ImmunoTools IT-Box-139 Award 2012

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CHARACTERIZATION OF CIRCULATING AND MONOCYTE-DERIVED DENDRITIC CELLS IN METABOLIC SYNDROME PATIENTS

Type 2 diabetes and Metabolic Syndrome (MetS) are strong predictors of severity of occlusive coronary disease. The underlying cause of CVD is atherosclerosis, a chronic inflammatory process. It has been demonstrated that dendritic cells (DCs), professional antigen-presenting cells, are present in the vascular wall with others immunity cells, and increase in the atherosclerotic plaques. However DCs role in the pathogenesis of atherosclerotic lesions and their relationship with vascular wall cells is still unclear. Clinical and preclinical data highlighted the role of adipose tissue in the development of a systemic inflammatory state. Adipocytes secrete adipokines such as TNFα and leptin, which increase inflammation leading to insulin-resistance and atherosclerosis. The role of adipokines on DC maturation and function is not known. Moreover, the relationship between DC and vascular wall cells, in particular smooth muscle cells (VSMCs), following endothelial injury/dysfunction are still unclear. The aim of the present project is to study the role of DCs in vascular remodeling pathogenesis, with respect to MetS. Patients with a NCEP III diagnosis of MetS and different stages of glucose intolerance (NGT, IGT, IFG, DMT2), in primary prevention (no cardiovascular events) will be enrolled from the Obesity Agency, Diabetes Agency and Diabetes and Metabolic Diseases Section, AUO-Careggi. EDTA venous blood samples will obtained (protocol approved by the local Ethical Committee N. 0011762), in order to:

1. Evaluate adipokine plasma levels in MetS patients with different glucose tolerance.
2. Assess the different subsets of circulating DCs in MetS patients with different glucose tolerance. Using a direct immunofluorescence, single-platform **flow cytometry technique**, peripheral blood mononuclear cells (PBMCs) will be stained immediately with monoclonal antibodies that recognize the different lineage markers: BDCA-2 for lymphoid DCs, BDCA-1 and BDCA-3 for two subsets of myeloid DCs (BDCA1,2,3). Negative lineage markers (Lin1), containing antibodies against CD3-14-16-19-20-56) and BDCA+ cells were considered as DCs.

3. Study the role of some adipokines and anti-diabetic drugs (metformin, GLP-1 analog, DPP-4 inhibitors) in *in vitro* differentiation of circulating DC precursors obtained from MetS patients, into mature DCs. The yield of mature Monocyte-derived DCs (Mo-DCs) will be assessed by cytofluorimetric analysis of their phenotype with a flow cytometer and by confocal and electron microscopy. The expression of specific dendritic markers such as HLA-DR, CD1a, CD1c, CD40, CD80, CD83, CD86 will be measured.
4.Study DC/VSMC interactions, in *in vitro* models of inflammation and endothelial dysfunction;
5. Evaluate the interaction between patient's DCs and human vascular smooth muscle cells, with or without adipokine and anti-diabetic drug stimulation. For this purpose, a co-culture model between DCs and human coronary smooth muscle cell (CASMC) will be used.