Histological and Functional Characteristics of a Novel Shiga Toxin-2 Secreting Citrobacter rodentium Model of Murine Shigellosis

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Background. Enterohemorrhagic *E. coli* (EHEC) cause life-threatening shigellosis with disease symptoms, including severe colitis, intense abdominal pain, hemorrhagic diarrhea, and hemolytic uremic syndrome (HUS). EHEC in murine models does not cause shigellosis. However, genetically similar *Citrobacter rodentium* (Cr) is a murine pathogen with common virulence strategies, but conveniently does not infect humans. Our objective was to utilize a novel Cr lysogenized by a Shiga toxin-2 phage (CrStx2) to produce a murine shigellosis model in which CrStx2-induced alterations to the gi tract, immune system, and kidneys can be delineated. And, to investigate the potential of microbiome manipulation to ameliorate the disease.

Methods. C3H/HeN 3mo mice were orally inoculated with 10^9 CFU of CrStx2 with sacrifice at onset of sickness behavior. MagPIX serum cytokine profiling was performed (N=5,5 each). Colon, kidney and urinary bladder were removed for structural and microscopic changes (N=3,3 each). Colonic mucosal barrier failure was assessed (N=4,4) and the whole-mount spatial distribution of neutrophil infiltrates within the colonic lamina propria (LP) and muscularis externa (ME) were characterize (N=4,4). Functional gastrointestinal transit was quantified using orally fed FITC-dextran (70kD, 80min)(p-values <0.05 for significance).

Results. On post-day 5, CrStx2 mice progressively lost weight and were sacrificed on day 8.1±0.19 (N=9). Colons exhibited histologically mild hemorrhagic mucosal sloughing with mucosal barrier failure due to the copious presence of 0.4μm fluorescent microspheres within the lamina propria after oral administration preferentially at lymph nodules. No microsphere transference in controls. Spatial analysis of neutrophil lamina propria infiltrates demonstrated increased clustering around enlarged and more numerous lymph nodules (8.0±1.07 vs. cntrl=5.6±1.09, N=7,4). CrStx2 did not increase the presence of neutrophils within the colonic (cntrl=2.5±0.97 vs. CrStx2=3.2±1.3, 100X) or jejunal ME vs. controls (N=3,3,4,4). Although, upper gastrointestinal transit was severely delayed (4.3±0.66 vs. cntrl=9.6±1.35, N=4,4). Serum demonstrated significant increases in circulating (VEGF=69.7±4.60 vs. 95.4±14.17 and IL-10=48.9±2.88 vs. 118.1±29.45) for control vs CrStx2, respectively. But, not IL-1α, IL-6, GM-CSF, KC, MCP-1 (MCAF), MIP-1α, RANTES or TNF-α) vs. control (N=5,5). The presence of IV injected 0.1μm microspheres in the urinary bladder lumen demonstrated renal failure. Microbiome manipulation by daily oral administration of probiotics (30,000 CFU) or a nonpathogenic Cr intimin mutant (10^9 CFU) did not alter the pathogenesis of shigellosis.

Conclusion. We developed a safe reliable murine model of shigellosis that encompasses intestinal colonization and severe Stx2-mediated tissue damage, which provides an opportunity to mechanistically explore its pathophysiology and potential treatments. Additionally, we concluded that microbiome manipulation was not successful in ameliorating the pathogenesis of shigellosis in this model.