ImmunoTools *FlowISiAM* Award 2024 -

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Detection of early trophoblast damage using epitope-detection in inflammatory monocytes as a marker of preterm labor

Background

Preterm labor (PTL) is defined as labor that begins before 37 completed weeks of pregnancy. It is estimated to affect between 15 and 18% of all pregnancies (1). PTL represents the leading cause of infant morbidity and mortality, associated with a significant risk of diverse, long-term health complications (2). The majority of PTLs are triggered by a chronic inflammatory process caused by interactions between the maternal immune system and the semi-allogeneic fetus (1,3,4).

Under normal circumstances, maternal-fetal tolerance is maintained on two levels. Firstly, at the systemic level, pregnancy-maintaining hormones skew immune responses from pro-inflammatory Th1 towards Th2 responses (5). Furthermore, they promote the expansion and function of Tregs (6).

Secondly, a tolerogenic microenvironment is established at the maternal-fetal interface, where direct contact occurs between maternal and fetal cells. This environment is characterized by a tightly regulated infiltration of anti-inflammatory immune cells, such as Tregs (7).

Additionally, tolerogenic antigen-presenting cells, such as M2 macrophages, which constitute 20 – 30% of decidual leukocytes, contribute to the local differentiation of Tregs (1,8). Although non-Treg $CD4^+$ and $CD8^+$ T cells are found to infiltrate the decidua, these cells have unique signatures characterized by high expression of exhaustion markers and represent only a minority of infiltrating lymphocytes (9,10). The most prevalent lymphocyte subsets present in the decidua are NK cells. In contrast to NK cells in the peripheral blood, these decidual NK cells lack cytotoxicity. They are characterized by the production of cytokines, chemokines, and growth hormones that help guide trophoblast invasion.

However, during PTL, the tolerogenic mechanisms fail, and fetal antigens activate the maternal immune system. Although no specific antigen has been defined, several fetal molecules acting as a danger-associate molecular pattern (DAMP) can activate maternal immune cells. For example, cell-free fetal DNA has been shown to activate maternal monocytes to produce IL-1β and CXCL10, which can stimulate the activation of T cells to produce cytokines such as IFNγ and TNFα (11). These can, in turn, lead to changes in the polarization of local macrophages to M1 and exuberate the inflammation, leading to tissue damage and the release of additional fetal antigens and DAMPs (8).

We hypothesize that the earliest steps in maternal immune system activation lead to local damage to the trophoblast, which releases antigens that are subsequently phagocytosed by pro-inflammatory monocytes. In this project, we will evaluate if the *FlowISiAM* technology can be used to assess early inflammatory events at the end of the first trimester and if it could be used as a biomarker for predicting PTL.

Experimental design and methods

Blood samples from high- and normal-risk women will be collected at the gestational age of 9 – 12 weeks as part of our ongoing screening program. The blood will be analyzed using *FlowISiAM* technology for the presence of pro-inflammatory monocytes that contain peptides derived from trophoblast-associated proteins, i.e., KRT7 and Syncytin-1. The analysis will be performed on fresh peripheral samples as well as samples cryopreserved using the Cytodelics Whole Blood Cell Stabilizer (Cytodelics AB, Sweden). Blood from healthy, age-matched, non-pregnant women will be used as a negative control, and peripheral blood samples from ovarian carcinoma patients will be used as a positive control for the *FlowISiAM* assay. Patients will be monitored on a regular basis, and anonymized clinical information on demographics and time of delivery will be obtained.

Aim

Currently, no early markers for predicting PTL are available. Our study aims to evaluate whether the *FlowISiAM* technology could be used for non-invasive detection of early trophoblast damage that could predict the occurrence of PTL. This could substantially improve the risk stratification of pregnant women and enhance the medical care provided.

Cooperation Partner

Dr. Musil and Dr. Laštůvka will work together with ImmunoTools to adjust the experimental and instrumental set-up to conduct *FlowISiAM* analysis at the Institute of Hematology and Blood Transfusion (Prague). ImmunoTools and its partner SME, INVIGATE, will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test results. ImmunoTools' partner SME, INVIGATE, will help develop peptide-specific monoclonal antibodies for detecting peptides derived from KRT-7 and Syncytin-1 in inflammatory monocytes by *FlowISiAM*. The partners hope to collect preliminary data that could be used to prepare a joint research grant application.

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ImmunoTools *FlowISiAM* AWARD for

Jan Musil and **Zdeněk Laštůvka** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.