36. **PMA-TREATMENT OF HUMAN MONOCYTES INDUCES A M1 PHENOTYPE IN ADHERENT MACROPHAGES.**

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https://www.youtube.com/watch?v=0JIMP5Fyl7s&t=1025s

INTRODUCTION: Human monocyte lines are widely used in basic research as model of inflammation, mostly following adherence with phorbol 12-myristate 13-acetate (PMA). However, the SC line, of normal human monocytes is not well documented, unlike tumour-derived cell lines, such as THP-1. **AIM:** The purpose of this study was to determine the phenotype of adherent macrophages, induced after the treatment with PMA in three different concentrations, starting from the most widely reported concentration in the literature. **METHODS:** Normal human monocytes SC (ATCC CRL-9855) were routinely maintained according to manufacturer's instructions. Cells were treated with Phorbol 12-myristate 13-acetate (PMA Sigma Aldrich P1585), in concentrations of 200 ng/mL, 100 ng/mL, 25 ng/mL and adhesion was documented using an Evos phase-contrast inverted microscope. Cell behaviour was validated by real-time impedance readings. The adhered cells were treated with bacterial lipopolysaccharide (LPS) in concentrations of 50 ng/mL (mimicking chronic inflammation) and 1 μ g/mL (mimicking acute inflammation). The supernatant was collected twice, after 4 hours, respectively after 18 hours of treatment with LPS. A screening of pro- and anti-inflammatory cytokines was performed using the multiplexing platform Luminex 200. ELISA tests were performed to validate the cytokines secretion: IL-6, IL-8, IL-10, IL-23 and TNF-α, using a LEGEND MAX Human ELISA kit specific to each cytokine. **RESULTS**: Cell adhesion was studied by time-lapse microscopy for 48 hrs. The lowest concentration of PMA which induced cell adherence was 25 ng/mL. Multiplex screening of cytokines showed a pro-inflammatory phenotype of macrophages stimulated with LPS. This finding was validated by ELISA tests for IL-6, IL-8, IL-23 and TNF-α (as pro-inflammatory cytokine) and IL-10 (an anti-inflammatory molecule). For the first category, we noticed a time-dependent response, present in adherent macrophages, but not in circulating monocytes. Regarding the second category of cytokines, the secretion is present only for the adhered and LPS treated cells. It is also present in a time-dependent manner (a higher concentration can be noticed in the collected supernatant after 18 hours of treatment compared with the one collected after 4 hours of treatment). **CONCLUSION**: The macrophages obtained from normal human monocytes with PMA are M1 type, regardless of the concentration used for differentiation.

Key words: SC Monocytes; Macrophages; Lipopolysaccharide; Phorbol 12-myristate 13-acetate; Inflammation.