Abstract

Three-dimensional organotypic co-culture model of intestinal epithelial cells and macrophages to study "Salmonella enterica" colonization patterns

Background: Three-dimensional/3-D organotypic models of human intestinal epithelium mimic the differentiated form and function of parental tissues often not exhibited by 2-D monolayers and respond to *Salmonella* in ways that reflect *in vivo* infections. To further enhance the physiological relevance of 3-D models to more closely approximate *in vivo* intestinal microenvironments during infection, we developed and validated a novel 3-D intestinal co-culture model containing multiple epithelial cell types and phagocytic macrophages, and applied to study enteric infection by different *Salmonella* pathovars.

Methods: NASA Rotating Wall Vessel (RWV) bioreactors were used to engineer a novel 3-D co-culture model of human colonic epithelial cells and macrophages. U937 cells were activated on collagen-coated porous scaffolds. HT-29 epithelial cells were then added and the model was cultured in the RWV under physiological fluid shear until optimal differentiation was reached, as assessed by immunohistochemical profiling and bead uptake assays. Contribution of macrophages to infection was assessed by colonization studies of *Salmonella* pathovars with different host adaptations/disease phenotypes (Typhimurium SL1344 and ST313 D23580, and Typhi Ty2). To recapitulate environments encountered prior to and during intestinal infection, *Salmonella* were cultured aerobically or microaerobically.

Results: The new model exhibited *in vivo*-like structural and phenotypic features, such as 3-D architecture, apical-basolateral polarity, well-formed tight/adherens junctions, mucin, multiple epithelial cell types, and functional macrophages. Phagocytic activity of macrophages was confirmed by uptake of inert, bacteria-sized beads. All *Salmonella* strains exhibited decreased colonization in co-culture relative to epithelial models, indicating antimicrobial function of macrophages. Multidrug resistant D23580 exhibited enhanced survival in both models following invasion. Pathovar-specific differences in colonization and co-localization patterns with different host cell types were observed.

Conclusions: A novel RWV-derived 3-D co-culture model of human colonic epithelium containing functional macrophages was developed and applied to study *Salmonella* infection. Our studies reinforce that mimicking the multicellular complexity of host tissues in the context of 3-D architecture is important for unveiling pathovar-specific infection properties, including visualization of co-localization patterns of pathogens within different host cell types. These findings emphasize the power of incorporating a series of related 3-D models within a study to identify microenvironmental factors regulating infection. These platforms can be used to study a variety of enteric diseases of both infectious (bacterial, viral) and non-infectious etiologies (IBS/IBD, drug toxicity, etc).