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Rotaviruses: Cause of vaccine-preventable disease yet many fundamental questions remain to be explored

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Editorial Overview

Discovered in 1973 as a major cause of life-threatening gastroenteritis in infants and young children, rotaviruses (RVs) also infect many mammalian and avian species. Less than 40 years later, two live-attenuated RV vaccines are now licensed in many countries and are increasingly being used in universal mass vaccination programs. However, vaccine effectiveness in the countries that need them most is suboptimal, requiring further work to increase immunogenicity. Post-marketing surveillance has to track whether new RV strains emerge, to monitor for possible zoonotic transmission, and to determine efficacy against unusual RV strains emerging in developing countries. New molecular tools and epidemiologic capabilities will help comprehend evolution of this global pathogen.

The contributors to this special issue of the journal review recent developments, outline topics of controversy and indicate areas of future RV research. RVs are fascinating models to understand complex virus structure, extensive genomic and antigenic diversity, zoonotic transmission, novel mechanisms of non-enveloped virus entry into cells, pathways regulating host and viral protein translation and the host innate responses, mechanisms of intestinal pathogenesis and viral immunity.

RVs form the genus *Rotavirus* of the *Reoviridae* family. Particles are non-enveloped and possess a triple-layered protein capsid surrounding the genome of 11 segments of dsRNA. The genome encodes 6 structural and 6 non-structural proteins. Near atomic resolution structures of RV particles have been achieved by combined x-ray crystallography and cryo-electron microscopy imaging [1, 2]. Early RNA structure work and to some extent *in silico* and biochemical studies remain to be pursued to resolve the detailed structure of the genomic RNAs within the particle. Here SD Trask, KM Ogden and JT Patton report new data on the structure of the RV RNA-dependent RNA polymerase (RdRp) VP1, its possible

interaction with the capping enzyme VP3 and the inner-layer protein VP2, and how this interaction directs the egress of RV mRNA transcripts from particles.

RV replication is well studied *in vitro* but remains incompletely understood. Attachment of RVs to susceptible cells is by a domain (VP8*) within the outer capsid VP4 spike protein, that interacts initially with sialic acid (SA), either in terminal or internal positions of cellular glycoproteins as receptors. Some human RVs bind to histo-blood group antigens at the same site on the VP8* spike domain where animal RVs bind sialic acid [3] mimicking known host glycan susceptibility factors characterized for infection of humans with noroviruses. RV replication occurs in the cytoplasm of cells with RNA replication and initial particle assembly is orchestrated within specialized virus factories that contain several of the non-structural proteins (NSP2/4/5/6). Many of these proteins function as oligomeric complexes that are critical for replicating the genome and encapsidation of the RNA segments within cytoplasmic inclusion bodies termed “viroplasms”. Details of how viroplasms form and function as complexes of several of the non-structural proteins, specifically NSP2 and NSP5, remain to be understood. Cellular proteins, e.g. tubulins, and lipid droplets are involved in viroplasm formation, and proteosome activity is essential for RV replication. Another non-structural glycoprotein, NSP4, interacts with endoplasmic reticulum (ER) membranes as well as with viroplasms, modulates viral transcription and links genome packaging with particle assembly. NSP4 functions as a viroporin [4], releasing Ca^{2+} from the ER stores and regulates cellular calcium homeostasis for viral replication in still unknown ways. All this indicates that RVs hijack multiple cellular pathways. Newly made (+)ssRNAs likely complexed with VP1, VP3 and possibly a VP2 pentamer are assorted and packaged into cores within the viroplasms in a process which is not understood at all. Cores interact with VP6 trimers to form DLPs, which become transiently enveloped particles in the ER acquiring VP4 and VP7, and are ultimately released as TLPs by lysis of non-polarized cells.. BVV Prasad and colleagues provide an overview of the replication cycle and the known structure-function relationships of the non-structural proteins.

Infection with RV has severe consequences for the host cell. The RV non-structural protein NSP3 binds to the 3’end of RV mRNAs (non poly-adenylated), mediating contact with components of the cellular translation machinery, i.e. acting like a polyA binding protein on cellular mRNAs. Thus, cellular mRNA translation is hindered since NSP3 can evict PABP from the translation complexes. RV-host cell interactions are reviewed by S Lopez and CF Arias, concentrating on the unique regulation of translation of RV (+)ssRNA *vs* cellular mRNAs but also discussing previously unidentified viral mechanisms to overcome cellular innate immune responses.

All viral studies are enhanced by robust genetic systems. RVs undergo extensive genetic exchanges by gene reassortment, and this technique has been exploited over the past 30 years to map specific gene functions. The genetics of RVs was also explored by producing temperature-sensitive (ts) mutants of several strains, and 9 of the 11 reassortment groups of these mutants have been mapped to specific genome segments [5]. However, only the availability of a generally tractable reverse genetics (RG) system will permit exact correlations of particular genotypes and phenotypes. For members of two other genera (*Orthoreovirus* and *Orbivirus)* of the *Reoviridae,* plasmid-only based RG systems have been

established since 2007. For RVs, some success has been achieved by incorporating RNAs transcribed from transfected cDNA plasmids into the genomes of helper virus [6–8]. The procedures require strong selections. In this issue, K Taniguchi and S Komoto describe the inaugural success of their laboratory in this area and the obstacles which remain to be overcome to establish an efficient and helper virus-free RG system.

The pathogenesis of RV infections is complex and multifactorial. Several RV genes are implicated in virulence in various animal models. A major symptom, diarrhea, is caused by a combination of maladsorption due to apoptosis of enterocytes, constriction of villous small arteries, activation of the enteric nervous system and the activity of the RV-encoded NSP4 that can function as an enterotoxin [9, 10]. Recent studies have found that enterochromaffin cells of the small intestine release 5-hydroxytryptamin (serotonin) upon RV infection or exposure to NSP4, which in turn activates nerve cells in the brain stem involved in controlling vomiting, another major symptom of RV disease. In this issue, M Hagbom, S Sharma, O Lundgren and L Svensson present a model of RV pathogenesis based on clinical and therapeutic observations in humans. Some practical implications emerge from these new analyses. Besides oral rehydration solutions (ORS), which are highly efficacious in the treatment of RV disease, encephalinase inhibitors, e.g. racecadotril, or serotonin receptor antagonists, such as Granisetron, are showing benefit for treatment.

The immune response to RV infection involves a combination of innate and acquired, humoral and cell-mediated responses. Since RV vaccines have been developed, there has been great interest in identifying the ‘correlates of protection’, and much work towards this goal has been carried out in animal models and humans after both, natural infection and vaccination. Whilst there is some correlation of protection from RV disease with the levels of neutralising (NT) antibodies (directed against VP7 and VP4), this correlation is not absolute [11, 12]. VP6-specific antibody of the IgA class, which can be transcytosed via enterocytes into the gut lumen, may play a role in protection [13], and antibody to NSP4 may also have an effect. For some time, the presence of high levels of RV-specific IgA antibodies in the gut lumen (fecal IgA) has been considered a good correlate of protection. However, this is not always the case. J Angel, M Franco and H Greenberg analyse innate immune responses against which RVs have developed defenses through the actions of NSP1. This story is complex because the innate immune responses to RV infection can vary according to RV strain and host species.

RVs occur in at least 7–8 different species (A-H, also termed groups). Most human infections are due to group A RVs, which have been further classified into types according to properties of the two outer capsid proteins (VP4 and VP7) that induce neutralizing antibodies. Thus, a bivalent classification system describes the antigenicity and gene composition of VP7 (G [glycoprotein] types) and VP4 (P[protease-sensitive protein] types), and currently 27 G and 35 P types are recognized. With the advent of whole genome sequencing, genetic clusters (genotypes) of the other 9 RNA segments are being differentiated [14]. Accordingly, whole genome sequencing explores specific constellations of genes in co-circulating RVs. In this issue, Matthijnssens and van Ranst review the genotype constellations of co-circulating human RV strains and conclude that beyond the G and P genotype diversities there is less variation in the other 9 genes. These results are

somewhat unexpected because in cell culture many different reassortants can be obtained after coinfection with two parental strains. Clearly not all possible reassortants adapt to new hosts and are transmitted efficiently.

The clinical significance of RV disease, resulting in significant morbidity in the US in the pre-vaccination era, and in almost half a million deaths worldwide, mainly in sub-Saharan Africa and SE Asia, motivated efforts to develop a human vaccine. Vaccine development faced multiple hurdles. The first live, attenuated vaccine (RotaShield™, quadrivalent), licensed in the USA in 1998, was epidemiologically linked with gut intussusception (IS); resulting in discontinuation of vaccine production. In 2006, two other live, attenuated RV vaccines, the monovalent Rotarix™ vaccine (called RV1) and the pentavalent human-bovine reassortant RotaTeq™ (termed RV5), were found to be efficacious in large phase III clinical trials with no apparent risk of gut IS [15, 16]. Both vaccines provide considerable protection from heterotypic infection. As a consequence, the isolation of RVs and admission to hospital for RV-associated acute gastroenteritis has significantly decreased in developed countries, and in some settings RV vaccination has also decreased the mortality from RV disease. In this issue B Lopman and members of UD Parashar's group report on the recent experience with RV vaccination in high-income and semi-developed countries, and S Babji and G Kang et al on the problems encountered with and challenges facing RV vaccination programs in low income countries.

Conclusions

Exciting scientific information should continue to emerge as the successes and challenges of global introduction of live-attenuated RV vaccines are pursued and new insights into virus-host-environment interactions and viral evolution emerge. Better or novel definitions of correlates of protection and recognition of the causes of decreased RV vaccine efficacy in low income countries are important research goals. The significance of herd immunity after RV vaccination requires further investigations. Many questions remain to be answered about the mechanisms of RV replication. A few include: Why is there so little intestinal inflammation after RV infection? Will the newly discovered human RV-HBGA interactions change our understanding of virus-host pathogenesis and virus entry into cells? Will advanced techniques such as single particle reconstruction combined with tomography provide additional structural insights into the molecular functioning of RV particles and oligomeric complexes during replication? What are the molecular mechanisms controlling the interactions of the non-enveloped RVs with host cell membranes? How are recruiting of lipid droplets by viroplasms and involvement of the autophagy pathway in virus replication regulated? How do the outer capsid proteins assemble onto DLPs in transiently enveloped particles? Finally and crucially, what molecular mechanisms are responsible for assortment of one of each of the 11 segments of genomic RNAs into replication intermediate particles? The answer to this question and to many others in RV research will likely arise from a breakthrough in the development of a tractable, helper virus-independent RG system.

Biography

Mary K. Estes is the Cullen Endowed Professor of Human and Molecular Virology in the Department of Molecular Virology and Microbiology at Baylor College of Medicine in Houston, TX, USA. She directs a multidiscliplinary research program on gastrointestinal viruses - rotaviruses and noroviruses- that focuses on understanding the molecular basis of viral replication and pathogenesis.

Ulrich Desselberger is a virologist at the Department of Medicine, University of Cambridge, Cambridge, U.K. His recent research has been on rotaviruses, focussing on the role of lipid droplets in viral replication, structural studies of viral RNA, and attempts to establish a helper virus-free reverse genetics system. The work is funded by The Wellcome Trust, with Professor Andrew Lever as a co-investigator.

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