ImmunoTools *special* Award 2015

Brigitta Buttari, BSc, PhD

Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy

Neuropeptide Y as regulator of macrophage phenotype and functions: a neuroimmune cue in atherosclerosis regression?

Atherosclerosis is a chronic inflammatory immune-mediated disease where neoangiogenesis, hypoxia and psychological stress are key pathological factors (*Sluimer JC and Daemen MJ. 2009; Everson-Rose SA et al. 2014*). Plaque is characterized by accumulation of macrophages within the atherosclerotic plaque which are subject to both retention and emigration signals (*Moore KJ, et al. 2013*). Macrophage numbers and phenotype influence plaque fate. Macrophage phenotype M1 and M2 represent two ends of a functional spectrum of polarization state, which may accelerate or decelerate atherosclerosis. M1 activity inhibits cell proliferation and causes tissue damage while M2 activity promotes remodeling and repair. Animal and clinical studies have been shown that during regression or stabilization of atherosclerosis, macrophages switch from to M1 pro-inflammatory phenotype to M2 anti-inflammatory reparative one (*Feig JE et al. 2012*).

Mechanisms underlying macrophage polarization and signals that guide cells to influx/exit plaque remain unclear. Therapies that alter macrophage inflammation by increasing polarization to M2 phenotype or macrophage emigration may be beneficial for atherosclerosis regression.

Neuropeptide Y (NPY), a major mediator of the stress system, is known to influence vascular tone and atherogenesis, as well as immune responses (*Elenkov IJ et al. 2000*). It modulates immune cells toward anti-inflammatory phenotype by signalling through NPY receptors and regulates leukocyte trafficking (*Buttari B et al. 2014*).

The topic of my research is to understand the role played by NPY or NPY receptor agonists on migration behaviour, phenotype and functions of human polarized M1 and M2 macrophage subsets in normoxic and hypoxic conditions. The main expected outcome is the characterization of angiogenic/angiolytic reparative macrophage responses to NPY in term of phenotype and functions.

Methods: Monocytes isolated from human blood of healthy donors, will be induced to differentiate for 6 days in the presence of either GM-CSF or IFN-gamma to obtain M1

macrophages, or M-CSF, IL-4, IL-10 alone or in combination to obtain M2 macrophages. Both phenotypical and functional activities of macrophages will be assessed after stimulation with NPY or NPY receptor agonists under normal or hypoxic conditions. Conditioned media of stimulated macrophages will be harvested to be tested for metalloprotease, cytokine, chemokine and growth factor expression. To analyse whether NPY influences macrophage migration *in vitro* chemotaxis of M1 and M2 macrophages in response to NPY, in the presence or absence of homing chemokines, will be performed.

The ImmunoTools selected products would be of great benefit to this project as they would be used to: 1) induce macrophage differentiation and polarization in the presence of recombinant human (rh) growth factors (**IFN-gamma; GM-CSF; M-CSF; IL-4; IL-10**); 2) evaluate the cytotoxic effects of NPY and NPY-receptor agonists on different macrophage subsets by **Annexin-V/Propidium Iodide** double staining and flow cytometric analysis; 3) control the phenotype of macrophage subsets by flow cytometric analysis (**CD1a, CD40, CD45, CD14, CD16, CD147, HLA-DR, CD80, CD86, CD62L, CD54, Control-IgG1, Control-IgG2a**; from other manufactures: CD163 CD36, CD68, CD206, CCR2, CX3CR1, CCR5, CCR7, adhesion receptor aVß3 integrin); 4) evaluate by ELISA assay and gelatin zymography the cell secretion capacity for cytokines, chemokines, growth factors (**hsCD147**; from other manufactures: IL-10, IL-1ra, IL-6, IL-12, TNF-alpha, G-CSF, GM-CSF, VEGF, MCP1, RANTES, MIP1a, MIP1b, IP-10, IL-8) and matrix metalloproteinases (MMP9, MMP2); 5) analyse macrophage migration *in vitro* chemotaxis in response to NPY, in the presence or absence of homing chemokines (**rhMCP-1 /CCL2, rhMIP-3b/CCL19, rhMIP-3a/CCL20**).

I will be very pleased to use ImmunoTools reagents for the analyses presented in this project.

ImmunoTools *special* AWARD for **Brigitta Buttari** includes 25 reagents

FITC - conjugated anti-human CD14, CD54, CD86, HLA-DR, Control-IgG1, Control-IgG2a, Annexin-V

PE - conjugated anti-human CD1a, CD40, CD62L, CD80, CD147

PerCP - conjugated anti-human CD45

APC - conjugated anti-human CD16

recombinant human cytokines: IFN-gamma; GM-CSF; M-CSF; IL-4; IL-10 rhMCP-1 /CCL2, rhMIP-3b/CCL19, rhMIP-3a/CCL20

human ELISA-set humana sCD147 for 96 wells, (each 3 reagents)