Abstract:

Previous studies from our group have identified that neutrophil infiltration during colitis results in mucosal hypoxia, due primarily to the neutrophil respiratory burst. Subsequently we demonstrated that the NADPH oxidase burst was primarily responsible for eliciting tissue hypoxia. Chronic granulomatous disease (CGD) patients exhibit mutations in neutrophil NADPH oxidase, resulting in an impaired ability to form reactive oxygen species, required for killing phagocytosed pathogens. Utilizing a murine CGD model we demonstrated an aggravated non-resolving inflammatory response to colitis coupled with diminished tissue hypoxia. CGD neutrophils fail to undergo apoptosis or externalize phosphatidylserine, resulting in diminished efferocytosis by macrophages and ultimately impaired resolution of inflammation. The principal aims of this project were to ascertain if and how pharmacological stabilization of HIF could overcome the resolution-deficit of NADPH oxidase-deficient mice.

Compared with wild type, CGD mice failed to induce mucosal hypoxia and developed a more severe and non-resolving colonic inflammation. Histological examination revealed extensive mucosal damage coupled with augmented infiltration of neutrophils and monocytes. Fluorescent in situ hybridization revealed mucosal infiltration of bacteria and 16S rDNA qPCR revealed dissemination of bacteria to mesenteric lymph nodes. To overcome the HIF deficit in CGD mice, we treated with a PHD-inhibitor and observed a restitution of inflammation resolution. Fewer bacteria were observed in both the mucosa and in the lymph nodes of PHD-inhibitor treated CGD mice. Moreover, histological and injury scores were improved. Analysis of PMN apoptosis in vitro by both flow cytometric Annexin-V/propidium iodide staining and Caspase 3/7 cleavage revealed augmented PMN apoptosis in the presence of PHD-inhibition. Finally, blocking NADPH oxidase reduced apoptosis, an effect abrogated by pharmacological HIF stabilization. In order to ascertain how HIF stabilization elicits resolution, CGD neutrophils were fluorescently labeled and adoptively transferred into wild type mice. TNBS colitis was induced in the recipient mice in the presence and absence of PHD-inhibitor. Flow cytometric analysis, gating on macrophages, revealed that HIF stabilization resulted in enhanced efferocytosis of CGD neutrophils.

In conclusion, pharmacological PHD-inhibition resulted in enhanced efferocytosis of CGDx PMN by macrophages and increased PMN apoptosis, independent of NADPH oxidase activity.

Research Category: Basic Science