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

Adam D. Price, BS1,2, Ingrid Jurickova, MD1, Lee A. Denson, MD1

Cincinnati Children’s Hospital Department of Gastroenterology, Hepatology, and Nutrition1
Medical Student Summer Research Program, University of Cincinnati College of Medicine2

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% O2 (physiologic distal gut hypoxia) and atmospheric O2, 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).

Acknowledgements:
Cincinnati Children’s Hospital Medical Center Division of Gastroenterology, Hepatology, and Nutrition, supported by grants P30 DK078392 (Flow Cytometry Core) and R01 DK098231.

University of Cincinnati College of Medicine Medical Student Summer Research Program Fellowship, supported by NIH grant T35DK060444.