A Characterization of the Response of Enteroids to LPS Provocation

S. Malin, N. Shroyer, J. Vallance
Cincinnati Children's Hospital Medical Center, Division of Gastroenterology, Hepatology and Nutrition

Introduction: Enteroids, self-replicating models of intestinal epithelium, provide a novel system to study the interactions of single bacteria and bacterial products on intestinal crypt-villus units. We sought to use murine intestinal crypt-derived enteroids to study the effects of LPS on the intestinal epithelium.
Methods: Proliferation, apoptosis, and differentiation were measured using immunohistochemistry and confocal microscope imaging, while inflammatory marker mRNA levels were quantified using qPCR.
Results: LPS had no effect on enteroid proliferation at a concentration less than 50 μg/mL. At 50 μg/mL, proliferation appeared to be increased, yet enteroid epithelial integrity was lost. Apoptosis proved difficult to measure, as cells are rapidly shed into the lumen and caspase-3 activation, a marker of all apoptotic cells, was found only occasionally in the intact epithelium. When examining cellular differentiation, higher concentrations (10 μg/mL) of LPS decreased the differentiation of stem cells into paneth cells ($P < 0.05$) as compared to controls (0 μg/mL). mRNA levels of CXCL-1, an IL-8 murine homolog, decreased with increasing levels of LPS ($P <0.05$). mRNA levels of RegIIIγ and IL-1β appeared to decrease, and mRNA levels of TLR4 appeared to increase, as the concentration of LPS increased, however the results were not significant. mRNA levels of TNFα and angiogenin4 did not appear affected by LPS concentration.
Conclusion: It was expected that enteroid exposure to LPS would result in an inflammatory-like state characterized by increased proliferation, apoptosis, and paneth cell differentiation as well as increased levels of inflammatory marker mRNAs. Instead, LPS exposure seemed to induce a quiescent state in which proliferation remained the same, differentiation decreased, and inflammatory markers either remained unchanged or decreased. TLR4 was the only marker that seemed to follow our initial hypotheses, as LPS exposure appeared to result in increased TLR4 levels, possibly indicating that TLR4 was indeed activated, but that the signaling cascade was inhibited. Epithelial TLR4 signaling might be dependent on an inflammatory primer, such as interferon, tumor necrosis factor, or T-cell interactions, in order to prevent excessive inflammation due to constant apical LPS exposure.
Acknowledgement: This study was supported in part by NIH grant: T35 DK 60444