Physiologic hypoxia and lipid peroxidation products modulate Granulocyte-Macrophage Colony Stimulating Factor-dependent neutrophil bacterial killing in Pediatric Crohn's Disease

Adam D. Price, BS[1,2], Ingrid Jurickova, MD[1], Lee A. Denson, MD[1]

Cincinnati Children's Hospital Department of Gastroenterology, Hepatology, and Nutrition[1]
Medical Student Summer Research Program, University of Cincinnati College of Medicine[2]

Introduction: Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) enhances neutrophil regulation of gut bacteria. Crohn's Disease (CD) patients with reduced neutrophil GM-CSF signaling due to GM-CSF receptor mutations and GM-CSF auto-antibodies are more likely to experience stricturing complications requiring surgery. While lipid peroxidation products including 4-hydroxynonenal (4-HNE) are known to inhibit neutrophil activation, effects of physiologic hypoxia and 4-HNE on GM-CSF stimulated neutrophil bacterial killing were not known.

Hypothesis: Bacterial killing by GM-CSF primed neutrophils will be enhanced by physiologic hypoxia and suppressed by 4-HNE.

Methods: HL-60 human promyelocytic leukemia cells were differentiated to a neutrophil phenotype via 7-day incubation in DMSO. Primary human control or CD patient neutrophils or differentiated HL-60 cells were exposed to GM-CSF and/or 4-HNE, at 5% O_2 (physiologic distal gut hypoxia) and atmospheric O_2, exposed to *S. Aureus*, and the frequency of bacterial killing was determined via light microscopy. Statistical analyses were performed using GraphPad Prism software.

Results: The frequency of intra-cellular bacterial killing increased in CD patient primary neutrophils when exposed to hypoxia (99(3)) vs. atmospheric oxygen tension (92(6), $p<0.0001$) as well as in non-IBD control primary neutrophils when exposed to hypoxia (99.75(1)) vs. atmospheric oxygen tension (93(3), $p<0.0001$). The frequency of intra-cellular bacterial killing also increased in HL-60 cells when exposed to hypoxia (88(11)) vs. atmospheric oxygen tension (80(10), $p=0.0089$) and when primed with GM-CSF (80(4)) vs. basal conditions (73(8), $p=0.0446$). The frequency of intra-cellular bacterial killing was markedly suppressed in HL-60 cells stimulated with GM-CSF under hypoxic conditions in the presence of 4-HNE (68(7)) vs. HL-60 cells stimulated with GM-CSF under hypoxic conditions in the absence of 4-HNE (84(4), $p=0.0171$).

Conclusions: The relationship between decreased GM-CSF bioactivity and stricturing complications in CD implicates inadequate neutrophil activation as a possible mechanism of increased disease severity. Differentiated HL-60 cells behave similarly to primary neutrophils with regard to higher levels of bacterial killing in response to hypoxia and GM-CSF stimulation. HL-60 cells in a simulated environment of a damaged gut (hypoxic, +GM-CSF, +4-HNE) exhibit markedly reduced bacterial killing compared to HL-60 neutrophils in a simulated environment of a healthy gut (hypoxic, +GM-CSF, -4-HNE).

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