Hypoxia promotes a detrimental neutrophil phenotype with increased capacity for tissue damage in ANCA-associated vasculitis

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Background: Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is characterized by neutrophil-mediated vascular inflammation. Inflamed tissues are profoundly hypoxic. Neutrophils are able to deform to squeeze through the microvasculature but the degree of cellular deformability can impact their ability to traffic through capillary networks. We investigated the effect of hypoxia and ANCA-IgG on neutrophil biomechanical, transcriptional and functional properties.

Methods: Whole blood (WB) or isolated neutrophils from patients with AAV or healthy controls (HC) were examined. Neutrophils were primed with TNF (2ng/mL, 15min) then stimulated with ANCA- or HC-IgG (100ug/mL, 1h) under normoxia (21% O2) or pathological hypoxia (1% O2). WB neutrophil biomechanical properties were analysed using real time deformability cytometry. Isolated neutrophil transcriptional profiles were examined by bulk RNA-sequencing. Neutrophils were stained with AF488-Phalloidin (polymerised F-actin) and imaged using confocal microscopy. Neutrophils were co-cultured with glomerular endothelial cells (EC), and secretion profiles and neutrophil-endothelial transmigration were assessed.

Results: Untreated and stimulated HC WB neutrophils exposed to hypoxia were stiffer (lower deformability) than WB cells incubated in normoxia. Similarly, WB neutrophils from patients with active AAV were stiffer than those from patients in remission or HC, potentially reflecting *in vivo* exposure to hypoxia. Neutrophil deformability negatively correlated with AAV disease activity (BVAS score), and patients with organ damage (lung or kidney) had the stiffest neutrophils. RNA-sequencing did not demonstrate any differentially expressed genes between normoxic versus hypoxic HC neutrophils treated with ANCA-IgG, suggesting that biomechanical responses in hypoxia are not transcriptionally regulated, but gene ontology analysis showed hypoxic dysregulation of the cytoskeleton. Hypoxia increased HC neutrophil actin polymerisation, both at baseline and in synergy with ANCA-IgG, to equivalent levels seen in normoxic AAV neutrophils. Functionally, hypoxia increased ANCA-IgG-induced secretion of pro-inflammatory cytokines (IL-8) and cell adhesion molecules (ICAM-1 and VCAM-1) in neutrophil-EC co-culture. Additionally, hypoxia promoted neutrophil transmigration through intact EC monolayers, potentiating the effect of ANCA-IgG.

Conclusions: Hypoxia may induce a detrimental biomechanical neutrophil phenotype in AAV, whereby increased cell stiffness through remodelling of the actin cytoskeleton leads to neutrophil retention in the pulmonary and renal microvasculature. Hypoxia-induced excessive secretion of pro-inflammatory cytokines and cell adhesion molecules, and enhanced neutrophil-endothelial transmigration, increases the potential for neutrophil-mediated microvascular and tissue injury in AAV.