Rotavirus Infection Elicits Host Responses via P2Y1 Purinergic Signaling

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Introduction: Rotavirus causes life-threatening diarrhea in children, resulting in ~130,000 deaths each year. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine. Yet, rotavirus dysregulates cells far away from the site of infection. We recently identified, using simian (SA11) and rhesus rotavirus (RRV), that infected cells release the purinergic signaling molecule ADP, which binds to the P2Y1 receptor on nearby uninfected cells. Furthermore, using the *in vivo* mouse model, mild rotavirus diarrhea in mouse pups was alleviated by daily treatment with a P2Y1 inhibitor.

Methods: To elucidate the role of purinergic signaling via P2Y1 receptors during rotavirus infection, we used the mouse-like rotavirus (D6/2) to investigate the effects of purinergic signaling in the context of severe rotavirus infection *in vivo*. C57Black6 mouse pups were orally gavaged D6/2 rotavirus at day 4-6 of age and assessed over the course of 5 days. Beginning at day 1 post infection, infected pups were treated daily by oral gavage with saline or 4mg/kg MRS2500, a selective P2Y1 antagonist. Stool was collected and scored for diarrhea daily prior to each treatment. Pups were euthanized and small intestine tissue was collected at 3 and 5 days post infection for immunostaining, qRT-PCR and luminal contents.

Results: Similar to mild rotavirus infection, treatment of D6/2-infected mouse pups with MRS2500 results in decreased severity and incidence of diarrhea. MRS2500 treated pups also exhibit decreased luminal serotonin and chloride content compared to control infected pups. Together, these results confirm that P2Y1 signaling is also involved in the pathogenesis of a homologous murine rotavirus strain. Viral stool shedding, assessed by qRT-PCR for rotavirus gene 11 levels, revealed that MRS2500 treated pups had significantly lower viral shedding starting at day 4 post infection compared to saline treated pups, which suggests P2Y1 signaling may amplify rotavirus replication.

Conclusion: Collectively these findings point to the conserved role of purinergic signaling in the pathophysiology of rotavirus infection, and indicate P2Y1 is a new candidate for host-targeted therapeutics that could have both antiviral and antidiarrheal effects against rotavirus pathophysiology.