

P003 Effects of hypoxia on transcriptional regulation in human neutrophils

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Introduction: Hypoxia causes a delay in neutrophil apoptosis via a ferroprotein oxygen-sensing mechanism [Blood 2002; 100:3008-16]. Here we identify the regulation of HIF and key apoptotic transcripts by hypoxia.

Methods: Neutrophils were purified using discontinuous Percoll gradients and cultured in normoxia (N) (19 kPa), hypoxia (H) (3 kPa) or anoxia (A) (0 kPa). Apoptosis was assessed by morphology and Annexin V / propidium iodide flow cytometry. RNA was prepared using Trizol/DNase digest. Samples were run on gene array filters comprising 988 unique cDNAs. Findings were confirmed by TaqMan and Western blotting.

Results: Stabilisation of HIF-1 α protein was observed with H or inhibition of the prolylhydroxylase enzymes (PHDs) with dimethylxaloylglycine (1 mM). No change in transcript abundance was observed at 3 hours of H or A however by 6 hours GAPDH, macrophage inhibitory factor and NF- κ B showed an increase ($p < 0.005$ Cyber T test) in gene expression. This was confirmed using real-time PCR; H also stabilised NF- κ B at protein level.

Conclusion: Neutrophil apoptosis is inhibited by H and A via a PHD dependent pathway. Reduced oxygen tension is associated with HIF-1 stabilisation, transcriptional regulation and modifications in the expression of proteins known to influence apoptotic thresholds.