Driving Macrophage Polarization towards M2 Phenotype: *in vitro* and *in vivo* Studies

Geetika Bajpai,[1,2] Jeannine M. Durdik[1,3] and Julie A. Stenken[1,2]

[1]Cell and Molecular Biology Program, [2] Department of Chemistry and Biochemistry, [3]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701

Statement of Purpose: Macrophages are highly versatile cells. Pro-inflammatory, classically activated, M1 phenotypes, are produced when exposed to bacterial endotoxin or type 1 cytokines. Alternatively activated, anti-inflammatory, M2 phenotypes are induced by different cytokines including interleukin-4 (IL), IL-10, and IL-13. These differentially polarized phenotypic states have generated enormous interest in the field of regenerative medicine and biomaterials.[1, 2]

Although several studies have been performed in murine and human derived macrophages to understand macrophage polarization biology, more studies need to be performed in rats which serve as important model systems for biomaterials implant studies. This work aims to determine the macrophage phenotypic changes in response to IL-4, IL-10 and dexamethasone in *in vitro* and *in vivo*.

Methods: Culture systems using spleen and peritoneal derived macrophages from male Sprague-Dawley rats were used in all *in vitro* studies. Cultures were induced with IL-10 (50 ng/mL), IL-4 (50 ng/mL or 100 ng/mL), LPS (50 ng/mL) and dexamethasone (50 nM) alone or in combination for 24 hrs or 48 hrs. Gene expression, flow cytometry and ELISA assays were performed. Polyvinyl alcohol (PVA) sponges treated with IL-10 (200 ng/mL), IL-4 (200 ng/mL) and LPS (200 ng/mL) were implanted in the dorsal and ventral subcutaneous tissue of male Sprague-Dawley rat (preliminary study). Sponges were harvested after 8 days of implantation, wound fluid was collected and analyzed with multiplexed cytokine assays. Histological analysis was performed on implanted sponge samples.

Results: 1. Cytokine analysis in cell culture supernatant and in wound fluid.

In vitro, IL-4 (100 ng/mL) with LPS (50 ng/mL) renders the TNF-α concentration below detection while IL-10 (50 ng/mL) brings down TNF-α level by 10 fold indicating the modulation of cellular phenotype by cytokines when challenged with LPS in our model system. Wound fluid collected *in vivo* from LPS treated sponge had more IL-1β (2000 pg/mL) and IL-6 (500 pg/mL) concentration when compared to wound fluid collected from an IL-10 (IL-1β at 520 pg/mL and IL-6 at 240 pg/mL) treated sponge.

2. Surface receptor analysis by flow cytometry.

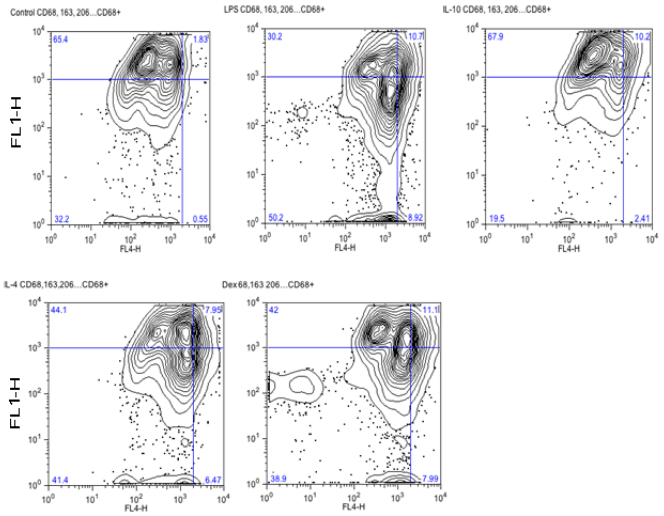

Fig 1: shift in Macrophage (CD68+) population between the FL1 (CD 163) and FL4 (CD 206). From top left to bottom right the cell poplulations are: Control, LPS, IL-10, IL-4 and dexamethasone.

CD68 positive (Pan macrophage marker) population was analyzed for CD163 and CD206 signal in the FL1 and FL4 channel, respectively. Based on a careful look at the epicenters of the population in contour plots, IL-10 causes maximum shift of the population towards the FL1 region showing high CD163 positives. For LPS treatment, the epicenter has moved below in the low FL-1 positive region. This is the expected shift with these treatments.

Conclusions: Cytokines modulate the secretory cytokine profile *in vitro* and *in vivo*. IL-10 treated cells have high CD163 positive population when compared to LPS and control samples. Future directions include understanding how *in vitro* macrophage polarization translates *in vivo*.

References:

1. Brodbeck et al. J Lab Clin Med. 2002;139:90-1002.
2. Higgins et al.,Am J Pathol. 2009;175:161-170.