

Host Intestinal Defenses Against *Clostridioides difficile* Infection in Chemotherapy Patients

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Introduction: *Clostridioides difficile* infection (CDI) is a common complication in patients undergoing cancer treatment with cytotoxic chemotherapy. Previous studies have shown that cancer patients are twice as likely to develop CDI compared to non-cancer patients. Exposure to antibiotics or chemotherapy disrupts the microbiome by killing protective intestinal flora which consequently promotes *C. difficile* spore germination and disease. The host has a multifaceted defense against CDI which includes colonization resistance conferred by the healthy microbiome and innate defenses provided by intestinal epithelial cells. One protective factor secreted by Paneth cells of the intestinal epithelium is lysozyme, an enzyme that degrades the cell walls of Gram-positive bacteria such as *C. difficile*. This project examines changes in intestinal epithelial cell-derived lysozyme resulting from CD toxin B (TcdB) and chemotherapy (melphalan) exposure.

Hypothesis: We hypothesized that chemotherapy-induced mucosal barrier injury and the resultant death of Paneth cells leads to decreased production of lysozyme.

Methods: Differentiated HIEs were exposed to TcdB and stained for nuclei, lysozyme, and necrosis. Live imaging was performed, and the fluorescent intensities for each stain were calculated. Additionally, stool samples from twelve patients undergoing cancer treatment were collected longitudinally at four different time points over the course of their treatment and recovery. The lysozyme concentration for each sample was determined via ELISA.

Results: A decrease in lysozyme staining was seen for both the toxin-treated and control (differentiation media-treated) samples; however, more cell death was seen in the toxin-treated HIEs. Analysis of stool lysozyme concentrations demonstrated that 11/12 (91%) patients experienced a drop in lysozyme levels during chemotherapy. Some patients, 5/12 (42%), showed recovery of lysozyme production with white blood cell recovery.

Conclusion: Our data indicate that TcdB causes increased toxicity of HIEs compared to an unexposed control. We have also shown that chemotherapy causes decreased concentrations of lysozyme in stool. Low lysozyme levels could in part account for the increased susceptibility to CDI during chemotherapy.

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