ImmunoTools *special* Award 2019

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Correlation between tryptophan catabolism and the intrauterine renin-angiotensin system in human placenta and maternal blood at the end of pregnancy

Background

Pregnancy is a symbiosis between partially different organisms, therefore it requires a complex regulation of the maternal and fetal immune system, whose purpose is to guarantee protection against possible infections and at the same time to allow the invasion of the embryonic tissue in the context of maternal ones.

The enzymes of tryptophan (Trp) metabolism are IDO 1, 2 (indoleamine-2,3-dioxygenase) and TDO (trp-2,3-dioxygenase), these enzymes are abundant in placenta [1]. The catabolism of the Trp is involved in immunoregulation, and one of its functions within the placenta is the regulation of maternal-fetal tolerance. In the placenta, Trp is degraded into nicotinamide adenine dinucleotide by of enzymatic reactions, called the kynurenine pathway (KP). The activity of the KP increases under inflammatory conditions and different metabolites produced by this pathway can exert potent immunoregulatory functions [2]. The first step of the pathway can be carried out by any of the following enzymes, IDO1, IDO2, or TDO, that reduce Trp level thus suppressing T cell activity or by inducing anergy of reactive T-cells [3].

Intrauterine renin-angiotensin system (RAS) is important for normal pregnancy progression in both the mother and the fetus, as it is involved in placental development through processes such as angiogenesis, modulation of placental blood flow. All components of the RAS have been identified in term human decidua, placenta, myometrium, and fetal membranes [4]. Ang-(1-7) acting on the Mas

receptor has actions that antagonize the Ang II/AT1R pathway, *i.e.* inhibits the pro-inflammatory actions of the AngII/AT1R interaction, maintaining the integrity of the fetal membranes during pregnancy via the interaction of decidual prorenin with amniotic prorenin receptor [4; 5].

Objective

This study aims to assess the correlation between Trp catabolism and the intrauterine RAS system, which have never been investigated together, in order to assess how immune and inflammatory reactions cooperate for maintaining an active tolerance towards an allogeneic fetus.

We evaluate the presence and co-expression of IDO, TDO and Ang-(1-7) in placenta of spontaneous vaginal delivery and the correlation between these elements in the placenta and in the maternal blood.

- The use of specific antibodies for the cells of the immune system, both for dendritic cells and for lymphocytes, will allow us which cells express the proteins indicated above and to understand the state (immature or mature) of these cells by flow cytometric analysis; this analysis will be performed on cells isolated from tissue, both on the maternal and fetal sides of the placenta, and will be performed on PBMC cells isolated from peripheral blood. The anti-human antibodies to use are: CD1a, CD3, CD4, CD8, CD11b, CD11c, CD14, CD25, CD40, CD54, CD80, CD86, HLA-DR, Annexin V (ImmunoTools);
- mRNA will be extracted to evaluate the expression of IDO, TDO and angiotensin 1-7 by real-time PCR from the tissue;
- Cells derived from placenta will be isolated from maternal samples and cultured in an inflammatory environment [6], to evaluate how expression of IDO, TDO, Ang-(1-7) changes. Recombinant human cytokines to use are: rh GM-CSF, rh IL-6, rh IL-1beta, rh IL-17A, rh TNFα (ImmunoTools); isolated cells will be grown with Ang II or Ang-(1-7) to evaluate how the expression of IDO, TDO changes, following an inflammatory or anti-inflammatory stimulus.

References

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FITC - conjugated anti-human	CD1a, CD3, CD11b, CD25, CD80, Annexin V
PE - conjugated anti-human	CD4, CD11c, CD54, HLA-DR.
PerCP - conjugated anti-human	CD14
APC - conjugated anti-human	CD8, CD40, CD86
recombinant human cytokines	rh GM-CSF, rh IL-1beta/IL-1F2 , rh IL-6, rh IL-17A rhTNFα

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