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REVIEW

Infection models of human norovirus: challenges and recent progress

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Abstract Human norovirus (hNoV) infections cause acute gastroenteritis, accounting for millions of disease cases and more than 200,000 deaths annually. However, the lack of *in vitro* infection models and robust small-animal models has posed barriers to the development of virus-specific therapies and preventive vaccines. Promising recent progress in the development of a norovirus infection model is reviewed in this article, as well as attempts and efforts made since the discovery of hNoV more than 40 years ago. Because suitable experimental animal models for human norovirus are lacking, attractive alternatives are also discussed.

Introduction

Human norovirus (hNoV) was discovered as the cause of an outbreak of acute gastroenteritis in an elementary school in 1968 in the city of Norwalk, Ohio, and in 1972, the gastroenteritis was confirmed by immunoelectron microscopy to have a viral etiology [47, 48]. Although gastroenteritis is caused by bacteria, protozoa and viruses, viral gastroenteritis is particularly problematic due to the lack of effective antiviral therapies. Acute gastroenteritis caused by hNoV is estimated to account for 90 % of cases of virus-mediated gastroenteritis. Approximately 800 fatalities among infants and the elderly due to hNoV infection are reported each year in the US alone, and 200,000 children under the age of 5 years in developing countries die annually from the disease [79]. Norovirus infection in healthy adults causes self-limiting acute disease including vomiting and diarrhea and typically resolves in 2-3 days. However, in immunocompromised patients, hNoV can establish chronic and potentially fatal infections [7].

Since the discovery of hNoV and the cloning of its genome [57, 111], much effort has been made to develop *in vitro* infection models for hNoV in cultured cell lines [19, 31, 58]. However, the lack of cell lines that can support hNoV infection poses a barrier to its *in vitro* culture. Therefore, hNoV stocks have been prepared from stool samples of human patients or volunteers for human infection trials. For these reasons, progress in the study of the pathological characteristics and mechanisms of viral replication and gene expression has been severely impeded for this virus compared to other positive-sense RNA viruses such as hepatitis C virus and poliovirus. The present review focuses on recent progress and challenges in the development of *in vitro* culture models and alternative models available to study hNoV.

In vitro infection and culture models of hNoV

De novo infection of established cell lines

A large number of established cell lines have been tested for *in vitro* infection with hNoV (Table 1). Many different

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Table 1 Norovirus infections in established human cell lines

Cell line	Culture method	Norovirus strain	References
Human intestinal epithelium			
AGS	Rotation	GGI.1, GGI.2, GGI.4, GGII.4	[18, 37]
	Low temperature (34 °C)		
Caco-2	Cell differentiation	GGI.1, GGI.2, GGI.4, GGII.3, GGII.4, GGII.5	[25, 80, 96]
	Co-culture		
	Various culture techniques		
	Infection by low-speed centrifugation		
	Treatment with Lipofectamine		
	Low temperature (34 °C)		
HT-29	Cell differentiation (I)	GGI.1, GGI.2, GGI.4, GGII.3, GGII.4, GGII.5	
	Long-term culture		
	Culture in transwell plates		
HCT-8	Cell differentiation	GGI.2, GGI.4, GGII.3, GGII.4, GGII.5	[23, 44, 82]
Detroit 562	DMSO (0.3-1 %)		
HuTu-80	Butyric acid (1-5 mM)		
	Insulin (0.5 U/ml)		
	Dexamethasone (0.1-10 uM)		
	Various culture techniques		
	Culture with rocking		
	Hypotonic shock		
	Infection by low-speed centrifugation		
I-407	Treatment with Lipofectamine	GGI.1, GGII.4	[18, 56]
	Low temperature (34 °C)		
Kato-3			
Other human cell lines			
A549			
CCD-18	Co-culture with Caco-2	GGI.1	[25]
Detroit 551	Rotation	GGI.1-4, GGII.1-4	[37]
HEp-2			
HEC	Infection by low centrifugation	GGI.1	[63, 90]
HeLa	Low temperature	GGI.1	[18]
RD	Rotation	GGI.1-4, GGII.1-4	[37]
293	Infection by low-speed centrifugation	GGI.1	[63, 90]

Adapted and modified from Duizer *et al.* [19]

animal cell lines as well as human epithelial cells from the gastrointestinal tract have been tested for susceptibility to hNoV infection, with no clear indications of infection. As simple and conventional infections have not been successful, various culture methods and manipulations of cellular phenotypes have also been attempted (Table 1). [18, 19, 23, 25, 37, 44, 56, 63, 80, 82, 90, 96]. However, there have been no clear indications of *de novo* hNoV infection observed [19]. A plausible explanation may include the inactivation of virus particles upon excretion in feces. This possibility can be tested if virus stocks can be prepared from sources other than feces. However, Schwab et al. [87] reported that hNoV forms very stable particles that can survive for an extended period outside the human body. In addition, other enteric viruses are not inactivated by similar preparations from feces. Therefore, noroviral inactivation upon excretion might not fully explain its inability to infect established cell lines [19, 88].

As some caliciviruses require the presence of intestinal contents for infection [22, 78, 86], the hypothesis that hNoV needs to be modified in a manner similar to that in the gastrointestinal tract of the human body was put forth and tested. For example, pre-treatment with trypsin and intestinal contents from a gnotobiotic pig has been reported

to be required for infection with and replication of feline and swine norovirus, respectively. However, similar pretreatment with supplements did not result in hNoV infection in the culture. Another possibility is that hNoV infection is inhibited or promoted by the presence of virus-specific antibodies. If hNoV particles are coated with neutralizing antibodies in the feces, viruses might lose infectivity in the subsequent infections in cultured cells. However, in some viral infections, the presence of virus-specific antibodies helps virions infect their target cells. Examples include, but are not limited to, human cytomegalovirus [65], foot-and-mouth disease virus [68, 84], and dengue virus [28, 29, 42]. Therefore, it would be intriguing to explore antibody-dependent enhancement of viral infection to test if the binding of neutralizing antibodies to hNoV renders them infectious under *in vitro* conditions.

Attempts have been made to infect not only established cell lines but also human macrophages and dendritic cells [58]. Murine norovirus (MNV) was first isolated and identified in immune-compromised mice [49]. MNV was found to infect and replicate in macrophages and dendritic cells from STAT1-deficient mice, and the same types of cells have been reported to be infectible *in vitro* [14, 109]. As the genome of hNoV is detected in the sera of pediatric patients [98], human macrophages and dendritic cells were tested for their ability to be infected *in vitro* [58]. However, no indications of infection were observed. Interestingly, it has been reported that infection with porcine enteric calicivirus (PEC) requires the presence of bile acids, which in turn inhibit the function of STAT1 [11]. Considering that type I and II interferons inhibit viral replication and protein expression of MNV, which can infect STAT1-deficient mice [14, 64], it is possible that STAT1 knockdown with specific siRNA can render otherwise resistant cells susceptible. However, the inhibition of STAT1 expression did not promote viral infection (Mary K. Estes, personal communication).

Most recently, a breakthrough has finally been made by Jones et al. in the development of an *in vitro* hNoV culture model [43] using B cells in the presence of a commensal bacterium, *Enterobacter cloacae*. In fact, it has been reported that B cells are required for replication of MNV [5, 73, 113]. Jones et al. [43] showed that while hNoV infection of B cells required the presence of *E. cloacae*, MNV could infect B cells *in vitro* even in the absence of the bacteria and that oral antibiotic administration reduced MNV replication *in vivo*. It has been established that norovirus infection in humans is correlated with the histo-blood group antigen (HBGA) expression profile [38, 66]; however, it remains unclear how the HBGA-expressing bacteria promote hNoV infection in B cells, especially considering the fact that HBGA-like molecules expressed on commensal bacteria would compete for hNoV binding with HBGA on hNoV target cells. The requirement for enteric bacteria for hNoV infection of B cells is a groundbreaking finding in the norovirus field, but it might not be surprising, as it is known that some enteric viruses, including mouse mammary tumor virus [46], poliovirus [55, 83], and reovirus [55] require bacteria for infection and replication.

Three-dimensional (3-D) cell culture methods

3-D culture techniques have been developed, helping to promote infections and cultures of various pathogenic bacteria and viruses. Infection of cells in a monolayer is very different from that in an *in vivo* environment. Proper differentiation of epithelial cells requires apical and basolateral polarization, and two-dimensional (2-D) culture techniques might not be able to support the same cellular differentiation found *in vivo*. 3-D organoid culture techniques were first developed and used to investigate the infection and pathogenicity of *Salmonella enteritica* in INT-407 human intestinal epithelial cells [32, 74, 75]. Similar culture techniques have been exploited for establishing infections with various bacteria (*Escherichia coli* [32, 74] and *Pseudomonas* species [9]) and viruses (Epstein-Barr virus [62], Kaposi's sarcoma-associated herpesvirus [16], rotavirus [21] and hNoV [93]). This culture technique was first developed by NASA [75], and the 3-D organoid culture is basically a bioreactor with a rotating cylinder. Cell culture medium is added to the cylinder along with collagen I-coated porous microcarrier beads. With the addition of cells, the cylinder continues to rotate to prevent the cells from binding to the walls of the cylinder. Cells grow in and on the porous beads to form 3-D structures that closely resemble physiological tissues or organs of the body. INT-407 cells in 3-D cultures have been shown to differentiate into various cell types, thus enabling 'co-cultures' in a bioreactor [74, 93]. The co-culture of various cell types in 3-D has been reported to allow noroviral infection [19, 93], which is not possible in the 2-D culture of the same cell type. In addition, Straub et al. [93] also reported that a type of histo-blood group antigen (i.e., Lewis antigen A), a cellular attachment receptor for hNoV, was expressed at the apical tip of the 3-D culture. When the 3-D culture of INT-407 was infected with hNoV, the authors observed a cytopathic effect, and an increase in the number of viral genome copies was detected by reverse transcription polymerase chain reaction (RT-PCR) and fluorescence *in situ* hybridization (FISH). Based on these findings, Straub et al. claimed that the 3-D culture of INT-407 was susceptible to hNoV. However, in the same report [93], Caco-2 and HT-29 cells were not susceptible to hNoV, even in 3-D organoid cultures. In 2011, the same group of researchers reported [94] that a

subclone of Caco-2 cells (C2bbe1) was susceptible. They claimed that C2bbe1 cells in the 3-D culture were able to fully differentiate in the 3-D culture, while the parental Caco-2 cells were not.

These findings, however, have been disputed by the other research groups: norovirus infection was not detected in 3-D culture [31]. A joint study conducted by the Nickerson and Estes groups reported that norovirus infection was not detected in a 3-D culture by real-time PCR or immunofluorescent assay. In addition, histo-blood group antigens such as Lewis antigen were not expressed. Based on these and other findings, the joint group concluded that the CPE in the 3-D culture that was observed upon inoculation with norovirus was likely due to the toxicity of contaminating lipopolysaccharides in the virus stock prepared from the fecal samples. The claims of the papers by Straub et al. [93, 94] could also not be verified by Takanashi et al. [99]. This discrepancy will only be resolved when virus stocks can be prepared from samples other than fecal samples.

Challenges to the development of *in vitro* cultivation of hNoV

HNoV is known to bind to HBGA to infect the host [38, 39, 50, 60, 66, 67, 77]. Interestingly, the same is true of rotavirus [40], one of the two major gastroenteritis-causing viruses in humans. When functional α (1,2) fucosyltransferase 2 (FUT2) was not expressed, norovirus infection was not detected in experimental infections in human volunteers. FUT2 is an enzyme required for the expression of HBGA on the surface of epithelial cells, some of which is secreted in bodily fluids, including saliva [54, 76]. HBGA type 1 (e.g., Lewis b [Le^b]) is mainly expressed on the epithelial cells at the junction of the stomach and duodenum, and HBGA type 2 is expressed at the glandular level [66, 71, 85, 92]. In an experiment using virus-like particles (VLPs), VLPs bind to HBGA, inducing their internalization [66]. In addition, VLPs seem to specifically bind to A, H1, and Lewis b antigens [17, 30, 35, 66]. Therefore, functional receptor expression appears to be critical for successful infection with hNoV in culture. The overexpression of FUT2 in Huh-7 cells, a human hepatoma cell line, resulted in strong binding of viruses to the cells; however, it did not influence viral internalization or replication. In fact, many other studies have led to the same conclusion: overexpression of FUT2 has a negligible effect on virus internalization, uncoating and viral genome replication [1, 11, 27, 31, 108]. These results imply that HBGA expression alone may not be sufficient for viral infection [31, 100]. Furthermore, the presence of other proteins may be required for norovirus infection. One interesting hypothesis is that not only HBGA but also a co-receptor may be required for norovirus infection [100], the discovery of which would lead to the development of an *in vitro* culture model of hNoV. In fact, this hypothesis has been proposed by many researchers in the field [31, 105]. Identification and characterization of co-receptor(s) would provide a breakthrough in the development of a convenient and reliable hNoV culture model. In addition, to develop a successful hNoV *in vitro* culture model, more experimental infection systems need to be investigated, including primary human intestinal cells or tissue explants and 3-D co-cultures of different cell types. An interesting alternative that has shown promising results in a recent study is the development of intestinal organoids using pluripotent stem cells [21].

One of the major barriers to hNoV study is the inability to prepare purified virus stocks. Virus stocks prepared from human feces are often contaminated with LPS or other enteric viruses (e.g., rotavirus), which makes it difficult to interpret the CPE observed in experimental infections [31]. Therefore, the development of a norovirus producer cell line that enables the preparation of a large quantity of purified hNoV will certainly provide a breakthrough in the field of hNoV research.

Research methods for hNoV

Utilization of norovirus replicons or infectious cDNA clones

Due to the lack of susceptible cell lines, the development of preventive vaccines and virus-specific therapies has been hampered. However, the use of virus replicons containing part of the viral genome enables screening of antiviral drugs and efficacy testing. The first of such replicons expresses neomycin in place of the VP1 capsid protein in Huh-7 and BHK21 cells (Fig. 1B) [12]. This replicon was stably maintained over extended passages, and viral protein expression was detected in those cells. Using the hNoV replicon, Chang et al. identified interferon alpha, interferon gamma, ribavirin [13], and peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) [6] as antiviral agents. Viral-replicon-containing cells are useful for identifying antiviral agents against non-capsid proteins but might not be adequate for studying the full life cycle of the virus due to the lack of VP1. To overcome these limitations, three independent groups of researchers have developed systems that harbor the full cDNA of hNoV [2, 45, 52]. The overall experimental designs of two of the systems are similar. First, the T7 promoter was added to the 5' end of the full viral cDNA with a poly(A) tract of 26 to 30 nucleotides at the 3' end (Fig. 1B and C). To regulate transcription by T7 polymerase, the T7 terminator

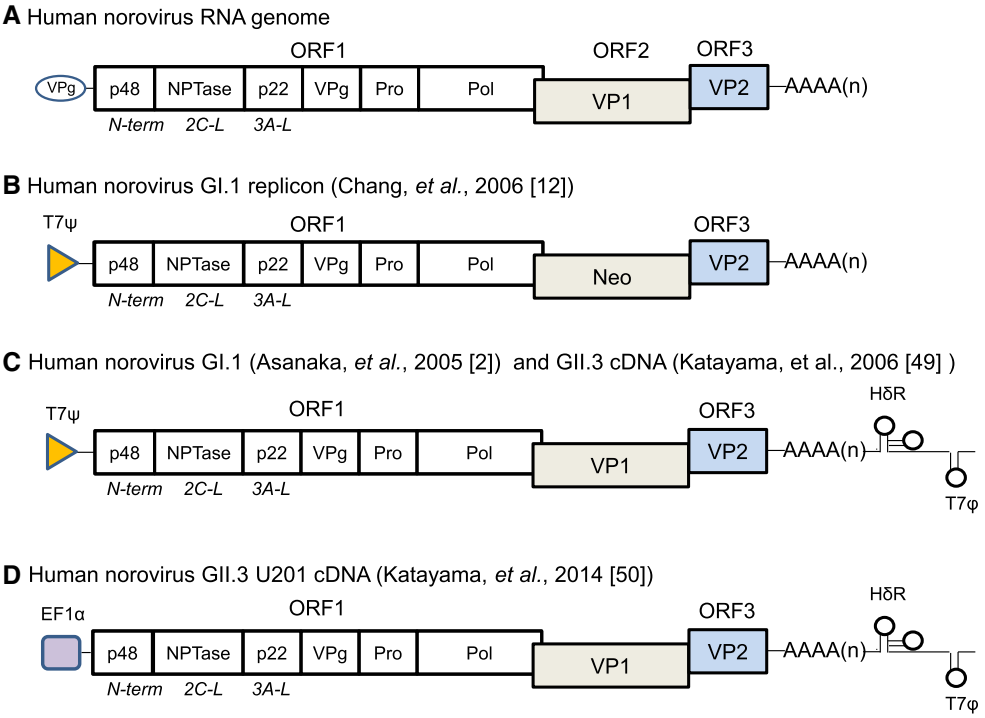

Fig. 1 HNoV replicons and infectious cDNAs

sequence was inserted at the far end of the 3' end of the construct (Fig. 1C and D). Between the poly(A) and T7 terminator, the ribozyme sequence was added so that the full viral genome was precisely processed (Fig. 1C and D). When the T7 polymerase was expressed using vaccinia virus, virus particle production was detected by electron microscopy at a density of 1.318 g/cm^3 as determined by gradient ultracentrifugation. These data suggest that complete virions were formed by the binding of the viral genome and capsid proteins. However, the infectivity of the virions could not be determined due to the lack of susceptible cell lines. Furthermore, these systems require the presence of a helper virus to express functional T7 polymerase. To overcome this inconvenience, Katayama et al. [53] developed a plasmid-based hNoV reverse genetics system (Fig. 1D), which was successfully exploited to produce GFP-expressing recombinant hNoV. Development of the long-awaited recombinant hNoV will certainly help identify susceptible cells *in vivo* and *in vitro*. For example, virus stocks prepared from cell culture without contamination with endotoxin, as is seen in stocks prepared from patient stool samples, will effectively help resolve the debate over whether contaminating endotoxin in hNoV stocks was the primary cause of the cytopathic effect observed in the 3-D organoid culture model. In addition, the use of GFP-expressing recombinant hNoV will enable investigators to detect low-level hNoV infections in both primary and established cells, allowing sensitive and high-throughput viral detection using fluorescence. Furthermore, with the availability of an *in vitro* B cell infection model and recombinant hNoV viruses, the requirement(s) for viral entry in cultured cell lines can be effectively analyzed and probed.

Utilization of virus-like particles (VLPs)

VLPs are suitable study materials for investigating the immunological aspects of hNoV infection. VLPs are particles made of self-assembled viral capsid proteins containing no viral RNA genome. The norovirus genome encodes two capsid proteins: VP1 and VP2. Of the two, VP1 alone can be assembled to form VLPs, and the function of VP2 has only begun to be revealed. Interestingly, VP2 is not required for VLP assembly but seems to enhance the expression of VP1 in cell culture and associate with VP1 within the shell domain, promoting the stability of VLPs [59, 106]. The outer structure of VLPs made of VP1 alone is known to be identical to that of complete virions containing the RNA genome. To date, many protein expression systems have been exploited, including insect cells [26, 41], human cells (293T [102], Caco-2 [4]), and plant cells (tomato [36], and potato [97]). In the absence of *de novo* infection systems for hNoV, VLPs have played a critical role in determining how norovirus interacts with host cells. VLPs have been shown to bind directly to HBGA molecules on the surface of host cells *in vitro* [66], including A, H type1, and Le^b carbohydrates [30]. These data indicate that the cellular receptor for hNoV is HBGA,

Table 2 Animal infection models for norovirus

Virus	Pathology	Host	Susceptible cells
GI & II NV (Hu)	Gastroenteritis	Human	N/A
Saporovirus	Gastroenteritis	Human	N/A
GII NV (Hu)	Gastroenteritis	Gnotobiotic piglets	Duodenal and jejunal enterocytes [15]
GI & II NV (Hu)	No	Dogs	Not known [8, 95]
Recombinant swine NV (GII)	Gastroenteritis	Pigs	N/A [91]
Bovine NV (GIII)	No	Cattle	N/A
Murine NV (GV)	No*	Mice	Macrophages/DCs [49, 109]
Tulane	Not known	Rhesus macaques	Kidney cells [20, 101, 112]
RHDV	Hemorrhagic	Rabbits	Hepatocytes [72], kidney cells [81] and alveolar macrophages [24]
FCV	Respiratory/systemic disease	Cats	Kidney cortex cells [70]

Adapted and modified from Vashist et al. [105]

N/A, not available; RHDV, rabbit hemorrhagic disease virus; FCV, feline calicivirus

* MNV can cause systemic disease in immune-compromised mice

which attests to the usefulness of VLPs. In this regard, VLPs are invaluable for the study of virus-host interactions.

Utilization of other animal noroviruses

Due to the lack of cell culture or animal models for hNoV, other caliciviruses that infect experimental animals represent useful alternatives. In fact, a large portion of the known mechanisms of regulation of viral gene expression, gene function, and genome structure have been extrapolated from animal noroviruses. Animal noroviruses that can be cultivated *in vitro* are listed in Table 2. Among them, the best-characterized model is MNV (MNV) [49, 107, 110]. MNV infection occurs through the same fecal-oral route as hNoV. MNV is easy to manipulate experimentally, and it infects murine macrophages and dendritic cells as well as RAW264.7[109], a macrophage cell line. MNV belongs to genogroup V, while hNoV belongs to genogroup I, II, or IV. MNV is especially useful for studying virus-specific immune responses in a variety of knockout mice [89]. Using these models, primary and memory responses to norovirus infection have been extensively studied [3, 69, 73]. Furthermore, MNV has been successfully used for development of vaccines against norovirus infection [10, 61]. Details are reviewed elsewhere [51, 103, 110].

However, despite its many advantages, the MNV model has clear limitations as an alternative to hNoV infection. First, MNV-infected mice do not show symptoms such as diarrhea or vomiting. Second, mice are chronically infected [33, 34], which is in stark contrast to acute infection by hNoV. Third, number of MNV genotypes is limited, and it thus may not be suitable for the development of vaccines [104]. Lastly, hNoV does not seem to be able to infect monocyte-derived macrophages and dendritic cells *in vitro* [10]. The availability of a small-animal model that mimics the pathology of hNoV infections will undoubtedly play a key role in the development of antivirals and vaccines in the future.

Conclusion

Since the discovery of hNoV, little progress has been made regarding its mechanism of infection, replication, or host immune responses, and the most important reason for this dearth of understanding is the lack of cell culture or animal infection models. Thus, it is of paramount importance to develop a cell culture model to identify antiviral agents and vaccines against hNoV. As such, the development of a cell culture model is the key to an explosive expansion of research on this virus.

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References

1. Amano J, Oshima M (1999) Expression of the H type 1 blood group antigen during enterocytic differentiation of Caco-2 cells. J Biol Chem 274:21209–21216
2. Asanaka M, Atmar RL, Ruvolo V, Crawford SE, Neill FH, Estes MK (2005) Replication and packaging of Norwalk virus RNA in cultured mammalian cells. Proc Natl Acad Sci USA 102:10327–10332
3. Bailey D, Thackray LB, Goodfellow IG (2008) A single amino acid substitution in the murine norovirus capsid protein is sufficient for attenuation in vivo. J Virol 82:7725–7728
4. Baric RS, Yount B, Lindesmith L, Harrington PR, Greene SR, Tseng FC, Davis N, Johnston RE, Klapper DG, Moe CL (2002)

Expression and self-assembly of norwalk virus capsid protein from venezuelan equine encephalitis virus replicons. J Virol 76:3023–3030
5. Basic M, Keubler LM, Buettner M, Achard M, Breves G, Schroder B, Smoczek A, Jorns A, Wedekind D, Zschemisch NH, Gunther C, Neumann D, Lienenklaus S, Weiss S, Hornef MW, Mahler M, Bleich A (2014) Norovirus triggered microbiota-driven mucosal inflammation in interleukin 10-deficient mice. Inflamm Bowel Dis 20:431–443
6. Bok K, Cavanaugh VJ, Matson DO, Gonzalez-Molleda L, Chang KO, Zintz C, Smith AW, Iversen P, Green KY, Campbell AE (2008) Inhibition of norovirus replication by morpholino oligomers targeting the 5'-end of the genome. Virology 380:328–337
7. Bok K, Green KY (2012) Norovirus gastroenteritis in immunocompromised patients. N Engl J Med 367:2126–2132
8. Caddy SL, de Rougemont A, Emmott E, El-Attar L, Mitchell JA, Hollinshead M, Belliot G, Brownlie J, Le Pendu J, Goodfellow I (2015) Evidence for human norovirus infection of dogs in the UK. J Clin Microbiol 53:1873–1883
9. Carterson AJ, Honerzu Bentrup K, Ott CM, Clarke MS, Pierson DL, Vanderburg CR, Buchanan KL, Nickerson CA, Schurr MJ (2005) A549 lung epithelial cells grown as three-dimensional aggregates: alternative tissue culture model for Pseudomonas aeruginosa pathogenesis. Infect Immun 73:1129–1140
10. Chachu KA, LoBue AD, Strong DW, Baric RS, Virgin HW (2008) Immune mechanisms responsible for vaccination against and clearance of mucosal and lymphatic norovirus infection. PLoS Pathogens 4:e1000236
11. Chang KO, Sosnovtsev SV, Belliot G, Kim Y, Saif LJ, Green KY (2004) Bile acids are essential for porcine enteric calicivirus replication in association with down-regulation of signal transducer and activator of transcription 1. Proc Natl Acad Sci USA 101:8733–8738
12. Chang KO, Sosnovtsev SV, Belliot G, King AD, Green KY (2006) Stable expression of a Norwalk virus RNA replicon in a human hepatoma cell line. Virology 353:463–473
13. Chang KO, George DW (2007) Interferons and ribavirin effectively inhibit Norwalk virus replication in replicon-bearing cells. J Virol 81:12111–12118
14. Changotra H, Jia Y, Moore TN, Liu G, Kahan SM, Sosnovtsev SV, Karst SM (2009) Type I and type II interferons inhibit the translation of murine norovirus proteins. J Virol 83:5683–5692
15. Cheetham S, Souza M, Meulia T, Grimes S, Han MG, Saif LJ (2006) Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. J Virol 80:10372–10381
16. Cheng F, Pekkonen P, Laurinavicius S, Sugiyama N, Henderson S, Gunther T, Rantanen V, Kaivanto E, Aavikko M, Sarek G, Hautaniemi S, Biberfeld P, Aaltonen L, Grundhoff A, Boshoff C, Alitalo K, Lehti K, Ojala PM (2011) KSHV-initiated notch activation leads to membrane-type-1 matrix metalloproteinase-dependent lymphatic endothelial-to-mesenchymal transition. Cell Host Microbe 10:577–590
17. Choi JM, Hutson AM, Estes MK, Prasad BV (2008) Atomic resolution structural characterization of recognition of histo-blood group antigens by Norwalk virus. Proc Natl Acad Sci USA 105:9175–9180
18. Cubitt WD, Barrett AD (1984) Propagation of human candidate calicivirus in cell culture. J Gen Virol 65(Pt 6):1123–1126
19. Duizer E, Schwab KJ, Neill FH, Atmar RL, Koopmans MP, Estes MK (2004) Laboratory efforts to cultivate noroviruses. J Gen Virol 85:79–87
20. Farkas T, Sestak K, Wei C, Jiang X (2008) Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. J Virolgy 82:5408–5416
21. Finkbeiner SR, Zeng XL, Utama B, Atmar RL, Shroyer NF, Estes MK (2012) Stem cell-derived human intestinal organoids as an infection model for rotaviruses. mBio 3:e00159–e00212
22. Flynn WT, Saif LJ (1988) Serial propagation of porcine enteric calicivirus-like virus in primary porcine kidney cell cultures. J Clin Microbiol 26:206–212
23. Gauthier R, Harnois C, Drolet JF, Reed JC, Vezina A, Vachon PH (2001) Human intestinal epithelial cell survival: differentiation state-specific control mechanisms. Am J Physiol Cell Physiol 280:C1540–C1554
24. Gelmetti D, Grieco V, Rossi C, Capucci L, Lavazza A (1998) Detection of rabbit haemorrhagic disease virus (RHDV) by in situ hybridisation with a digoxigenin labelled RNA probe. J Virol Methods 72:219–226
25. Goke M, Kanai M, Podolsky DK (1998) Intestinal fibroblasts regulate intestinal epithelial cell proliferation via hepatocyte growth factor. Am J Physiol 274:G809–G818
26. Green KY, Kapikian AZ, Valdesuso J, Sosnovtsev S, Treanor JJ, Lew JF (1997) Expression and self-assembly of recombinant capsid protein from the antigenically distinct Hawaii human calicivirus. J Clin Microbiol 35:1909–1914
27. Guix S, Asanaka M, Katayama K, Crawford SE, Neill FH, Atmar RL, Estes MK (2007) Norwalk virus RNA is infectious in mammalian cells. J Virol 81:12238–12248
28. Halstead SB, Chow JS, Marchette NJ (1973) Immunological enhancement of dengue virus replication. Nat New Biol 243:24–26
29. Halstead SB, O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med 146:201–217
30. Harrington PR, Lindesmith L, Yount B, Moe CL, Baric RS (2002) Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. J Virol 76:12335–12343
31. Herbst-Kralovetz MM, Radtke AL, Lay MK, Hjelm BE, Bolick AN, Sarker SS, Atmar RL, Kingsley DH, Arntzen CJ, Estes MK, Nickerson CA (2013) Lack of norovirus replication and histo-blood group antigen expression in 3-dimensional intestinal epithelial cells. Emerg Infect Dis 19:431–438
32. Honerzu Bentrup K, Ramamurthy R, Ott CM, Emami K, Nelman-Gonzalez M, Wilson JW, Richter EG, Goodwin TJ, Alexander JS, Pierson DL, Pellis N, Buchanan KL, Nickerson CA (2006) Three-dimensional organotypic models of human colonic epithelium to study the early stages of enteric salmonellosis. Microbes Infect Institut Pasteur 8:1813–1825
33. Hsu CC, Riley LK, Wills HM, Livingston RS (2006) Persistent infection with and serologic cross-reactivity of three novel murine noroviruses. Comp Med 56:247–251
34. Hsu CC, Riley LK, Livingston RS (2007) Molecular characterization of three novel murine noroviruses. Virus Genes 34:147–155
35. Huang P, Farkas T, Zhong W, Tan M, Thornton S, Morrow AL, Jiang X (2005) Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. J Virol 79:6714–6722
36. Huang Z, Elkin G, Maloney BJ, Beuhner N, Arntzen CJ, Thanavala Y, Mason HS (2005) Virus-like particle expression and assembly in plants: hepatitis B and Norwalk viruses. Vaccine 23:1851–1858
37. Hughes JH (1993) Physical and chemical methods for enhancing rapid detection of viruses and other agents. Clin Microbiol Rev 6:150–175

38. Hutson AM, Atmar RL, Graham DY, Estes MK (2002) Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis 185:1335–1337
39. Hutson AM, Airaud F, LePendu J, Estes MK, Atmar RL (2005) Norwalk virus infection associates with secretor status genotyped from sera. J Med Virol 77:116–120
40. Imbert-Marcille BM, Barbe L, Dupe M, Le Moullac-Vaidye B, Besse B, Peltier C, Ruvoen-Clouet N, Le Pendu J (2014) A FUT2 gene common polymorphism determines resistance to rotavirus A of the P[8] genotype. J Infect Dis 209:1227–1230
41. Jiang X, Wang M, Graham DY, Estes MK (1992) Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 66:6527–6532
42. Johnson KM, Halstead SB, Cohen SN (1967) Hemorrhagic fevers of Southeast Asia and South America: a comparative appraisal. Prog Med Virol 9:105–158
43. Jones MK, Watanabe M, Zhu S, Graves CL, Keyes LR, Grau KR, Gonzalez-Hernandez MB, Iovine NM, Wobus CE, Vinje J, Tibbetts SA, Wallet SM, Karst SM (2014) Enteric bacteria promote human and mouse norovirus infection of B cells. Science 346:755–759
44. Joshi SS, Jackson JD, Sharp JG (1985) Differentiation inducing effects of butyrate and DMSO on human intestinal tumor cell lines in culture. Cancer Detect Prev 8:237–245
45. Kamata K, Shinozaki K, Okada M, Seto Y, Kobayashi S, Sakae K, Oseto M, Natori K, Shirato-Horikoshi H, Katayama K, Tanaka T, Takeda N, Taniguchi K (2005) Expression and antigenicity of virus-like particles of norovirus and their application for detection of noroviruses in stool samples. J Med Virol 76:129–136
46. Kane M, Case LK, Kopaskie K, Kozlova A, MacDearmid C, Chervonsky AV, Golovkina TV (2011) Successful transmission of a retrovirus depends on the commensal microbiota. Science 334:245–249
47. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM (1972) Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol 10:1075–1081
48. Kapikian AZ (2000) The discovery of the 27-nm Norwalk virus: an historic perspective. J Infect Dis 181(Suppl 2):S295–S302
49. Karst SM, Wobus CE, Lay M, Davidson J, Virgin HWt (2003) STAT1-dependent innate immunity to a Norwalk-like virus. Science 299:1575–1578
50. Karst SM (2010) Pathogenesis of noroviruses, emerging RNA viruses. Viruses 2:748–781
51. Karst SM, Wobus CE, Goodfellow IG, Green KY, Virgin HW (2014) Advances in norovirus biology. Cell Host Microbe 15:668–680
52. Katayama K, Hansman GS, Oka T, Ogawa S, Takeda N (2006) Investigation of norovirus replication in a human cell line. Arch Virol 151:1291–1308
53. Katayama K, Murakami K, Sharp TM, Guix S, Oka T, Takai-Todaka R, Nakanishi A, Crawford SE, Atmar RL, Estes MK (2014) Plasmid-based human norovirus reverse genetics system produces reporter-tagged progeny virus containing infectious genomic RNA. Proc Natl Acad Sci USA 111:E4043–E4052
54. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB (1995) Sequence and expression of a candidate for the human Secretor blood group alpha(1,2)fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem 270:4640–4649
55. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, Dermody TS, Pfeiffer JK (2011) Intestinal microbiota promote enteric virus replication and systemic pathogenesis. Science 334:249–252
56. Lamartina S, Roscilli G, Rinaudo D, Delmastro P, Toniatti C (1998) Lipofection of purified adeno-associated virus Rep68 protein: toward a chromosome-targeting nonviral particle. J Virol 72:7653–7658
57. Lambden PR, Caul EO, Ashley CR, Clarke IN (1993) Sequence and genome organization of a human small round-structured (Norwalk-like) virus. Science 259:516–519
58. Lay MK, Atmar RL, Guix S, Bharadwaj U, He H, Neill FH, Sastry KJ, Yao Q, Estes MK (2010) Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans. Virology 406:1–11
59. Lin Y, Fengling L, Lianzhu W, Yuxiu Z, Yanhua J (2014) Function of VP2 protein in the stability of the secondary structure of virus-like particles of genogroup II norovirus at different pH levels: function of VP2 protein in the stability of NoV VLPs. J Microbiol 52:970–975
60. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R (2003) Human susceptibility and resistance to Norwalk virus infection. Nat Med 9:548–553
61. LoBue AD, Thompson JM, Lindesmith L, Johnston RE, Baric RS (2009) Alphavirus-adjuvanted norovirus-like particle vaccines: heterologous, humoral, and mucosal immune responses protect against murine norovirus challenge. J Virol 83:3212–3227
62. Long JP, Hughes JH (2001) Epstein-Barr virus latently infected cells are selectively deleted in simulated-microgravity cultures. Cell Dev Biol Anim 37:223–230
63. Maitreyi RS, Broor S, Kabra SK, Ghosh M, Seth P, Dar L, Prasad AK (2000) Rapid detection of respiratory viruses by centrifugation enhanced cultures from children with acute lower respiratory tract infections. J Clin Virol 16:41–47
64. Maloney NS, Thackray LB, Goel G, Hwang S, Duan E, Vachharajani P, Xavier R, Virgin HW (2012) Essential cell-autonomous role for interferon (IFN) regulatory factor 1 in IFN-gamma-mediated inhibition of norovirus replication in macrophages. J Virol 86:12655–12664
65. Manley K, Anderson J, Yang F, Szustakowski J, Oakeley EJ, Compton T, Feire AL (2011) Human cytomegalovirus escapes a naturally occurring neutralizing antibody by incorporating it into assembling virions. Cell Host Microbe 10:197–209
66. Marionneau S, Ruvoen N, Le Moullac-Vaidye B, Clement M, Cailleau-Thomas A, Ruiz-Palacois G, Huang P, Jiang X, Le Pendu J (2002) Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. Gastroenterology 122:1967–1977
67. Marionneau S, Airaud F, Bovin NV, Le Pendu J, Ruvoen-Clouet N (2005) Influence of the combined ABO, FUT2, and FUT3 polymorphism on susceptibility to Norwalk virus attachment. J Infect Dis 192:1071–1077
68. Mason PW, Baxt B, Brown F, Harber J, Murdin A, Wimmer E (1993) Antibody-complexed foot-and-mouth disease virus, but not poliovirus, can infect normally insusceptible cells via the Fc receptor. Virology 192:568–577
69. McCartney SA, Thackray LB, Gitlin L, Gilfillan S, Virgin HW, Colonna M (2008) MDA-5 recognition of a murine norovirus. PLoS Pathogens 4:e1000108
70. Milek M, Wooley RE, Blue JL (1976) Replication of feline herpesvirus and feline calicivirus in cell and organ cultures. Am J Vet Res 37:723–724
71. Mollicone R, Bara J, Le Pendu J, Oriol R (1985) Immunohistologic pattern of type 1 (Lea, Leb) and type 2 (X, Y, H) blood group-related antigens in the human pyloric and duodenal mucosae. Lab Investig J Tech Methods Pathol 53:219–227
72. Moussa A, Chasey D, Lavazza A, Capucci L, Smid B, Meyers G, Rossi C, Thiel HJ, Vlasak R, Ronsholt L et al (1992)

Haemorrhagic disease of lagomorphs: evidence for a calicivirus. Vet Microbiol 33:375–381
73. Mumphrey SM, Changotra H, Moore TN, Heimann-Nichols ER, Wobus CE, Reilly MJ, Moghadamfalahi M, Shukla D, Karst SM (2007) Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon responses. J Virol 81:3251–3263
74. Nickerson CA, Goodwin TJ, Terlonge J, Ott CM, Buchanan KL, Uicker WC, Emami K, LeBlanc CL, Ramamurthy R, Clarke MS, Vanderburg CR, Hammond T, Pierson DL (2001) Three-dimensional tissue assemblies: novel models for the study of Salmonella enterica serovar Typhimurium pathogenesis. Infect Immun 69:7106–7120
75. Nickerson CA, Richter EG, Ott CM (2007) Studying host-pathogen interactions in 3-D: organotypic models for infectious disease and drug development. J Neuroimmune Pharmacol 2:26–31
76. Oriol R (1990) Genetic control of the fucosylation of ABH precursor chains. Evidence for new epistatic interactions in different cells and tissues. J Immunogenet 17:235–245
77. Parrino TA, Schreiber DS, Trier JS, Kapikian AZ, Blacklow NR (1977) Clinical immunity in acute gastroenteritis caused by Norwalk agent. N Engl J Med 297:86–89
78. Parwani AV, Flynn WT, Gadfield KL, Saif LJ (1991) Serial propagation of porcine enteric calicivirus in a continuous cell line. Effect of medium supplementation with intestinal contents or enzymes. Arch Virol 120:115–122
79. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD (2008) Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 14:1224–1231
80. Pinto RM, Diez JM, Bosch A (1994) Use of the colonic carcinoma cell line $CaCo_2$ for in vivo amplification and detection of enteric viruses. J Med Virol 44:310–315
81. Prieto JM, Fernandez F, Alvarez V, Espi A, Garcia Marin JF, Alvarez M, Martin JM, Parra F (2000) Immunohistochemical localisation of rabbit haemorrhagic disease virus VP-60 antigen in early infection of young and adult rabbits. Res Vet Sci 68:181–187
82. Quaroni A, Tian JQ, Goke M, Podolsky DK (1999) Glucocorticoids have pleiotropic effects on small intestinal crypt cells. Am J Physiol 277:G1027–G1040
83. Robinson CM, Jesudhasan PR, Pfeiffer JK (2014) Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. Cell Host Microbe 15:36–46
84. Robinson L, Windsor M, McLaughlin K, Hope J, Jackson T, Charleston B (2011) Foot-and-mouth disease virus exhibits an altered tropism in the presence of specific immunoglobulins, enabling productive infection and killing of dendritic cells. J Virol 85:2212–2223
85. Ruvoen-Clouet N, Mas E, Marionneau S, Guillon P, Lombardo D, Le Pendu J (2006) Bile-salt-stimulated lipase and mucins from milk of 'secretor' mothers inhibit the binding of Norwalk virus capsids to their carbohydrate ligands. Biochem J 393:627–634
86. Schaffer FL, Soergel ME, Black JW, Skilling DE, Smith AW, Cubitt WD (1985) Characterization of a new calicivirus isolated from feces of a dog. Arch Virol 84:181–195
87. Schwab KJ, Estes MK, Neill FH, Atmar RL (1997) Use of heat release and an internal RNA standard control in reverse transcription-PCR detection of Norwalk virus from stool samples. J Clin Microbiol 35:511–514
88. Schwab KJE, Atmar RL (2000) Norwalk and other human caliciviruses: molecular characterization, epidemiology, and pathogenesis. In: JW Cary JELDB (ed) Microbial foodborne diseases. Technomic Publishing, Lancaster, pp 460–493
89. Scipioni A, Mauroy A, Vinje J, Thiry E (2008) Animal noroviruses. Vet J 178:32–45
90. Seno M, Takao S, Fukuda S, Kanamoto Y (1991) Enhanced isolation of influenza virus in conventional plate cell cultures by using low-speed centrifugation from clinical specimens. Am J Clin Pathol 95:765–768
91. Shen Q, Zhang W, Yang S, Yang Z, Chen Y, Cui L, Zhu J, Hua X (2012) Recombinant porcine norovirus identified from piglet with diarrhea. BMC Vet Res 8:155
92. Shirato H, Ogawa S, Ito H, Sato T, Kameyama A, Narimatsu H, Xiaofan Z, Miyamura T, Wakita T, Ishii K, Takeda N (2008) Noroviruses distinguish between type 1 and type 2 histo-blood group antigens for binding. J Virol 82:10756–10767
93. Straub TM, Honerzu Bentrup K, Orosz-Coghlan P, Dohnalkova A, Mayer BK, Bartholomew RA, Valdez CO, Bruckner-Lea CJ, Gerba CP, Abbaszadegan M, Nickerson CA (2007) In vitro cell culture infectivity assay for human noroviruses. Emerg Infect Dis 13:396–403
94. Straub TM, Bartholomew RA, Valdez CO, Valentine NB, Dohnalkova A, Ozanich RM, Bruckner-Lea CJ, Call DR (2011) Human norovirus infection of $CaCo_2$ cells grown as a three-dimensional tissue structure. J Water Health 9:225–240
95. Summa M, von Bonsdorff CH, Maunula L (2012) Pet dogs—a transmission route for human noroviruses? J Clin Virol 53:244–247
96. Svensson L, Finlay BB, Bass D, von Bonsdorff CH, Greenberg HB (1991) Symmetric infection of rotavirus on polarized human intestinal epithelial ($CaCo_2$) cells. J Virol 65:4190–4197
97. Tacket CO, Mason HS, Losonsky G, Estes MK, Levine MM, Arntzen CJ (2000) Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. J Infect Dis 182:302–305
98. Takanashi S, Hashira S, Matsunaga T, Yoshida A, Shiota T, Tung PG, Khamrin P, Okitsu S, Mizuguchi M, Igarashi T, Ushijima H (2009) Detection, genetic characterization, and quantification of norovirus RNA from sera of children with gastroenteritis. J Clin Virol 44:161–163
99. Takanashi S, Saif LJ, Hughes JH, Meulia T, Jung K, Scheuer KA, Wang Q (2014) Failure of propagation of human norovirus in intestinal epithelial cells with microvilli grown in three-dimensional cultures. Arch Virol 159:257–266
100. Tan M, Jiang X (2010) Norovirus gastroenteritis, carbohydrate receptors, and animal models. PLoS Pathogens 6:e1000983
101. Tan M, Wei C, Huang P, Fan Q, Quigley C, Xia M, Fang H, Zhang X, Zhong W, Klassen JS, Jiang X (2015) Tulane virus recognizes sialic acids as cellular receptors. Sci Rep 5:11784
102. Taube S, Kurth A, Schreier E (2005) Generation of recombinant norovirus-like particles (VLP) in the human endothelial kidney cell line 293T. Arch Virol 150:1425–1431
103. Taube S, Kolawole AO, Hohne M, Wilkinson JE, Handley SA, Perry JW, Thackray LB, Akkina R, Wobus CE (2013) A mouse model for human norovirus. mBio 4:e00450–e00513
104. Thackray LB, Wobus CE, Chachu KA, Liu B, Alegre ER, Henderson KS, Kelley ST, Virgin HWt (2007) Murine noroviruses comprising a single genogroup exhibit biological diversity despite limited sequence divergence. J Virol 81:10460–10473
105. Vashist S, Bailey D, Putics A, Goodfellow I (2009) Model systems for the study of human norovirus Biology. Future Virol 4:353–367
106. Vongpunsawad S, Venkataram Prasad BV, Estes MK (2013) Norwalk virus minor capsid protein VP2 associates within the VP1 shell domain. J Virol 87:4818–4825
107. Ward JM, Wobus CE, Thackray LB, Erexson CR, Faucette LJ, Belliot G, Barron EL, Sosnovtsev SV, Green KY (2006)

Pathology of immunodeficient mice with naturally occurring murine norovirus infection. Toxicol Pathol 34:708–715
108. White LJ, Ball JM, Hardy ME, Tanaka TN, Kitamoto N, Estes MK (1996) Attachment and entry of recombinant Norwalk virus capsids to cultured human and animal cell lines. J Virol 70:6589–6597
109. Wobus CE, Karst SM, Thackray LB, Chang KO, Sosnovtsev SV, Belliot G, Krug A, Mackenzie JM, Green KY, Virgin HW (2004) Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol 2:e432
110. Wobus CE, Thackray LB, Virgin HWt (2006) Murine norovirus: a model system to study norovirus biology and pathogenesis. J Virol 80:5104–5112
111. Xi JN, Graham DY, Wang KN, Estes MK (1990) Norwalk virus genome cloning and characterization. Science 250:1580–1583
112. Zhang D, Huang P, Zou L, Lowary TL, Tan M, Jiang X (2015) Tulane virus recognizes the A type 3 and B histo-blood group antigens. J Virol 89:1419–1427
113. Zhu S, Regev D, Watanabe M, Hickman D, Moussatche N, Jesus DM, Kahan SM, Napthine S, Brierley I, Hunter RN 3rd, Devabhaktuni D, Jones MK, Karst SM (2013) Identification of immune and viral correlates of norovirus protective immunity through comparative study of intra-cluster norovirus strains. PLoS Pathogens 9:e1003592