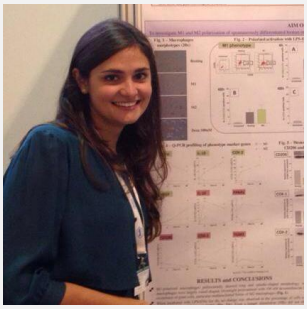
ImmunoTools *special* Award 2014



Serena Tedesco, PhD-student

Supervisor: Andrea Cignarella, PharmD, PhD

Department of Pharmaceutical and Pharmacological Sciences, School of Medicine, University of Padua, 35131 Padua, Italy

Modulation of human macrophage polarization by 17beta-estradiol

Young women have much lower rates of cardiometabolic disease than men. However, midlife women lose this apparent protection during the menopausal transition, so cardiometabolic disease is most common in post-menopause than any other stage of a woman's lifespan. Estrogen has a number of effects on the cardiovascular system including the modulation of inflammatory response and immune cell function (1,2). Estrogen receptors (ERs) are expressed on monocytes, the cells of the immune system that give rise to tissue macrophages. It's well established that monocytes and macrophages exist in at least two distinct phenotypes of differentiation/activation: classical/proinflammatory (M1) and alternative/anti-inflammatory (M2). Their activation state can be influenced by a variety of cytokines and microbial products (3).

Human monocytes were obtained from buffy coat of healthy donors or blood samples taken from pre- (Pre-MW) and post-menopausal (Post-MW) women. In particular, specific exclusion criteria were considered when collecting blood samples from women, as medication with anti-inflammatory drugs or antibiotics, oral contraceptive, and also chronic inflammatory diseases, infections, cardiovascular events or recent surgeries. PBMCs were isolated by density gradient centrifugation using a Ficoll-Paque solution. Cells were seeded at $1x10^6$/ml in serum-free RPMI 1640 medium. After 2 h, non-adherent cells were removed by repeated washing and the remaining adherent fraction was cultured over 7 days CO_2 in the presence of 10% FBS, supplemented with either M-CSF or GM-CSF for 6 days, to promote cell differentiation into resting macrophages (M0). Resting macrophages were polarized to M1 ($CD68^+/CCR2^+$) or M2 ($CD163^+/CD206^+/CX3CR1^+$) phenotypes by treating cells with LPS (1 µg/ml)/IFN-γ (10 ng/ml) or IL-4 (20 ng/ml)/IL-13 (5 ng/ml) for 48h, respectively, in the presence or absence of 17β-estradiol (E_2; 100 nM). Macrophage phenotypes were determined by flow cytometry using labeled antibodies.

Our central hypothesis is that the worsening of cardiometabolic risk profile in postmenopausal women is associated with a progressive shift of the heterogeneous monocyte/macrophage population towards more inflammatory subsets (M1) versus those involved in remodeling and repair (M2), leading to alterations inflammatory profile. So the ImmunoTools reagents would be of great benefit to this project as it would be used to correctly differentiate cells, which in turn will be used to asses expression of surface antigens in order to evaluate the effect of estrogen and how ER-mediated pathways control polarization of monocyte-derived macrophages in relation to gender and menopausal status.

References

1. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Hum Reprod Update* 2005; 11:411-23.
2. Harkonen PL, Vaananen HK. Monocyte-macrophage system as a target for estrogen and selective estrogen receptor modulators. *Ann N Y Sci* 2006; 1089:218-227.
3. Murray PJ, Wynn t. Protective and pathogenic functions of macrophages subsets. *Nat Rev Immunol* 2011; 11:411-23.

ImmunoTools *special* AWARD for **Serena Tedesco** includes 24 reagents

FITC - conjugated anti-human CD11a, CD11b, CD86,

PE - conjugated anti-human CD11a, CD11b, CD80,

APC - conjugated anti-human IL-6,

human IL-4 ELISA-set for 96 wells, (each 3 reagents),

recombinant human cytokines: rh EGF, rh FGF-a / FGF-1, rh FGF-b / FGF-2, rh GM-CSF, rh IFNgamma, rh IL-3, rh IL-4, rh IL-5, rh IL-6, rh IL-10, rh IL-13, rh MCP1 / CCL2, rh TNFα, rh VEGF-A/VEGF-165 DETAILS more AWARDS