PROJECT 1

DoS: Alessandra Petrelli

Title: Investigation of CD8 T cell-mediated mechanisms of insulin-resistance in human obesity and type 2 diabetes

Curriculum: BAIO

Link to OSR/UniSR personal page: https://research.hsr.it/en/institutes/diabetes-research-institute/units/tissue-and-systemic-immunity/alessandra-petrelli.html

Project description (*Number of characters, including spaces*: 2.000 - 3.000):

Insulin-resistance (IR), tightly associated with obesity, precedes the onset of type 2 diabetes (T2D) and is triggered by chronic low-grade inflammation, with a prominent role for pro- inflammatory macrophages infiltrating the visceral fat [1]. Although unexpected in a context of non-specific inflammation, the adaptive immune system has been shown to be a crucial for the development of IR. Furthermore, CD8 T cells are enriched in obese visceral adipose tissue (VAT) [2], their selective depletion ameliorates IR [3] and the T Cell Receptor (TCR) repertoire is restricted in the VAT of obese mice [4], suggesting the presence of antigen(s)-specific immune response. On the other hand, several studies now show that elevated BMI is associated with increased risk to develop (autoimmune) type 1 diabetes (T1D) [5-6], which is supported by the evidence that higher cumulative excess BMI confers greater risk of progressing to T1D in autoantibody-positive T1D relatives [7]. This further suggests that autoimmunity may underlie the pathogenesis of IR.

Our hypothesis is that, in human obesity, CD8 T cells localized in the VAT are primed by unknown autoantigens and exert metabolic activity, thus inducing IR. Therefore, our objective is to assess if pro-inflammatory CD8 T cells infiltrating the VAT of patients with coexisting obesity and dysglycemia induce IR in an antigen-specific manner.

In this project VAT and peripheral blood (PB) will be collected from obese patients with and without T2D undergoing bariatric surgery, lean non-diabetic controls and type 1 diabetes patients. In this 3-year project we propose to accomplish the following specific aims:

AIM I: Determine the presence and the profile of CD8 T cell clonotypes expanding towards VAT-derived antigen(s) in obese patients

- CFSE-labeled CD8 T cells from PB or VAT will be co-cultured with dendritic cells from PB of the same subjects pulsed in vitro with VAT-derived protein lysate.
-TCR deep sequencing of the Vß CDR3 region will be performed on total PB and VAT-derived CD8 T cells.
- In case clonal VAT-derived CD8 T cells will be identified, an attempt to identify the autoantigen(s) potentially driving CD8 T cell local responses will be performed by testing the expression of autoantigens described in other autoimmune diseases.
-Characterization of bulk and Ag-specific VAT-CD8 T cells will be performed by single cell RNA sequencing to assess CD8 T cell heterogeneity and identify pathways involved in CD8 T cell (dys)function.
-Once defined the phenotype of IR-inducing VAT-derived CD8 T cells, the presence of "pro-diabetogenic" CD8 T cells will be investigated in the circulation.

AIM II: Elucidate whether CD8 T cells isolated from VAT of obese patients are pathogenic and induce IR

-Control and patient-derived CD8 T cells will be co-cultured with differentiated pre-adipocytes.
- Differentiated pre-adipocytes will be co-cultured with CD8 T cells and serial concentrations of blocking antibodies against candidate molecules selected from RNA-seq analysis.
-ER stress level of adipocytes will be tested by WB, rt-PCR and Immunofluorescence (IF).

Skills to be acquired by the student (*Number of characters, including spaces*: max 600):

- Isolation of the stromal vascular fraction from the visceral fat of patients and controls
- In vitro co-culture assays with VAT-derived T cells
- FACS Sorting of VAT-derived CD8 T cells
- Supervised and unsupervised flow cytometry analysis
- RNA extraction
- Single cell RNA sequencing analysis
- TCR sequencing analysis
- rt-PCR
- Western Blot
- Immunohistochemistry and Immunofluorescence

References (max. 15)

1. Kahn SE et al, Nature, 2006. PMID: 17167471
2. McLaughlin T. et al., Arterioscler Thromb Vasc Biol. 2014. PMID: 25341798
3. Nishimura S et al, Nat Med, 2009. PMID: 19633658
4. McDonnell WJ et al, Diabetes. 2018. PMID: 30181158
5. Libman, I. M., et al. Diabetes Care 26 2003. PMID: 14514594
6. Hypponen, E., et al., Diabetes Care 2000. PMID: 11128347
7. Ferrara, C. T., et al., Diabetes Care 2017. PMID: 28202550