FasL expression by DM could be a part of an M2-like polarization. FasL-expressing DM could induce apoptosis to Fas-bearing activated T-cells reducing potentially harmful immune responses against the semi-allogenic embryo. FasL-expressing DM could mediate the T-cell triggered trophoblast apoptosis highlighting an alternative way of inducing apoptosis in SM. Whether the expression of FasL is linked to stress related peptides like shown for CRH and Urocortin in T-cell FasL expression is still to be explored. DM and their FasL expression in regulating apoptosis play possibly a role in normal

pregnancies and spontaneous abortions. However, the answer whether the increased apoptosis is causing the embryos death or whether it is just a reaction to apoptosis as a process against the trophoblast, is still missing.

Preeclampsia

16 placental tissue samples from women who gave birth at the Department of Obstetrics and Gynaecology of the LMU Munich were collected. These samples included 8 specimens from women with preeclampsia (mean week of gestation 34+/-3.2) and 8 age matched patients (mean week of gestation 35+/-2.3) with a normal course of pregnancy. Patients with chorioamnionitis, chronic hypertension, chronic renal disease, cardiac disease, pre-existing diabetes mellitus and gestational diabetes were excluded. Analysis was done by immunohistochemistry, immunofluorescence and western blot technique. Placental biopsies showed an elevated corticotropin releasing hormone (CRH) protein expression in preeclamptic placentas compared to the control samples. Preeclamptic placentas revealed to have a higher rate of early (up to 25%) and late (up to 15%) apoptosis compared to normal placentas with rates of below 10% and 1-5% respectively. DM in control placentas were not identified expressing FasL, however preeclampsia samples were rich in FasL positive macrophages in contrast to NK and T-cells, which did not show to express FasL. Both control placentas and preeclampsia samples showed Fas peptide expression at the same level in the EVT. A 6 h incubation with CRH showed an 1.5 fold rise in FasL expression at DM, which is comparable to the effect of LPS derived from E.coli. An increased macrophage population in the placenta has been associated with preeclampsia before in terms of hampering trophoblast invasion and re-modelling of uterine spiral arteries²⁵ and as mechanism of triggering apoptosis of trophoblast cells²⁶. We were able to show larger numbers of apoptotic EVT in preeclamptic placentas compared with normal samples. Additionally in preeclamptic placentas, decidual macrophages, that are increased in number,

were found to be FasL positive and an upregulation of CRH in EVT was noted. The generated data shows a possible pathway by which CRH could increase the potential for apoptosis of the Fas expressing EVT cells by influencing DM via FasL expression. The pathogenesis of preeclampsia and especially of insufficient placentation seems to be partially based on a combination of macrophages, CRH and Fas/FasL interaction. The effect and origin of CRH in pregnancies has already been described as vaso-dilatory response improving fetomaternal blood circulation released by the placenta as a consequence of pathologic stress conditions^{27,28}. Highlighting the positive aspects of CRH effects on the fetus like accelerated organ maturation, improved placental blood flow and preterm labor in case of endangered fetal survival are mentioned in literature²⁸. These contrary statements raise the question whether CRH is part of a compensatory mechanism in preeclampsia and whether it is a part of the primary pathophysiology of preeclampsia by inducing changes in the Fas/FasL system and therefore triggering macrophages promoting apoptosis. The aberrant expression of CRH in preeclampsia may activate the FasL-positive DM, impair the physiological turnover of EVT and eventually disturb placentation. Concluding from our findings we propose that CRH potentiates the cytotoxic effect of macrophages not only by up-regulation of FasL but also by inducing the production of pro-inflammatory cytokines by macrophages.

Conclusion

Our results highlight new aspects of macrophage biology in endometriosis and pregnancy physiology and patho-physiology. Concerning endometriosis the involved population of macrophages found was in accordance with previous studies²⁵. However this is the first time that significant GPER up-regulation is demonstrated not just in tertiary follicles but also in macrophages. This highlights a potential alternative way through which estrogen may modulate immune response of macrophages in endometriosis. Further studies employing larger series and functional

approaches are needed to prove this hypothesis and evaluate the role of GPER concerning the pathogenesis of endometriosis and in order to evaluate therapeutic approaches. Looking at our findings in cases of spontaneous miscarriages the number of DM appeared to be increased and these macrophages showed an up-regulation of FasL expression⁷. A regulatory function of these macrophages in early pregnancy can be suggested, though these results need to be viewed cautiously due to the small number of samples. For any further studies concerning the function of DM further studies with larger samples are needed to verify the current results and evaluate their clinical impact. Our data strongly indicate that macrophages hold key roles in various gynaecological disorders and might be crucial to further elucidate their pathophysiology. In analogy to the immunohistochemical findings in cases of spontaneous abortion large numbers of DM were found in preeclamptic placentas^{24,25}. DM showed also an up-regulation of FasL which is supposedly caused by up-regulated CRH. In its immunomodulatory role CRH may alter trophoblast invasion and hamper re-modelling spiral arteries and therefore cause placenta malfunction in the sense of preeclampsia²⁷. In order to develop therapeutic interventions for pregnancy disorders associated with insufficient placentation the mechanism of early placentation needs to be understood entirely.

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