Pathogenesis and replication of norovirus: following the mouse tail?

Jennifer L Hyde[a] *and Jason M Mackenzie*[b]

[a]Department of Medicine, Washington University School of Medicine, St Louis, Missouri 63110, USA
Tel +1 314 362-2847
Email jhyde@DOM.wustl.edu
[b]Department of Microbiology
La Trobe University, Melbourne
VIC 3086
Tel (03) 9479 2225
Fax (03) 9479 1222
Email j.mackenzie@latrobe.edu.au

The emergence of human noroviruses (NoV) as significant human pathogens over the last decades has highlighted the need to research and understand the replication and pathogenesis of this group of viruses. One of the major hurdles faced by researchers in this field has been the lack of a viable tissue culture system or small animal model with which to study human NoV replication. The discovery of a murine NoV in 2003 and the identification of its tropism for macrophage and dendritic cell lines has provided the opportunity to study aspects of NoV replication and pathogenesis that were previously closed to researchers.

Noroviruses

Noroviruses (NoV; family *Caliciviridae*) are a group of small (27-35nm), icosahedral, non-enveloped viruses which contain a single-stranded positive sense RNA genome. NoVs comprise one of five genera within the *Caliciviridae* (Norovirus, Sapovirus, Vesivirus, Lagovirus, Nebovirus) and can be divided into five genogroups (GI-GV). Like other members of the *Caliciviridae*, NoVs are genetically diverse and exhibit broad host specificity, infecting a wide range of mammalian species including humans (GI, GII, GIV), swine (GII), bovine (GIII), canine and lion (GIV), and murine (GV). Initially identified in 1968 following an outbreak of gastroenteritis in a school in Norwalk, Ohio[1,2], human NoV (HuNoV; type species Norwalk) have long been recognized as etiologic agents of gastroenteritis, and are now known to be a major cause of acute non-bacterial gastroenteritis worldwide. Appreciation of the medical importance of this group of viruses has consequently prompted rigorous research efforts into understanding the biology of these viruses, with the goal of developing effective vaccines and antiviral therapies. However, in spite of this relatively little progress has been made towards understanding the replication and pathogenesis of these viruses, a fact that can largely be attributed to the inability to culture these viruses within the laboratory[3,4]. In 2003, the discovery of a novel murine NoV (MNV) provided the first opportunity to explore previously understudied aspects of NoV biology[5]. The subsequent identification of the tropism of MNV for cells of a mononuclear origin furthermore led to the establishment of a conventional MNV tissue culture system[6] which, together with reverse genetics systems[7,8], has provided a crucial step forward in the field of NoV replication and biology.

Intracellular replication

NoVs possess a single stranded RNA genome of positive polarity measuring approximately 7.2-7.5 kb in length. Like other members of the *Caliciviridae*, the NoV genome is protein linked at the 5' end to a viral encoded protein NS5 (VPg)[9] and polyadenylated at the 3' end[5]. The genome consists of four open reading frames (ORFs) designated ORF1-4 (**Fig. 1**) which encode the non-structural proteins (ORF1) and structural proteins (ORF2, ORF3) and a recently identified protein proposed to function as a virulence factor[33] (ORF4).

Following virus entry and release of the genome from the capsid structure, the positive-sense RNA genome acts as a messenger RNA and is translated by the host machinery in the cytoplasm into a single polypeptide encoding all the viral non-structural proteins (NS1-2 – NS7). The viral polypeptide is co- and post-translationally cleaved by the viral protease (NS6) to release functional polyprotein species and mature viral proteins which then facilitate subsequent rounds of replication.

Following initial translation and the proteolytic release of viral proteins, the positive-sense genome functions as a template for negative-strand RNA synthesis. Replication is mediated via the viral RNA-dependent RNA polymerase (NS7). Negative-sense RNA transcripts are then used as a template for synthesis of more full-length genomic RNA (gRNA) as well as positive-sense

subgenomic RNA (sgRNA) from which the NoV structural proteins are translated. Following subsequent rounds of replication, full-length gRNA is packaged into virions in what is believed to be a VP2-dependent manner[10]. At present, it is unknown what maturation steps packaged virions undergo during egress, and by what mechanism progeny viruses are released from infected cells, but the induction of apoptosis during feline calicivirus and MNV-1 infection suggests this as a possible mechanism[11,12].

Replication and membranes

Like all positive RNA viruses, the intracellular replication of MNV is intimately associated with cellular membranes[6] (**Fig 2A**). We identified the subcellular localisation of the MNV replication complex and showed by immuno-electron microscopy that MNV replication associates with unique virus-induced membrane clusters derived from components of the secretory pathway[13]. In addition, we have observed that MNV appears to utilise the host cytoskeleton network, particularly microtubules, to establish virus replication proximal to the microtubule organising centre[14] (**Fig 2B**). These observations are in agreement with previous studies identifying roles for the HuNoV proteins when expressed as individuals proteins[15,16]. Thus there appears to be homologous roles for both the MNV and HuNoV proteins during the replication cycle.

Pathogenesis

The initial discovery of MNV occurred after an observed lethal infection of mice that essentially had no immune system[5]. It was subsequently further shown that innate immunity is critical in protecting mice against lethal infection[5,17]. Accordingly MNV and HuNoV replication is sensitive to treatment with both type I and type II IFNs[18,19]. In addition to providing a means to study the host immune pathways that are critical for restricting NoV replication, the MNV model has facilitated the detailed study of NoV pathogenesis by allowing researchers to dissect the relationship between MNV genetics and host disease. To date numerous MNV strains have been identified, and comparison of the replication and pathogenesis of these variants has led to the identification of both viral and host determinants that impact viral fitness and pathogenesis[20-22]. Importantly, these comparative studies have revealed these viruses to exhibit significant differences in replication and pathology, despite displaying limited variability within their genetic sequences[23]. These results unequivocally highlight the importance of understanding how virus and host genetics influence pathology and disease outcome. While the advantage of using MNV as a surrogate model for study of HuNoVs is apparent, the significance of MNVs as pathogens of laboratory mice should not be overlooked. Recent surveys of animal facilities worldwide have revealed that as many as 20-100% of laboratory mice are seropositive for MNV[24,25]. The potential of MNV to impact the study of disease models in mice is of concern, and has been illustrated in recent work by Cadwell et al. (2010), who demonstrated an interaction between the ATG16L1 risk allele of Crohn's disease (a type of inflammatory bowel disease), and a persistent strain of MNV which together triggers the onset of pathologies similar to Crohn's disease[26].

Future of NoV biology

In the absence of a viable human NoV tissue culture system, MNV provides a valuable surrogate model for the study of NoV replication. The ability to use a small animal model for the study of MNV provides an ideal opportunity to explore important aspects of NoV pathogenesis, virus dissemination, and host immunity that would be otherwise difficult to study. While there is still much to learn regarding MNV and, in general, NoV replication, the current research has provided an important starting point for future focused research. Further research into this field will afford great insight into NoV biology and replication and will no doubt aid in the development of vaccines and antiviral therapies for use in preventing and controlling this medically important group of viruses.

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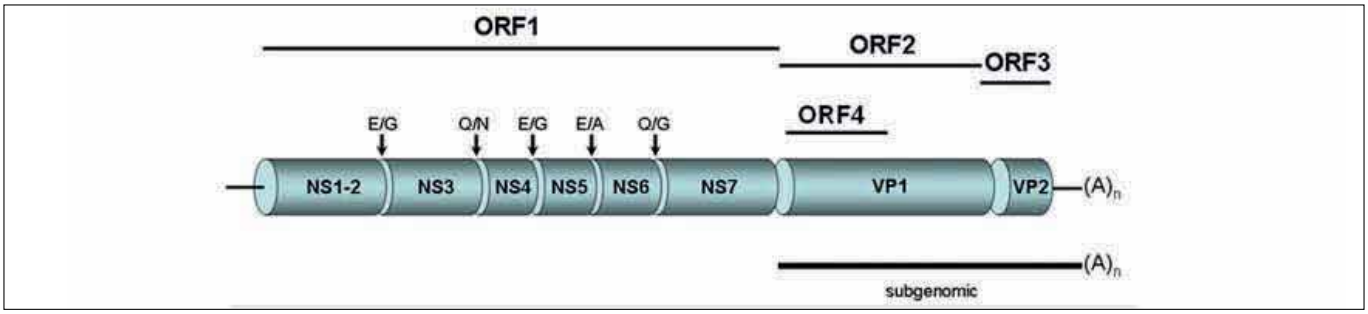

Figure 1. Genome structure and organisation of NoVs. The NoV genome is polyadenylated at the 3' end and protein linked at the 5' end to the viral encoded protein NS5 (VPg). Open reading frame 1 (ORF1) encodes six non-structural proteins which are translated as a single polypeptide and co- and post-translationally cleaved by the viral encoded protease (NS6) at the cleavage sites indicated. ORF2 and ORF3 encode the structural proteins VP1 and VP2 respectively and are translated from a sgRNA species which is also polyadenylated and NS5-linked. ORF4 encodes for a recently identified protein thought to aid in immune evasion.

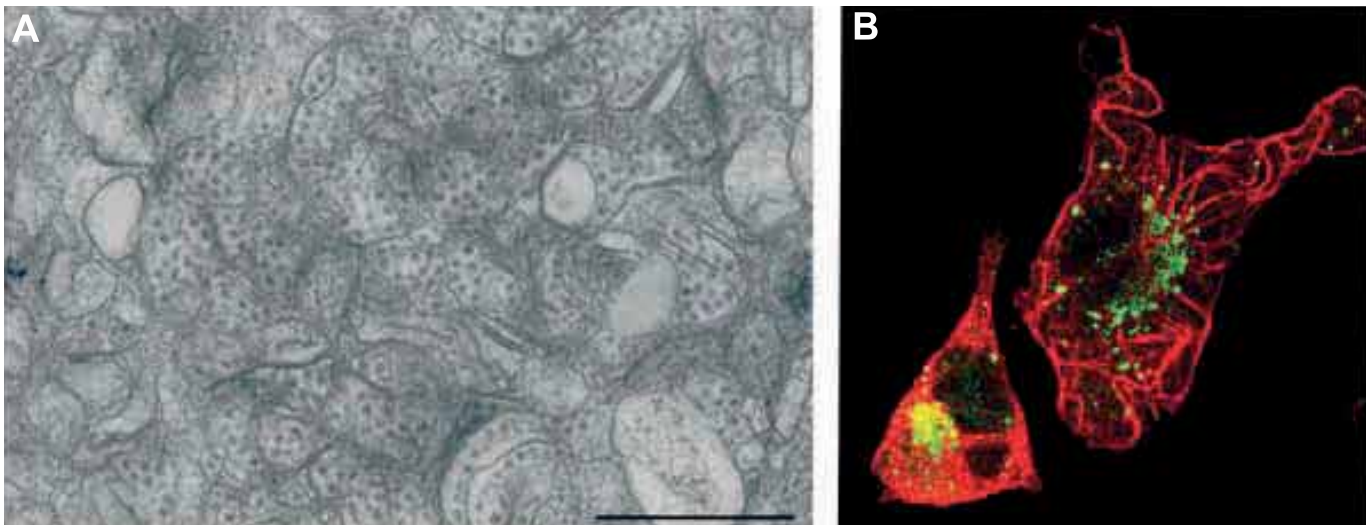

Figure 2. Morphological changes in cellular architecture induced by MNV replication in murine macrophage cells. Dramatic ultrastructural changes in membrane morphology (panel A) and microtubule dynamics (panel B) are observed after 24 hours infection of murine macrophage cells. Panel A, resin-embedded MNV-infected RAW264.7 cells; magnification bar represents 500 nm. In panel B, IF analysis was performed with anti-NS7 (green) and anti-tubulin (red) antibodies.

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Biographies

Jennifer Hyde is a Postdoctoral Research Associate within the department of Internal Medicine and Infectious Diseases at Washington School of Medicine. She received her PhD from the University of Queensland in 2011. Her major interests are in the intracellular replication of RNA viruses and the effects on immune signalling.

Jason Mackenzie is an Associate Professor within the Department of Microbiology at La Trobe University. He investigates the replication mechanism of two positive-stranded RNA viruses (West Nile virus [a flavivirus] and Mouse Norovirus [a Norovirus]) that are highly pathogenic to humans and cause outbreaks of encephalitis and gastroenteritis, respectively. He aims to correlate this abuse of host with the pathogenic outcomes associated with viral infection.