17beta-estradiol modulates monocyte heterogeneity and macrophage polarization

A. Toniolo[1], S. Tedesco[1], G.P. Fadini[2], E. Vegeto[3], A. Cignarella[1], C. Bolego[1]

1. Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua
2. Dept. of Medicine, University Hospital of Padua
3. Dept. of Pharmacological and Biomolecular Sciences, University of Milan

Mononuclear phagocytes respond to environmental cues with the acquisition of distinct functional phenotypes, M1 (classical) or M2 (alternative), which in turn characterize different pathological conditions (1). 17β-estradiol (E$_2$) is known to mediate profound effects on monocyte and macrophage immune function (2, 3) acting through estrogen receptors (ER). Data from our laboratory indicate that ERα mediates most of the beneficial effects of estrogens in the cardiovascular system (4). We hypothesized that estrogen-dependent effects on the monocyte/macrophage system protect postmenopausal women from cardiovascular disease (5). Therefore, we investigated the effects of E$_2$ on mouse circulating monocytes and human monocyte-derived macrophage subsets in resting state (M0) and after M1 or M2 polarized activation.

METHODS: Monocytes from ERα-knockout and wild-type mice were stained with antibodies against surface antigens for classical (Ly6C^{high}/CCR2$^+$) and alternative (Ly6C^{low}/CX3CR1$^+$) phenotypes, respectively, and analysed by flow cytometry. Macrophages were obtained by culturing monocytes isolated from buffy coats of healthy donors. After 7 days, macrophages were polarized to M1 or M2 phenotypes for 48 h using LPS (1 μg/ml)/IFNg (10 ng/ml) or IL-4 (20 ng/ml)/IL-13 (5 ng/ml), respectively, in the presence or absence of 17β-estradiol or dexamethasone (both 100 nM). M1 and M2 phenotypes were characterized by flow cytometry as percentage of CD68$^+$/CCR2$^+$ or CD163$^+$/CD206$^+$/CX3CR1$^+$ cells, respectively. In selected experiments cells were further analyzed for CD14 and CD16 expression. ERα expression was assessed by quantitative real-time PCR and Western blot analysis in M0, M1 and M2 polarized macrophages. Intracellular TNF-α and IL-10 production was measured by flow cytometry.

RESULTS: Genetic deletion of ERα in mice was associated with a decrease in the anti-inflammatory (M2) monocyte subset, suggesting that ER-mediated pathways are involved in the development of inflammatory phenotypes. Resting macrophages displayed a predominant M2 phenotype (36.8±7.1% vs 8.1±2.2%, p<0.05). M1 polarization significantly increased the % of CD68$^+$ macrophages compared with M0 cells (17.8±3.3% vs 8.9±2.1%, p<0.05). The same activation pattern was observed for CD14^{high}/CD16^{low}/CD68$^+$ cells (27.8±5.3 vs 7.4±1.4%, p<0.05). Interestingly, following pro-inflammatory stimulus, M2 cells dropped from 36.8±7.1% to 20.5±4.7% (p<0.05), suggesting a dynamic M2-to-M1 switch. Overnight pretreatment with dexamethasone enhanced CD163 surface expression in resting (M0: 83.3±5.5% vs 49.6±9.5%) and M2-polarized macrophages (78.8±8.8% vs 50.7±11.2%, p<0.05), and reversed LPS/IFNγ-induced CD163 down-regulation (64.4±10.3% vs 23.4±8.3%, p<0.05). Similarly, treatment with 17β-estradiol reversed M1-induced down-regulation of the alternative phenotype through a 25% increase in CD163$^+$/CD206$^+$ cells. After 48 h, ERα was down-regulated in M1 compared with M0 macrophages, as assessed at both protein (-23%, p<0.005) and mRNA levels. By contrast, no differences in ERβ expression were observed between macrophage subsets. Finally, overnight pre-incubation with 17β-estradiol reduced LPS-induced TNFα production compared with untreated macrophages (13.8±2.3% vs 24.4±1.7%, p<0.05).

CONCLUSIONS: Overall we demonstrated that M1 activation with LPS/IFNγ down-regulate M2 markers and ERα expression, whereas E$_2$ and dexamethasone promote M2 macrophage signature counteracting the effect of pro-inflammatory stimuli on both M2 surface marker expression and cytokine production.

1. Mantovani A. et al. *J Pathol* 2013; 229:176-85
2. Bouman A. et al. *Hum Reprod Update* 2005; 11:411-23.
3. Harkonen PL, Vaananen HK. *Ann N Y Acad Sci* 2006; 1089: 218-227.
4. Cignarella A. et al. *Trends Pharmacol Sci* 2010; 31:183-89.
5. Bolego C. et al. *Arterioscler Thromb Vasc Biol* 2013; 33:1127-34