UNIVERSITÀ DEGLI STUDI DI PADOVA

PROJECT

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1) Project title **Sex and estrogen signaling via GPER to control PD-L1: implications for slowing inflammaging**

Background

The endothelium plays an important role in initiating and shaping the immune response. We recently showed that in HUVECs from female donors, the immune checkpoint PD-L1 can be upregulated and released in soluble form (sPD-L1) in response to inflammatory cytokines and growth factors (GF), including IL-1β and VEGF. PD-L1 is important to control T cell activation, tolerance, and immune-mediated tissue damage in the context of inflammatory conditions. sPD-L1 is released by membrane PD-L1 or exosomes and likely behaves as an immunosuppressive molecule mimicking the effects of PD-L1.

Estrogens contribute to sex differences in immunity in a complex way, where low 17β-estradiol (E2) levels tend to increase while high levels inhibit the inflammatory response. The aging-related decrease in estrogen levels is accompanied by chronic, low-grade inflammation (inflammaging), which may contribute to increased risk of atherosclerosis complications. In this setting, the role of genetics and E2 in the functional regulation of PD-L1 in immune and endothelial cells remains largely unexplored. E2 signaling is mediated by nuclear receptors (e.g. ERα) and the G protein-coupled receptor GPER, whose role in immunity is emerging.

Objective

Based on this background, the **general aim** of the project is to further investigate factors (e.g. estrogens) and functional mechanisms underlying the sexual dimorphism of PD-1/PD-L1 in human ECs of different origins as well as in monocyte-derived macrophages.

The specific aims will be as follows: 1) to assess gender differences and the response to estrogenic agents in EC models and monocytes exposed to inflammatory mediators and GFs; 2) to define mechanisms of immune checkpoint release (exosomes, matrix metalloproteinases); 3) to define the functional role of endothelial PD-L1 and its pharmacological modulation, and 4) to assess sPD-L1 levels using blood samples from male and female patients with chronic inflammatory disease stratified by sex and age.

Methods

Experiments will be carried out in primary cultures of foetal and mature human endothelial cells (e.g. HUVECs, microvascular ECs) and monocyte-derived macrophages from male and female donors. Monocytes will be isolated from peripheral blood mononuclear cells. The expression and trafficking of immune checkpoints will be investigated by western blot, flow cytometry and ELISA. Cells will be challenged with estrogenic agents (GPER agonists including tamoxifen and raloxifen) in the presence

or absence of IL-1β or VEGF. Selected experiments will be carried out with anti-VEGF/ anti-IL-1β agents. The exosome content of immune checkpoints will be also measured after sequential centrifugation of cell culture medium and further spun on a sucrose gradient. Functional assays will include endothelial-leukocyte co-culture experiments and cytokine release.

Anticipated output

The project will extend current knowledge on how steroid hormones modulate vascular inflammation and immune responses according to sex and ageing.