A Murine Model of Shigellosis: Pathophysiology of Shiga Toxin-2 Secreting Citrobacter rodentium

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Background. Enterohemorrhagic E. coli (EHEC) causes life-threatening shigellosis with 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. Our objective was to study the pathophysiology of Shiga toxin-2 producing Citrobacter rodentium (CrStx2) in a murine model and also the effect of CrStx2 broth on primary cultured human intestinal endothelial cells.
Methods. Adult C3H/HeN mice were orally inoculated with 10^9 CFU of CrStx2 with sacrifice at onset of sickness behavior at post-inoculation day 5. Colons were removed for structural and microscopic changes. Colonic mucosal barrier failure was assessed (N=6) and the whole-mount spatial distribution of neutrophilic and mast cell infiltrates within the colonic lamina propria (LP) and muscularis externa (ME) were characterize. Functional gastrointestinal transit was quantified using orally fed FITC-dextran (70kD, 80 min) (N=5-6 each, p-values <0.05 for significance). Human intestinal microvascular endothelial cells were isolated for primary culture from human colonic surgical waste tissue.
Results. On post-day 5, CrStx2 mice progressively lost weight and were sacrificed on day 8.1±0.19. Shiga toxin-2 levels indicated the selective presence of 1.51 ng/ml of Stx2 toxin within the colonic lumen. Levels were undetectable in the jejunal lumen or serum. Morphologically, CrStx2 infected colons exhibited a histologically mild hemorrhagic mucosal sloughing and mucosal barrier failure to fluorescent microspheres (0.4µm). In addition, CrStx2 induced colonic mucosal lymphonodular hyperplasia (control nodules=4.2±0.31 vs. 8.4±0.55/colon) and hypertrophy (control area=0.42±0.287 mm^2 vs. 1.70±0.430 mm^2/lymph nodule). Spatial analysis of myeloperoxidase$^+$ neutrophil and avidin-Texas Red labeled mast cell infiltrates demonstrated increased clustering of the leukocytes around the enlarged, hyperplastic lymph nodules and submucosal microvasculature. Isolated colonic circular muscle strips displayed significantly diminished contractile activity to the cholinergic agonist bethanechol (30µM). And, interestingly, proximal gastrointestinal transit was severely delayed (geometric center calculation: 4.1±0.56 vs. cntrl=9.7±1.15). C3H/HeN mice orally infected with a Citrobacter rodentium containing a mutated intimin adhesin virulence factor did not display any structural or functional changes. We also tested the cytotoxicity of the CrStx2 culture broth on primary cultured human intestinal endothelial cells. Lactate dehydrogenase injury assay indicated a significant increase in LDH release with a CrStx2 concentration of 25% (4.8±4.19 vs. 54.8±5.42%) and a significant decrease in MTT cell viability (0.40±.041 vs. 0.23±0.007).
Conclusion. The development of a safe reliable human cell culture model and murine model of shigellosis that encompasses colonic colonization and severe CrStx2-mediated tissue damage provides an opportunity to mechanistically explore its pathophysiology in the hope of facilitating the development of potential therapeutic interventions.