

# Immunomodulation during human pregnancy

## Placental exosomes as vehicles of immune suppression

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### Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i Astrid Fagraeus hörsal A103, byggnad 6E, Umeå Universitet, fredagen den 25 april, kl. 13:00.

Avhandlingen kommer att försvaras på engelska.

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Clinical Microbiology/Clinical Immunology  
Umeå University  
Umeå 2014

**Organization**  
Umeå University  
Clinical Microbiology

**Document type**  
Doctoral thesis

**Date of publication**  
4 April 2014

**Author**  
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**Title**  
Immunomodulation during human pregnancy -  
Placental exosomes as vehicles of immune suppression

### **Abstract**

The mammalian pregnancy comprises a challenge to the maternal immune system since the fetus is semi-allogeneic and thus could be rejected. Pregnancy success is associated with the placenta that is not only essential for oxygen supply, nourishment and pregnancy hormones but also in the protection of the fetus against maternal immunologic attack. The aim of the current studies was to elucidate the role of human placenta as an immunomodulatory organ with a special focus on placental exosomes as means for establishment of maternal tolerance to the fetus.

By immunoelectron microscopy we discovered that human syncytiotrophoblast constitutively produces and secretes exosomes. Exosomes are 30-100 nanometer sized membrane microvesicles of endosomal origin that convey intercellular communication. Exosomes are secreted through the endosomal compartment and reflect the type and the activation state of the cells that secrete them. They carry cytosolic and membrane-bound proteins and ribonucleic acids and can influence and re-program the recipient cells. We developed methods for isolation and culture of trophoblast and placental explants from human normal first trimester pregnancy and isolated exosomes from the culture supernatants. The following aspects with potential importance in the establishment of maternal tolerance towards the fetus were studied: 1) exosomal modulation of the NKG2D receptor-ligand system, a major cytotoxic pathway for NK- and cytotoxic T cells; 2) placental exosome-mediated apoptosis of activated immune effector cells; and 3) Foxp3-expressing T regulatory (Treg) cells in human pregnant uterine mucosa, the decidua.

Our results show that human early syncytiotrophoblast constitutively expresses the stress-inducible NKG2D ligands, MICA/B and ULBP1-5, and the apoptosis inducing molecules FasL and TRAIL. While MICA/B were expressed both on the cell surface and intracellularly on the limiting membrane of multivesicular bodies (MVB) and on exosomes, the ULBP1-5, FasL and TRAIL were solely processed through the endosomal compartment's MVBs and secreted on exosomes. The NKG2D ligand-expressing placental exosomes were able to internalize the cognate receptor from the cell surface of activated NK- and T cells thus downregulating their cytotoxic function. In our studies of apoptosis we found that placental exosomes carry the proapoptotic ligands FasL and TRAIL in their active form as a hexameric complex of two homotrimeric molecules, required for triggering of the apoptotic signaling pathways. This finding was supported by the ability of isolated placental FasL/TRAIL expressing exosomes to induce apoptosis in activated peripheral blood mononuclear cells (PBMC) and Jurkat T cells. We also studied Foxp3-expressing CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in paired human decidua and blood samples from pregnant women compared to non-pregnant controls. The CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells were 10 fold enriched in the decidua mucosa compared to peripheral blood of pregnant women and non-pregnant controls. We discovered a pool of Foxp3-expressing, CD4<sup>+</sup>CD25<sup>-</sup> cells in human decidua, a phenotype consistent with naïve/precursor Foxp3<sup>+</sup> Treg cells. These results, together with our preliminary finding that placental exosomes express PD-L1 and membrane bound TGFβ might suggest that placental exosomes are providers of induction signals and possible promoters for generation of adaptive Treg cells. Our results provide evidence that placental exosomes are immunosuppressive and underline their role in immune modulation during pregnancy. The constitutive production and secretion of immunosuppressive placental exosomes create a protective exosomal gradient in the blood surrounding the foeto-placental unit. This "cloud of immunosuppressive exosomes" conveys immunologic privilege to the developing fetus and contributes to the solution of the immunological challenge of pregnancy.

**Key words:** exosomes, microvesicles, pregnancy, placenta, syncytiotrophoblast, immune privilege, apoptosis, cytotoxicity, T regulatory cells, NKG2D, MICA/B, ULBP, FasL, TRAIL

**Language**  
English

**ISBN**  
978-91-7601-034-1

**ISSN**  
0346-6612-1640

**Number of pages**  
73 + 4 papers