Methods: We consented patients with uncomplicated term pregnancy undergoing cesarean section at the University of Washington Medical Center, obtaining antepartum maternal blood, cord blood, and placentas. In order to determine single-cell phenotypes of leukocytes, we implemented multiple 30-parameter flow cytometry panels interrogating adaptive and innate immune cell subsets. In addition to *ex vivo* phenotyping of bulk leukocytes, we flow-sorted conventional lymphocyte populations for single-cell RNA sequencing.
Results: Major conventional and innate-like lymphocyte populations were similarly detected in circulation and placenta. Despite this, a substantial fraction of placental lymphocytes express biomarkers indicative of altered migration and tissue residence. These signatures are absent from lymphocytes recovered from maternal and fetal blood. Transcriptional analyses confirm that a subset of placental lymphocytes undergo a tissue-resident program.
Conclusion: Simultaneous high-parameter flow cytometry and single-cell RNA sequencing provides less biased analyses of tissue-specific modifications that immune cells undergo. We report a substantial fraction of lymphocytes that enter the placenta undergo a tissue-resident program, which has been largely understood in the context of skin and other barrier tissues. Our data suggest immune cells may fail to exit the placenta and recirculate in the periphery, highlighting the placenta is more than a circulatory interface. Despite observed phenotypic and transcriptomic differences of tissue-resident placental lymphocytes. further analyses will be required to understand their functional differences and role during disorder or disease.

P2.61.
TROPHOBLAST CELLS PREVENT NEUTROPHIL EXTRACELLULAR TRAP-INDUCED DAMAGE AND PROMOTE VASCULAR TRANSFORMATION SIGNALS IN NEUTROPHILS

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Objectives: Normal placentation entails a highly regulated interaction of maternal leukocytes and invasive trophoblast cells. Circulating neutrophils are activated during pregnancy and even more during pathological pregnancies. Vasoactive Intestinal Peptide (VIP) has immunomodulatory effects through its action on VPAC1 and VPAC2 receptors, both expressed by neutrophils. Our aim was to explore the effects of neutrophils and neutrophil extracellular traps (NET) on trophoblast cell function and whether trophoblast cell-derived factors and VIP condition neutrophils to favor angiogenesis and promote an anti-inflammatory environment required for normal pregnancy.
Methods: Peripheral blood neutrophils were isolated from healthy volunteers and cultured with human first trimester trophoblast cell line (Swan 71) conditioned media (CM) or with VIP. NET formation was induced with monosodium urate crystals (MSU). Trophoblast cell and neutrophil profiles were assessed by RT-qPCR, flow cytometry and confocal microscopy. Trophoblast migration was evaluated by wound healing assays and angiogenesis with the chorioallantoic membrane of quail embryos model assay.
Results: NETs isolated from MSU-activated neutrophils hindered trophoblast cell migration (%±SE Tb: 92.5±1.4; Tb+NETs: 70.0±3.3; $P<0.05$). CM and VIP prevented NET formation and thus reversed the effect on migration (%±SE Tb+neu+MSU+CM: 97.5±1.4; Tb+neu+MSU+VIP: 87.1±0.8; $P<0.05$). NETs increased CXCL8 and decreased TGF-β expression in trophoblast cells. On the other hand, factors released by trophoblast cells and VIP shaped neutrophils (Neu) to a proangiogenic profile with increased VEGF, Arginase-1, TGF-β and CCL2 expression ($P<0.05$) and increased vascular transformation as shown by the CAM assay (i.e. N° of segments, X±SE: Neu: 36±4, Neu+CM: 87±22, Neu+VIP: 62±15 $P<0.05$).
Conclusion: NETs adversely affect trophoblast cell function whereas factors released by trophoblast cells and VIP reverse this effect. Conditioning neutrophils with Tb cells stimulate angiogenic processes and might influence vascular transformation required during placentation.

P2.62.
HOMEOSTASIS LOSS IN VIP DEFICIENT MICE IS ASSOCIATED WITH ALTERED REGULATORY T CELLS RECRUITMENT

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Objectives: In order to have a successful pregnancy, it is required a balance between proinflammatory and tolerogenic mediators. Since VIP (vasoactive intestinal peptide) is a master regulator associated with Tregs induction and recruitment, we aim to understand the relevance of VIP in normal pregnancy with focus on the Tregs.
Methods: We used two mice models: VIP Knockout and Foxp3-IRES-GFP, both in a C57 background. The serum and tissues obtained were analyzed by flow cytometry, RT-PCR and histological staining. We also performed Foxp3-adoptive cell transfer and migration assays.
Results: On the day of estrus we found histological differences between the ovaries and uterus of a WT mice vs VIP knockout mice (-/-). In uterus, these changes were accompanied with undetectable levels of expression Foxp3 in the (-/-) group, higher expression of RORγt and a decrease in IL-10 ($p<0.05$ Wilcoxon). Therefore, to study Tregs effects and trafficking, we performed adoptive transfer of Treg cells in VIP(+/-), and we found that they were mainly recruited into the uterus in relation to all other tested tissues accompanied with an increase in IL-10 expression. When pregnant mice at d3.5 were injected with VIP antagonist and sacrificied at d5.5, we found a similar profile that (-/-) mice, lower expression of TGFb, IL-10, VEGFc and Foxp3 in comparison with WT mice ($p<0.05$ Mann Whitney). Finally, we performed *ex vivo* migration assays using CD4+ sorted cells towards conditioned media from WT-explants at d5.5 cultured in the absence/presence of VIP with or without VIP-antagonist. VIP induced an enrichment of CD4+Foxp3+ cells while restrain total CD4+ recruitment; and VIP-antagonist prevented these effects.
Conclusion: VIP may contribute to an immunetolerant environment associated with normal pregnancy, in particular with the selective recruitment of Tregs to the uterus during the estrous cycle and in early embryo implantation.

P2.63.
POSSIBLE ROLE OF POLYCLONAL IMMUNOGLOBULIN G (IGG) OF PATIENTS WITH DIFFERENT CLINICAL MANIFESTATIONS OF THE ANTIPHOSPHOLIPID SYNDROME ON ACTIVATION OF INFLAMMATION BIOMARKERS

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