## S79 REDUCED CD200 RECEPTOR EXPRESSION ON MONOCYTES IN SARCOIDOSIS

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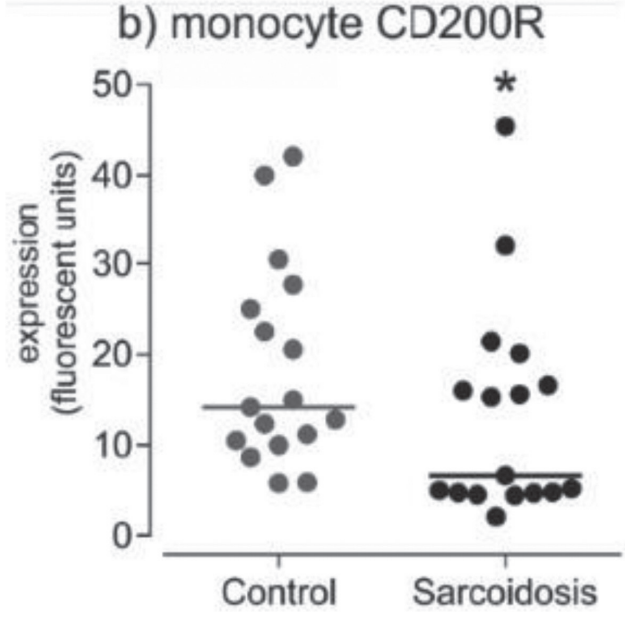
**Background** Sarcoidosis is characterised by release of pro-inflammatory cytokines in affected tissues. Lung macrophages, derived from blood monocytes, are potent producers of tumour necrosis factor (TNF) and interleukin-6 (IL-6) which contribute to the formation of sarcoid granulomata. Abnormalities of regulatory pathways that normally act to dampen inflammation could explain the hyper-active immunological state seen in sarcoidosis. The aim of the study was to assess the role of regulatory receptors in modulating monocyte cytokine production in sarcoidosis.
**Methods** Patients with sarcoidosis and healthy controls were recruited. Whole blood cytokine release in response to stimuli was measured by ELISA. Expression of the regulatory molecules IL-10R, SIRP-α/β, CD47, CD200R, and CD200L was measured by flow cytometry, and functional activity was determined using blocking antibodies.
**Results** Patients with sarcoidosis had less than half the number of T-lymphocytes in blood compared with healthy controls ($p < 0.0001$). Despite this, patients with sarcoidosis produced higher concentrations of TNF and IL-6 from whole blood in response to stimulation with phytohaemagglutinin. Kinetic analysis of TNF was consistent with release from monocytes. Expression of the monocyte regulatory receptor CD200R in patients with sarcoidosis showed a bimodal distribution (Figure 1), with 52.9% patients of patients having a CD200Rlow phenotype compared with 11.7% of healthy control subjects ($p < 0.0001$). CD200Rlow subjects produced more IL-6 in whole blood assays compared with CD200Rhigh subjects ($p < 0.05$). Experimental blockade of the CD200R axis increased pro-inflammatory cytokine responses, recapitulating the hyperactive monocyte phenotype seen in sarcoidosis.
**Conclusions** Reduced expression of CD200R on monocytes may be a mechanism contributing to monocyte and macrophage hyper-activation in sarcoidosis.




**Abstract S79 Figure 1** CD200R expression on monocytes in sarcoidosis and control subjects. Medians are indicated by horizontal lines; *p<0.05 using the Kolmogorov–Smirnov test; n = 17 healthy controls, n = 17 sarcoidosis patients.

## S80 MESENCHYMAL STROMAL CELLS (MSC) MODULATE HUMAN MACROPHAGES IN ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) VIA SECRETION OF EXTRACELLULAR VESICLES (EV) WHICH ENHANCE OXIDATIVE PHOSPHORYLATION AND REGULATE JAK/STAT SIGNALLING

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**Background** ARDS remains a major cause of respiratory failure in critically ill patients with no effective treatment. MSC are a promising candidate for therapy. However the mechanisms of MSC effects in lung injury are not well understood. We have recently shown that alveolar macrophages are critical cellular mediators of the therapeutic effect of MSC in the mouse model of *E.coli* pneumonia.[1] Here we focused on the paracrine effect of MSC on macrophage polarisation and intracellular signalling.
**Methods** Primary human macrophages were co-cultured with human bone marrow derived-MSC, without contact, at a 5:1 ratio, MSC-conditioned medium (CM) or EV with or without LPS or bronchoalveolar lavage fluid (BALF) from ARDS patients. A phospho-kinase array was performed on lysates for analysis of signalling cascades. Levels of pSTAT, Suppressor of Cytokine Signalling (SOCS) 1 and 3 proteins were tested by Western blot. Cell metabolism was investigated using Seahorse technology.
**Results** Cytokine and surface marker expression show that MSC promote an M2-like macrophage phenotype with enhanced phagocytic activity. MSC-CM enhanced mitochondrial respiration in macrophages and oligomycin inhibited the effect of MSC-CM on cytokine secretion and phagocytosis, suggesting that MSC-CM induced a metabolic switch to oxidative phosphorylation, characteristic of M2 macrophages. Consistently with the M2 phenotype, MSC induced a high SOCS1:SOCS3 protein expression ratio, accompanied with activation of STAT6 and inhibition of STAT1 phosphorylation. MSC effects were reversed by anti-CD44 antibody (important for internalisation of MSC-derived EV) suggesting that EV in MSC-CM are mediators of their effect. Importantly, adoptive transfer of EV-treated alveolar macrophages conferred protection in the mild model of murine LPS-induced pneumonia. EV contents responsible for these effects are currently being investigated.
**Conclusion** MSC promote M2-like macrophage polarisation via secretion of EV. This effect is associated with enhanced oxidative phosphorylation and altered JAK/STAT signalling, potentially regulated by differential expression of SOCS1 and 3 proteins.

### REFERENCE

1 Jackson MV, Morrison TJ, Doherty DF, *et al*. Mitochondrial Transfer via Tunnelling Nanotubes (TNT) is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS. *Stem Cells* 2016;**34**(8):2210–23.