M1 and M2 macrophages display profoundly different metabolic profiles and IL-10 production under hypoxic conditions

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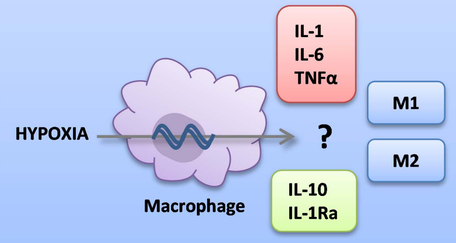
Introduction

Macrophages have a wide range of immunological and non-immunological functions, ranging from clearance of apoptotic cells, tissue remodelling, and release of pro and anti-inflammatory mediators at sites of tissue damage or infection. Subsets of phenotypically distinct macrophages may be uniquely adapted to perform these roles. Phenotypically and functionally distinguishable monocyte-derived cell lines also express unique metabolic profiles, suggesting metabolism may have a role in regulating function.

Aims

To determine the effect of oxygen, hypoxia (1%) and reperfusion conditions on the differentiation, metabolism and activation of blood derived CD14+ monocytes.

- Does hypoxia preferentially drive M1 or M2 differentiation?
- Are macrophage subsets differentially adapted to hypoxia?
- Does hypoxia favour a pro-inflammatory phenotype?

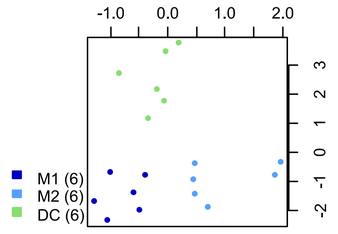


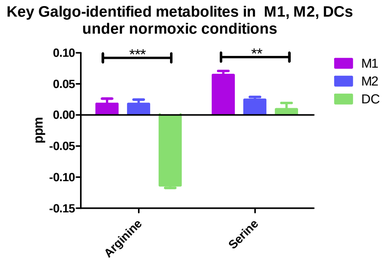
Methods

Primary human blood monocytes were differentiated to day 7 under hypoxia (1% O_2), normoxia (20% O_2) and reperfusion (1% O_2 culture, 20% O_2 feed). Samples of media were taken at each time point. On day 7 cells were stimulated with LPS and cultured overnight, with media and cell extracts collected the following day. Media samples and cell extracts were analysed for metabolic fingerprints using NMR spectroscopy and for cytokine profile by ELISA. NMR data was normalised and binned using ProMetab. Identification of key metabolites was subsequently performed by Galgo (genetic algorithm) and principal components analysis. Confirmatory glucose assays were performed to determine energy usage.

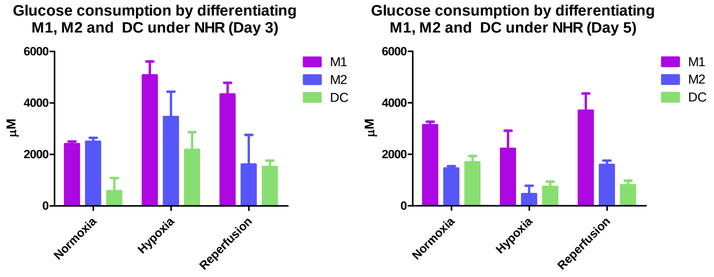
Results

Significant differences were seen in the metabolic profiles of M1s, M2s and DCs undergoing differentiation, with complete separation of cell types via serine and arginine levels alone.

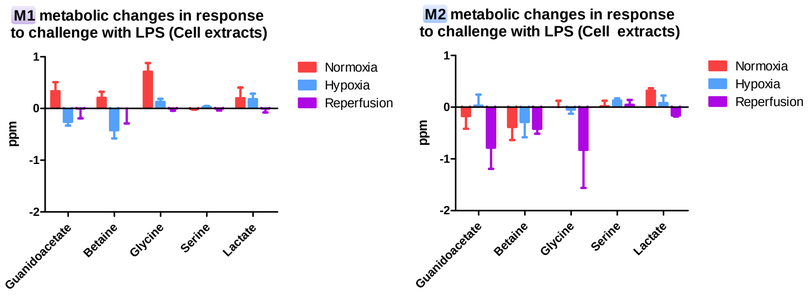




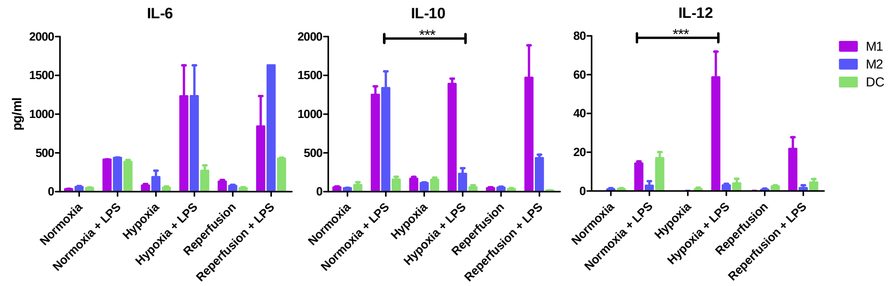
Further significant differences in energy usage profile were observed during the early stages of differentiation with M1s displaying much increased glucose consumption under hypoxia and reperfusion conditions, with M2s showing smaller increases. Consumption of glucose by differentiating DCs was minimal. These distinct metabotypes persisted out to completion of differentiation at day 5.



Post-differentiation M1s were demonstrated to be constitutively metabolically active under oxygen reperfusion conditions, with minimal metabolic differences between un-stimulated and LPS stimulated cultures. M2s in contrast showed large reductions in a number of metabolites under reperfusion conditions, suggesting up-regulation of cellular processes.



Under normoxia stimulation of macrophages with LPS is associated with increased IL-6, IL-10 in M1s and M2s, and increased IL-12 in M1s. Under hypoxic conditions this balance of pro and anti-inflammatory cytokines is reversed, with significantly reduced IL-10 and increased IL-6 from both subsets. Additionally, IL-12 is further increased in M1s, although slightly reduced in DCs. The overall profile under hypoxia is of a pro-inflammatory phenotype in differentiated M1 and M2 macrophages.



Conclusions

While differentiation under hypoxic or reperfusion conditions does not inhibit the development of distinct M1/M2 subsets, the resulting phenotypes are vastly altered. Results demonstrate a tendency towards a pro-inflammatory phenotype in M1 and M2 macrophages, with increases in IL-6 and IL-12, and concomitant reductions in IL-10. Results show clear metabolic profiles in M1, M2s and DCs. Stimulation of post-differentiation macrophages with LPS resulted in significant alternations in M2 metabolism but had no such effects in M1s under reperfusion. This suggests metabolic priming in this subset following exposure to reperfusion conditions as might occur during migration to inflamed tissues.