ImmunoTools IT-Box-Cy55M-Award 2013

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Modulation of macrophage polarization by 17beta-estradiol

Macrophages are an essential component of innate immunity and play a central role in inflammation and host defense. Cells of the monocyte-macrophage lineage are characterized by considerable diversity and plasticity (1). 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 (2); 17β-estradiol (E_2) is known to mediate profound effects on monocyte and macrophage immune function (3,4) acting through estrogen receptors (ER). Data from our laboratory demonstrated that ERα mediates most of the beneficial effects of estrogens in the cardiovascular system (5).

Bone marrow-derived macrophages (BMM) will be obtained from tibias and femurs of wild type and estrogen receptor alfa knock out (ERKO) mice. Bone marrow cavities will be flushed with complete medium (RPMI 1640; 11,2 mmol/L glucose) and cells recovered by centrifugation at 400xg for 5 min and will be plated in 6-well plates at $2x10^6$ per well in RPMI supplemented with either M-CSF or GM-CSF for 6 days, to promote cell differentiation. At day 6 medium will be switched to low-glucose (5,5mmol/L) DMEM with 30% L-conditioned, at the following day macrophages will be activated for 4 to 48 hours using either LPS (5ng/ml) and IFNγ (12ng/ml) for M1 polarization, or IL-4 (10ng/ml) plus IL-13 (5ng/ml) for M2 polarization, according to the protocol by Odegaard et al (2008).

This study is aimed at studying the effects of E_2 on bone marrow-derived macrophages subsets, in resting state and after M1 or M2 polarization. So, the ImmunoTools *IT-Box-Cy55M* would be of great benefit to this project as it would be used to correctly differentiate cells, which in turn will be used to assess expression of surface antigens for classical and alternative phenotype and to determine intracellular cytokines production by flow cytometry.

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