ROTAVIRUS SYMPOSIUM

MARCH 14-16 2023 BALI INDONESIA

Learn more on www.sabin.org

14th International Rotavirus Symposium The Ruth Bishop Keynote Lecture Bali, Indonesia March 16, 2023

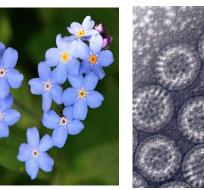
New Insights into Rotavirus Pathogenesis and Vaccines

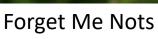


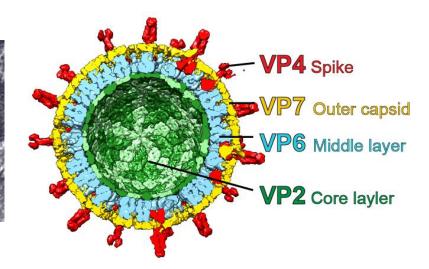
Ruth Bishop

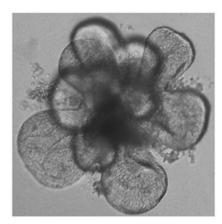
Mary K. Estes, PhD **Baylor College of Medicine**

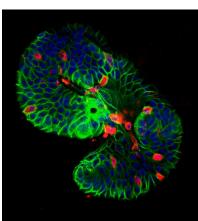




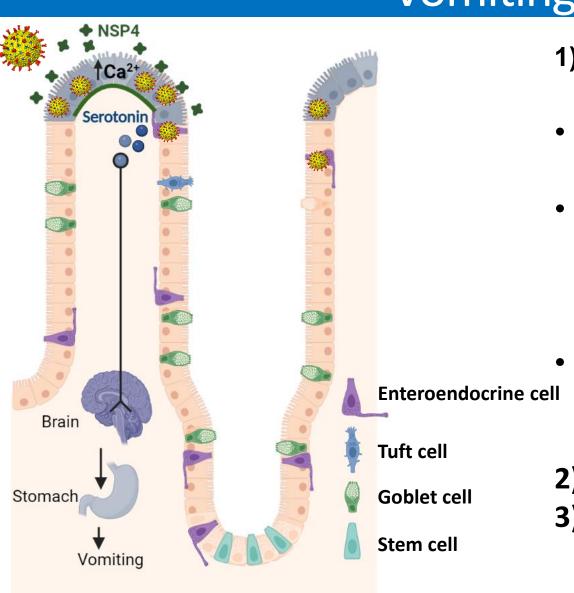








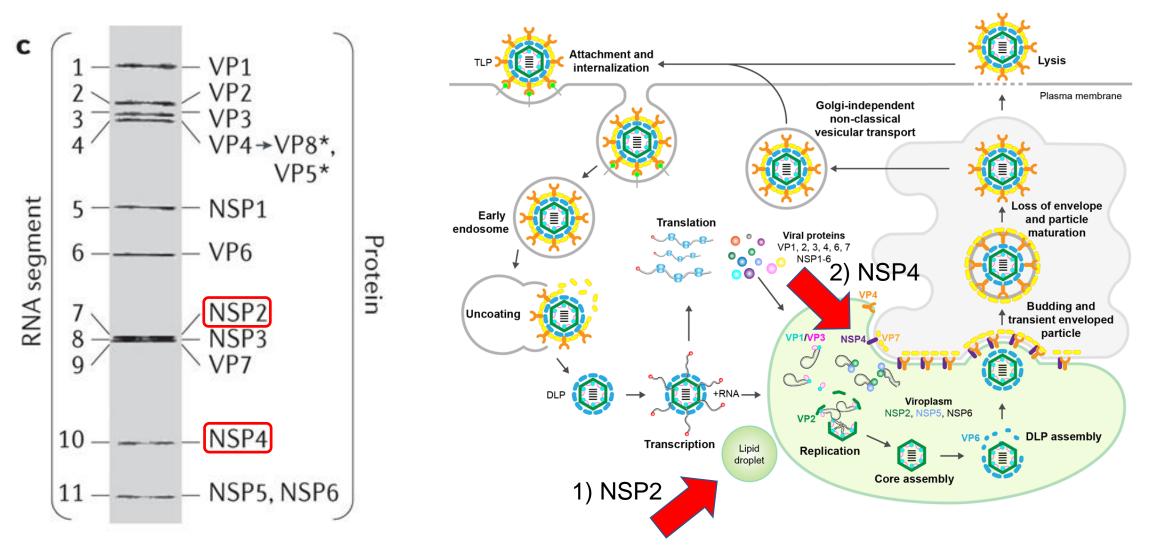
Multifactorial Mechanisms of Rotavirus-induced Vomiting and Diarrhea



Created with BioRender.com

- 1) Multifactorial mechanisms of rotavirus-induced vomiting and diarrhea from studies of RV NSPs
- Vomiting: Serotonin-activation of the enteric nervous system
- Malabsorptive Diarrhea: Loss of polarized epithelial cell tight junctions and cell integrity; downregulation of host proteins; cell death
 - Degradation of DGAT1
- Secretory Diarrhea: Viral enterotoxin; increased
 cytoplasmic calcium
 - ADP signaling
- 2) Enteroid models for rotavirus vaccine studies
- 3) Non-replicating RV vaccine and correlates of protection

Unexpected Functions of Nonstructural Proteins in Pathogenesis



Crawford, Ding, Greenberg, Estes, Fields Virology, 7th edition, 2022

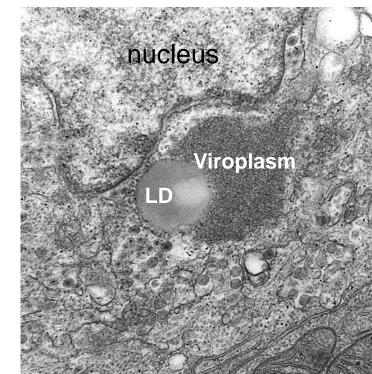
Rotavirus Replicates in Viroplasm/Lipid Droplets

 Viroplasms (virus factories) are cytoplasmic sites of genome replication and immature particle assembly

- Viroplasm formation requires:
 - RV non-structural proteins NSP2 and NSP5
 - Lipid droplets induced by infection HOW?

dNSP2 vNSP2 NSP5 Merge

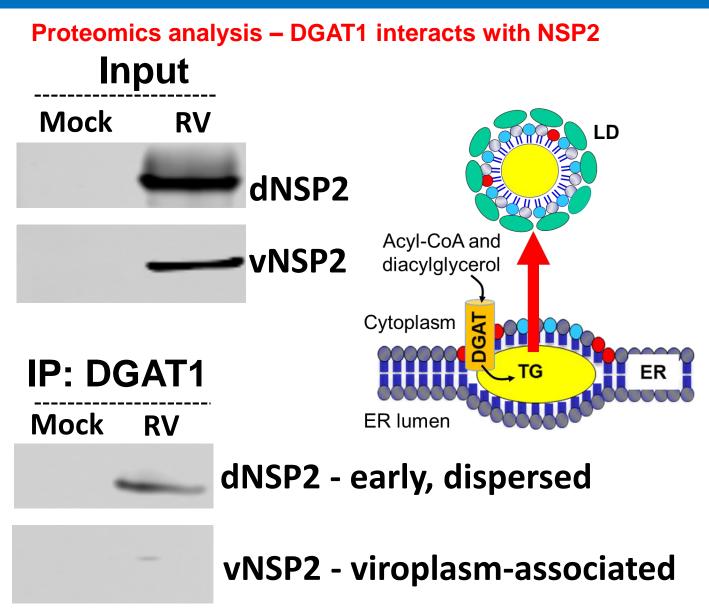
2 hpi
3 hpi
3 hpi





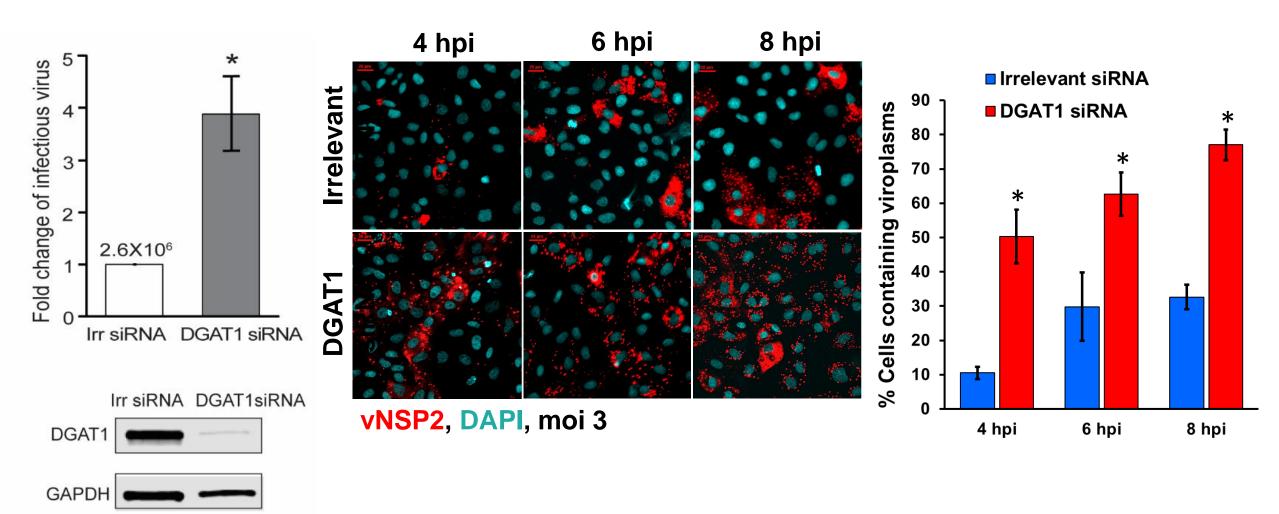
Adapted from Criglar, 2014

dNSP2, but Not vNSP2, Interacts with Host DGAT1



- Diglyceride acyltransferase 1 (DGAT1) is an ER membrane protein
- DGAT1 is the rate-limiting enzyme in triglyceride synthesis
- Newly synthesized TGs accumulate between the ER lipid bilayer and form a lens-like structure
- LDs bud into the cytoplasm by an unknown mechanism

DGAT1 Silencing Increases RV Yield and Viroplasm Formation



Increased viral yields correlate with increased disease?

DGAT1 Deficiency in Humans



Identification and characterization of a novel DGAT1 missense mutation associated with congenital diarrhea

Nina L. Gluchowski,**, Chandramohan Chitraju, Soseph A. Picoraro, Niklas Mejhert, Shirly Pinto, Minis Winis, Solar Daniel S. Kamin, Marland S. Winter, Merchant Winnis C. Walther, Solar and Robert V. Farese, Jr. 1,2,1,3,8



Reversible deficits in apical transporter trafficking associated with deficiency in diacylglycerol acyltransferase

Cameron Schlegel, Lynne A. Lapierre, Victoria G. Weis, Janice A. Williams, Izumi Kaji, Carolina Pinzon-Guzman, Nripesh Prasad, Braden Boone, Angela Jones, Hernan Correa, Shawn E. Levy, Xianlin Han, Miao Wang, Kelly Thomsen, Sari Acra, James R. Goldenring

Gastroenterology 2018;155:130-143

Gastroenterology

Intestinal Failure and Aberrant Lipid Metabolism in Patients With DGAT1 Deficiency



Jorik M. van Rijn, 1,2,* Rico Chandra Ardy, 3,4,* Zarife Kuloğlu, 5,* Bettina Härter, 6,* Désirée Y. van Haaften-Visser, 1,2,* Hubert P. J. van der Doef, Marliek van Hoesel, 1,2 Aydan Kansu, ⁵ Anke H. M. van Vugt, ^{1,2} Marini Thian, ^{3,4} Freddy T. M. Kokke, ¹ Ana Krolo, ^{3,4} Meryem Keçeli Başaran, Neslihan Gurcan Kaya, Aysel Ünlüsoy Aksu, Buket Dalgıç, Figen Ozcay, ¹⁰ Zeren Baris, ¹⁰ Renate Kain, ¹¹ Edwin C. A. Stigter, ¹² Klaske D. Lichtenbelt, ¹³ Maarten P. G. Massink, ¹³ Karen J. Duran, ¹³ Joke B. G. M Verheij, ¹⁴ Dorien Lugtenberg, ¹⁵ Peter G. J. Nikkels, ¹⁶ Henricus G. F. Brouwer, ¹⁷ Henkjan J. Verkade, ⁷ René Scheenstra, ⁷ Bart Spee, 18 Edward E. S. Nieuwenhuis, 1 Paul J. Coffer, 2 Andreas R. Janecke, 19 Gijs van Haaften, ¹³ Roderick H. J. Houwen, ¹ Thomas Müller, ^{19,§} Sabine Middendorp, 1,2,§ and Kaan Boztug 3,4,20,21,§



Congenital Diarrhea From DGAT1 Mutation Leading to Electrolyte Derangements, Protein-losing Enteropathy, and Rickets

*Thomas L. Ratchford, †Amelia J. Kirby, †Hailey Pinz, and *Dhiren R. Patel

DGAT1 Deficiency in Humans









Identification and characterization of a novel DGAT1



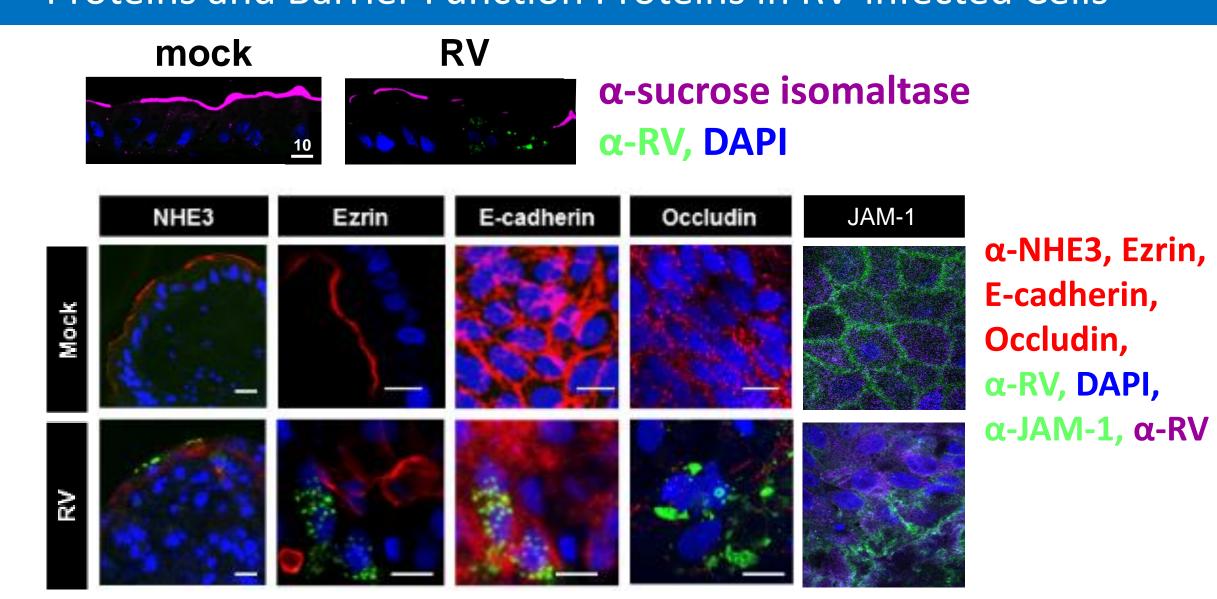


- Intractable, sometimes life-threatening, diarrhea and nutrient malabsorption
- Intestinal biopsies from DGAT1 deficient children showed:
 - Loss of apical trafficking of key ion transporters to the enterocyte brush border
 - Mislocalization of tight junctional proteins
 - Increased levels of diacylglycerol and phospholipids
 - Villus blunting
- Low-fat diet reverses these deficits

Peter G. J. Nikkels, ¹⁶ Henricus G. F. Brouwer, ¹⁷ Henkjan J. Verkade, ⁷ René Scheenstra, ⁷ Bart Spee, ¹⁸ Edward E. S. Nieuwenhuis, ¹ Paul J. Coffer, ² Andreas R. Janecke, ¹⁹ Gijs van Haaften, ¹³ Roderick H. J. Houwen, ¹ Thomas Müller, ^{19,§} Sabine Middendorp, ^{1,2,§} and Kaan Boztug^{3,4,20,21,§}

*Thomas L. Ratchford, [†]Amelia J. Kirby, [†]Hailey Pinz, and *Dhiren R. Patel

Mislocalized Brush Border Enzymes, Ion transporters, Actin Binding Proteins and Barrier Function Proteins in RV-infected Cells



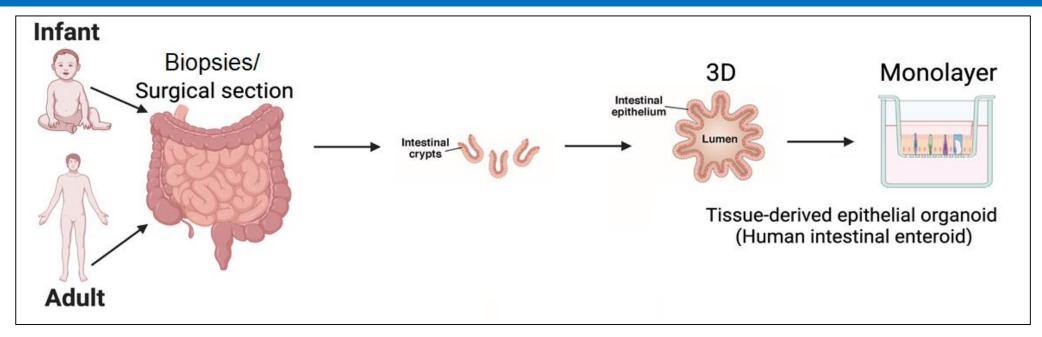
RV-mediated DGAT1-degradation in HIEs Mimics DGAT1 Deficiency in Children

Pathology	DGAT1- deficiency biopsies	RV-infected HIEs
Increased levels of diacylglycerol and phospholipids	\checkmark	✓
Villus blunting (epithelial thinning)	\checkmark	✓
Mislocalized brush border transporter & junctional proteins	✓	✓
Trafficking of these proteins is altered	\checkmark	✓
DGAT degradation shuts off host protein translation (eIF2 α -P)	unknown	✓

RV-mediated DGAT1 degradation is a previously unrecognized mechanism of RV-induced diarrhea

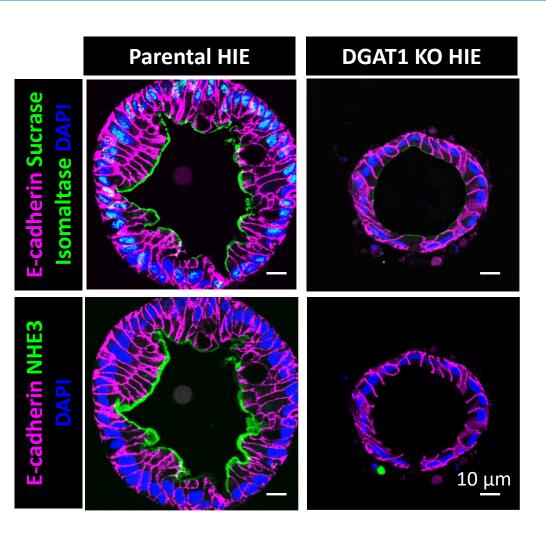
Under revision, PNAS

Human Intestinal Enteroids (HIEs)



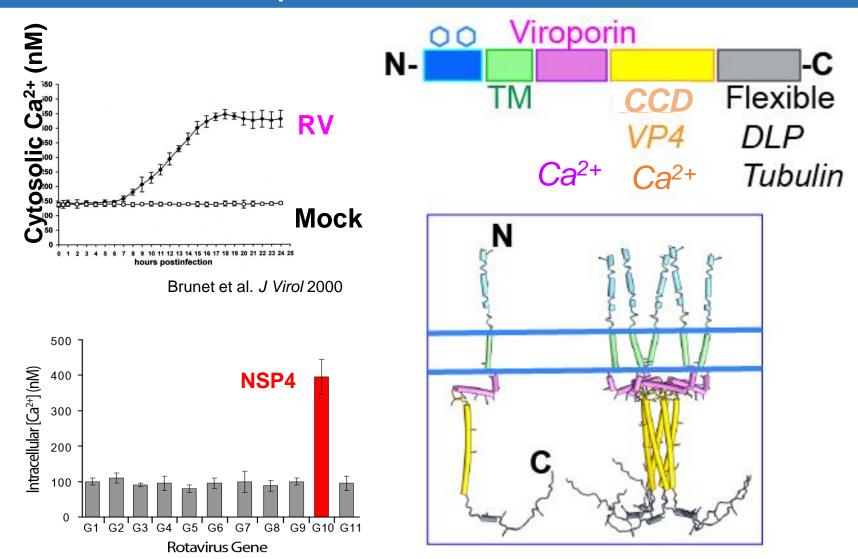
- Pioneered by Sato and Clevers (The Netherlands)
- •Tissue-derived cultures **HIEs**
- Non-transformed, indefinite passaging, reflect genetics of donor (miniguts, avatars)
- •All epithelial cell types enterocytes, goblet cells, enteroendocrine, Paneth cells
- Physiologically active; made from different intestinal segments
- Complexity and organization of the epithelium
- Able to be genetically modified

DGAT1 KO Enteroids: a New Tool to Understand Human Intestinal Physiology



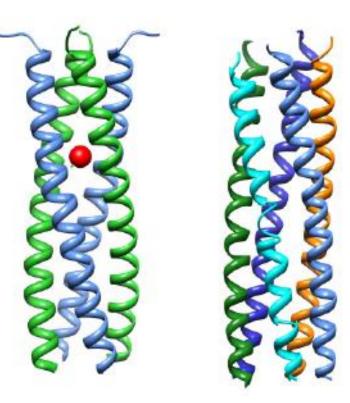
- Grow more slowly
- How does DGAT1 degradation mediate reprogramming of lipid metabolism?
- Are DGAT1 degradation and altered lipid metabolism responsible for the severity of rotavirus-induced disease?

NSP4 – A Membrane Anchored Calcium Agonist, Viroporin Calcium Channel, and Enterotoxin.



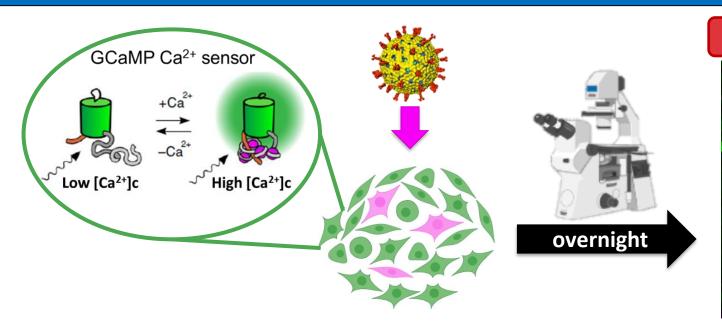
Tian et. al. JVI 1994

NSP4 CCD tetramer vs pentamer

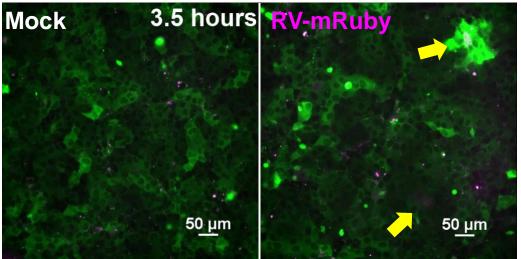


Sastri et al., J. Virol. 2014

Studying Ca²⁺ Signaling During Virus Infections

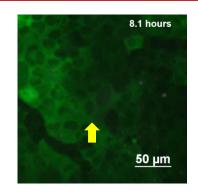


Time lapse Ca²⁺ imaging during RV infection

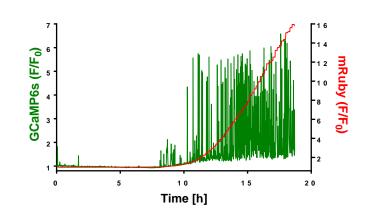


Increased Spatial Resolution

- Subcellular signaling
- Infected vs. uninfected cells



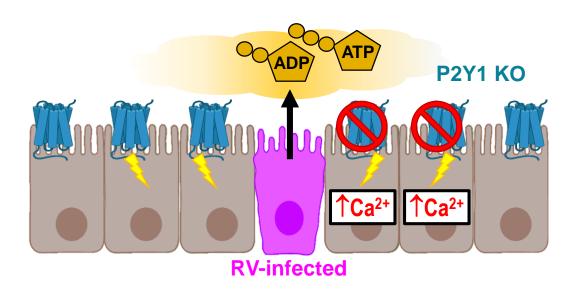
Expanded Temporal Resolution





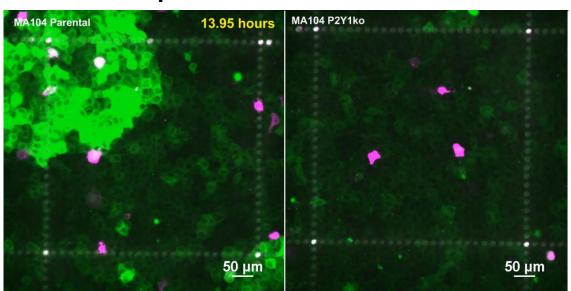
J. Hyser

Rotavirus Ca²⁺ Waves are Mediated by Paracrine Signaling to P2Y1 Purinergic Receptors

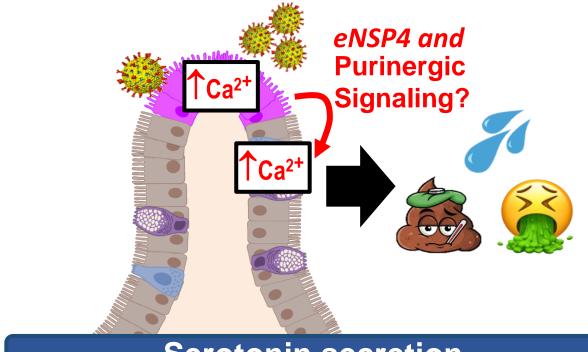


RV MA104 parental

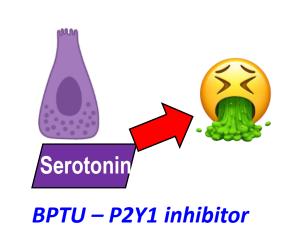
RV MA104 P2Y1-KO

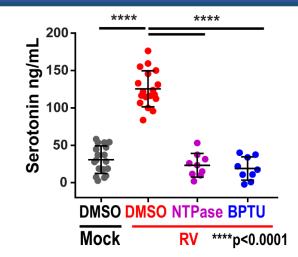


Does P2Y1 activation regulate mechanisms of RV gastroenteritis?

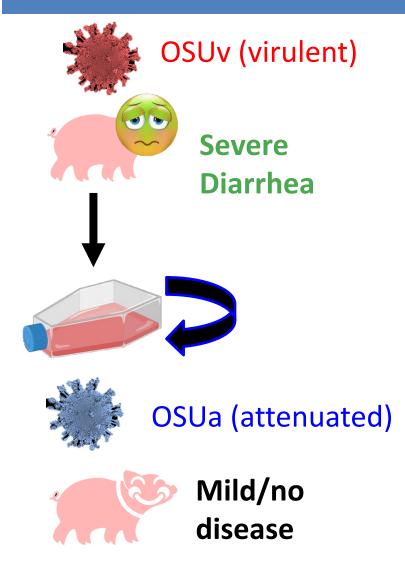


Serotonin secretion

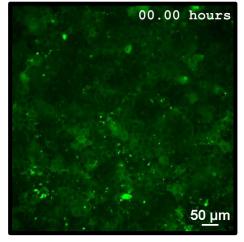




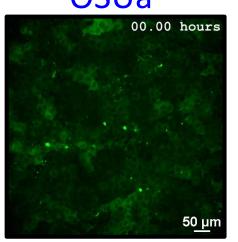
Attenuated Porcine RV NSP4 Produces Fewer Ca²⁺ Waves Correlates with Reduced Rotavirus-induced Fluid Secretion



OSUv

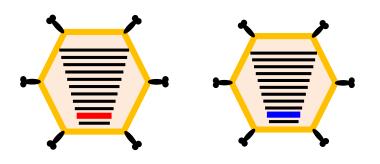


OSUa



Recombinant mono-reassortant

rSA11-mRuby: G10 OSUv rSA11-mRuby: G10 OSUa





Successful Expression of Full-Length Functional NSP4

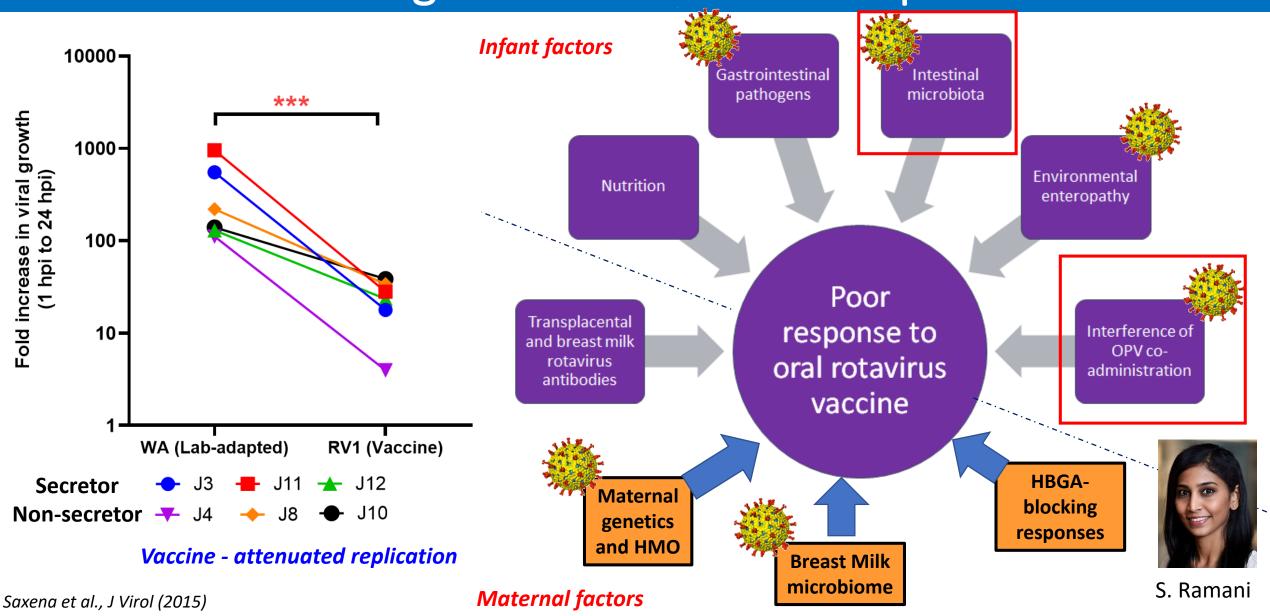
Studies to determine the structure of the Full-Length NSP4 protein are in progress

Will this be a pentamer or tetramer?

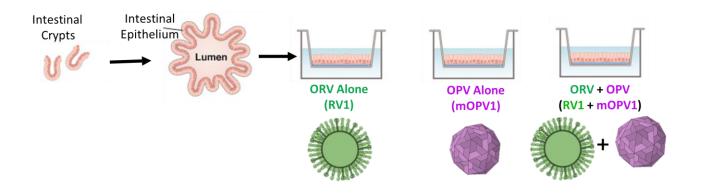


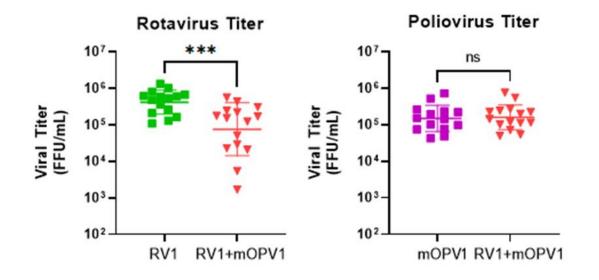
B.Prasad

Enteroids are a Pre-clinical Platform for Studying Factors Affecting Rotavirus Vaccine Response



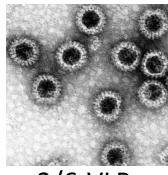
HIEs Mimic Vaccine Interference Seen in Infants



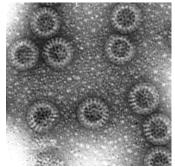


Poliovirus vaccine induces an innate response that inhibits RV replication

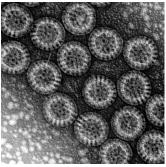
Virus-Like Particles (VLPs) as Vaccines



2/6-VLPs



2/6/7-VLPs



2/4/6/7-VLPs

Routes: Oral, Intranasal, Intramuscular, Intramammary

Diarrhea Models

Active Immunization



Passive Immunization



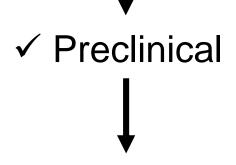


Infection Models





√ VLPs

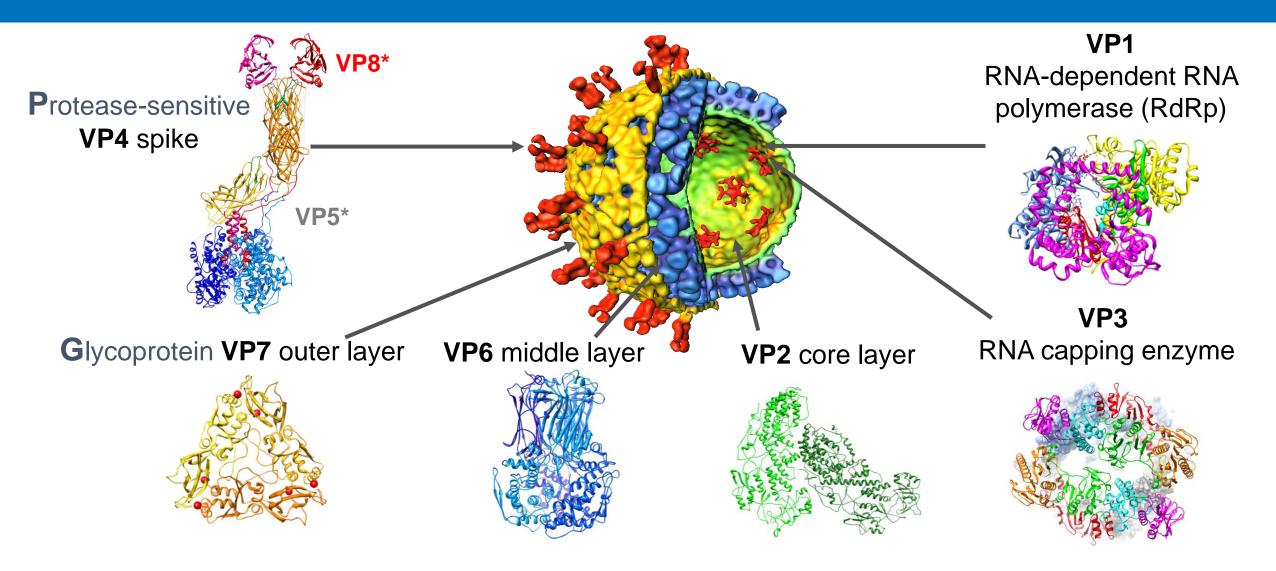


First Defense® Tri-Shield™

Immucell – Veterinary
Biotechnology Company
USDA approved, Nov 2017
Colostrum from cows
immunized with 2/6/7 VLPs

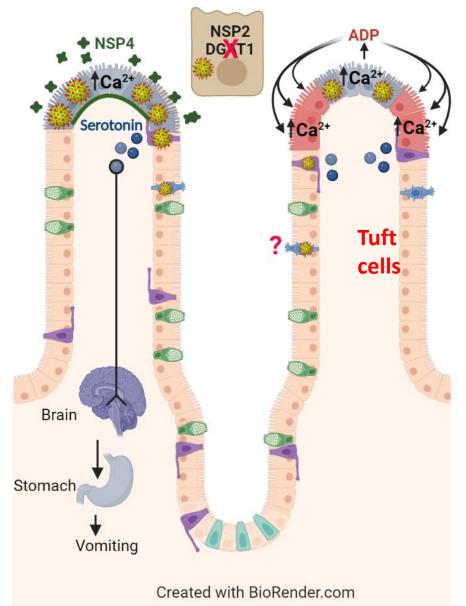
First approved rotavirus VLP vaccine!

Can a Correlate of Protection be Found Among the Structural Components of the Rotavirus Triple-Layered Particle or NSP4?



Prasad et al., Nature, 1996; Aoki et al., Science, 2009; Settembre et al., EMBO J, 2011; Kumar et al., Sci Adv. 2020

Multifactorial Mechanisms of Rotavirus-induced Vomiting and Diarrhea



- 1) Multifactorial mechanisms of rotavirus-induced vomiting and diarrhea from studies of RV NSPs
 - Vomiting: Serotonin-activation of the enteric nervous system
 - Malabsorptive Diarrhea: Loss of polarized epithelial cell ion transporters; downregulation of host proteins
 - Degradation of DGAT1
 - Secretory Diarrhea: Viral enterotoxin; increased cytoplasmic calcium
 - Novel ADP signaling
- 2) Enteroid models for rotavirus vaccine studies
- 3) Non-replicating VLP vaccine & correlates of protection
- 4) Viral Tropism- enterocytes, enterondocrine & Tuft cells

Awesome Collaborative Research Teams!







Z. Liu



J. Criglar



H. Smith

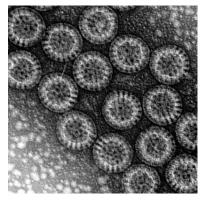


A. Valentin



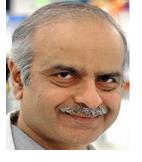
S. Zeng

Other Trainees and Staff









B. Prasad



J. Hyser



S. Blutt



S. Ramani



NIH Grants R01 Al080656, RO1 Al158683, R37 Al36040, UO1 DK103168 and P30 DK056338

