

Elucidating the Role of Host Purinergic Signaling During Rotavirus Infection

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Diarrheal diseases are among the top morbidity in children worldwide, with an estimated 1.7 billion cases of diarrhea and resulting in ~525,000 deaths each year. The enteric virus, rotavirus, accounts for ~27% of diarrheal disease in children, however the current pediatric suitable treatment is oral rehydration solution which does not alleviate diarrhea volume or symptoms. This highlights the need to better understand the pathophysiology of viral diarrhea to develop new therapeutics. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine. Yet, rotavirus influences cell types far away from the site of infection. We recently identified, using monkey and human rotavirus, that rotavirus exploits host purinergic signaling via the P2Y1 receptor to increase uninfected epithelial cell responses during infection. To assess if this dysregulation during rotavirus infection is conserved among all rotavirus strains and relies upon purinergic signaling, we used the recently developed cell culturable murine-like D6/2 rotavirus to assess the role of purinergic signaling *in vitro* and *in vivo*. Using MA104 cell lines that stably express cytosolic genetically-encoded calcium indicators, we characterized calcium signaling throughout D6/2 rotavirus infection by time-lapse imaging and determined if purinergic signaling is required using various small molecule signaling inhibitors. *In vitro*, we found that D6/2 infected cells have increased intracellular calcium signals which results in intercellular calcium waves that originate from the infected cells to neighboring cells. Preliminary data shows removal of ADP by Apyrase or blocking the P2Y1 receptor by BPTU results in decreased intercellular calcium waves, suggesting D6/2 infection relies upon purinergic signaling similar to other mammalian rotaviruses. Furthermore, treatment of D6/2 infected neonatal mice with a P2Y1 blocker (MRS2500) results in decreased incidence of diarrhea by day 4 post infection, confirming that P2Y1 receptor is also involved in rotavirus pathogenesis of a homologous virus strain. Collectively, these findings point to the conserved role of purinergic signaling and calcium waves in the pathophysiology of rotavirus infection.