ImmunoTools *special* Award 2023

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Impact of Endoplasmic Reticulum Stress from Stromal Cells on Dendritic Cell Conditioning

Embryo implantation is a complex process that triggers an inflammatory response. This response is associated with the rupture of the uterine epithelium, invasion of the endometrium, and remodeling of maternal vessels to meet the increased oxygen demand. This inflammation is sterile and can be initiated by endogenous damage signals released during tissue remodeling, which is a defining characteristic of the maternal-placental interface formation. This cyclic reprogramming of the endometrium is known as decidualization. Decidualization is a critical program for placental development. It involves not only morphological changes in endometrial stromal cells but also alterations in their secretome. This secretome expansion is associated with the expansion of the endoplasmic reticulum, leading to a physiological response known as endoplasmic reticulum stress (ERS) and unfolded protein response (UPR).

Specifically, the decidualization program enables the secretion of immunoregulatory factors, influencing maternal leukocytes to adopt a regulatory profile. Notably, myeloid dendritic cells (DCs), though constituting only 1–2% of decidual leukocytes, play a pivotal role in initiating adaptive immunity and are therefore crucial for establishing immunological tolerance. DCs in human decidua represent a complex population, and their number fluctuates through different phases of the menstrual cycle and during pregnancy.

In recent years, it has been reported that tumor cells have the ability to transmit ERS from cell to cell. The ERS suffered by tumor cells present in a hypoxic microenvironment is transmitted both to other tumor cells and to myeloid cells found in the microenvironment. As a result, they activate their own UPR pathways. In the case of DCs, macrophages, and suppressing myeloid cells, ERS transmission would modulate their phenotype towards a profile that favors tumor escape. However, not all factors and mechanisms involved in ERS transmission have been studied in the maternal-fetal interphase.

We previously showed an altered ERS/UPR in endometria of women with reproductive complications. Considering that stromal cells condition maternal monocytes to a tolerogenic dendritic cell (DC) profile and ERS can be cell-to-cell transmitted

modulating immune profiles, we will evaluate the impact of ERS/UPR triggered on stromal cells on DCs differentiation and the involvement of ERS-transmission in their conditioning.

For achieving this aim we will use human endometrial stromal cell line (HESC) treated or not with a potent ERS-inducer (Thapsigargin). Then, the conditioned media (CM) will be collected after 48 h. Then, isolated monocytes from peripheral blood mononuclear cells from healthy women will be cultured with rhGM-CSF + rhIL-4 for 5 days in the absence/presence of CM. To confirm the ERS-transmission, we will evaluate the expression of ERS-sensors on DCs cultures. Then, we will determine the expression of the DCs differentiation markers, **CD1a** and **CD14**, in these cultures by flow cytometry. Next, we will evaluate the **CD86** activation marker. The secretome will be investigated in the supernatant after differentiation by measuring **IL-1B, IL-6, IL-8, IL-10, IL-12p40, and TNF-alpha** using ELISAs. Additionally, DCs survival in the absence/presence of CM will be compared by flow cytometry using FITC conjugated **Annexin V**-PI.

The ImmunoTools special Award will enable us to answer some of our research questions to gain a better understanding of how endoplasmic reticulum stress could be transmitted by endometrial stromal cells to DCs and define their differentiation.

ImmunoTools *special* AWARD for **Elizabeth Soczewski** includes 9 reagents

FITC - conjugated anti-human CD1a, CD16, CD45, CD80, HLA-DR, Annexin V

PE - conjugated anti-human CD11c, CD86

PerCP - conjugated anti-human CD14

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