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Advances in understanding of the innate immune response to human norovirus infection using organoid models

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Abstract

Norovirus is the leading cause of epidemic and endemic acute gastroenteritis worldwide and the most frequent cause of foodborne illness in the United States. There is no specific treatment for norovirus infections and therapeutic interventions are based on alleviating symptoms and limiting viral transmission. The immune response to norovirus is not completely understood and mechanistic studies have been hindered by lack of a robust cell culture system. In recent years, the human intestinal enteroid/human intestinal organoid system (HIE/HIO) has enabled successful human norovirus replication. Cells derived from HIE have also successfully been subjected to genetic manipulation using viral vectors as well as CRISPR/Cas9 technology, thereby allowing studies to identify antiviral signaling pathways important in controlling norovirus infection. RNA sequencing using HIE cells has been used to investigate the transcriptional landscape during norovirus infection and to identify antiviral genes important in infection. Other cell culture platforms such as the microfluidics-based gut-on-chip technology in combination with the HIE/HIO system also have the potential to address fundamental questions on innate immunity to human norovirus. In this review, we highlight the recent advances in understanding the innate immune response to human norovirus infections in the HIE system, including the application of advanced molecular technologies that have become available in recent years such as the CRISPR/Cas9 and RNA sequencing, as well as the potential application of single cell transcriptomics, viral proteomics, and gut-on-a-chip technology to further elucidate innate immunity to norovirus.

Keywords

enteroids; innate immunity; norovirus

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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INTRODUCTION

Norovirus is the leading cause of epidemic and endemic acute gastroenteritis worldwide and the most frequent cause of foodborne illness in the United States [1]. Norovirus gastroenteritis causes approximately 21 million cases of illness in the United States and over 600 million cases worldwide every year [2]. Although the disease is typically self-limiting, some cases result in severe illness, with an estimated 56 000 to 71 000 hospitalizations and 570 to 800 norovirus related deaths reported in the United States each year [2]. Human norovirus spreads primarily via the oral-faecal route or through contact with contaminated food, water, or surfaces. As few as 18–1018 genome equivalents are sufficient to cause infection [3–5], and an estimated 50% human infectious dose (HID_{50}) ranges between 1320 and 2800 genome equivalents [5]. Norovirus disease is characterized by stomach pain, nausea, explosive vomiting and diarrhoea within 12–48 h of exposure [6]. In immune-competent individuals these symptoms typically resolve within 1–3 days although the virus might be detectable for several weeks [7]. There is no specific treatment for norovirus infection and therapeutic interventions are based on alleviating symptoms [8]. Outbreak management relies heavily on early identification of cases, isolation of infected individuals, and strict disinfection and decontamination protocols [7, 8]. Several norovirus vaccine candidates are under development, four of which have undergone clinical trials [9]; however, none have currently been licensed. In this review, we will summarize the current information on the innate immune response to norovirus infection in human intestinal enteroids (HIE) and advanced molecular technologies that may help to better understand the molecular mechanisms that regulate norovirus replication allowing the development of effective prophylactic and therapeutic interventions.

Human volunteer challenge studies have provided important information on aspects of virus infection such as the environmental conditions that affect virus stability [10], the HID_{50} [3, 5], host genetic factors that govern susceptibility to infection [11], as well as insights on the immune response during infection [12–14]. Several studies have shown association between susceptibility to norovirus infection and expression of a functional FUT2 gene [11, 15–17]. FUT2 encodes α–1,2-fucosyltransferase, an enzyme that is important for expression of human blood group antigen (HBGA) molecules on mucosal surfaces. α–1,2-fucosyltransferase transfers a second fucose molecule to the H blood group antigen precursor, thereby generating H antigen [17, 18]. Norovirus infection requires HBGA which function as binding ligands to facilitate virus attachment to cells [19]. Individuals that express a functional FUT2 gene are termed secretor-positive and are therefore susceptible to norovirus infection [17]. Secretor-negative individuals lack functional FUT2 alleles, consequently, they do not express H-antigen structures on their mucosa and are resistant to infection by most norovirus strains, including the globally predominant genotype GII.4 [20], although exceptions have been reported [6, 17].

Human volunteer challenge studies have also given initial insights into the antiviral response that restricts norovirus infections. Analysis of serum collected during the first 4 days after infection in two human volunteer challenge studies showed that norovirus induces T-helper 1 and T-helper 2 (Th1 and Th2) cytokines, chemokines, and inflammatory cytokines including IFN-γ, IL-6, IL-8, IL-12p70, MCP-1 and TNF-α, as part of the acute response, with peak

detection at 2 days post-infection [12]. Assessment of norovirus-specific antibody responses during infection using saliva collected from elderly individuals in 43 long-term care facilities showed that virus-specific salivary IgA titers increase beginning at 5 days after symptom onset, with peak titers at 14 days [21]. Together, these studies show that both the innate and adaptive immune responses are important for controlling norovirus infection [22, 23]. What remains to be elucidated are the specific molecular mechanisms and signalling pathways involved in the antiviral response against human norovirus.

Models for norovirus infection

For many years mechanistic studies were hampered by lack of a robust cell culture system. Efforts to grow norovirus in a number of well-established cell lines, including primary kidney cell lines, primary intestinal cell lines, and colon carcinoma cell lines, failed [24]. Despite the established tropism of murine norovirus (MNV) for innate immune cells, efforts to replicate human norovirus in the same cell types derived from peripheral blood mononuclear cells (PBMCs) also failed [25]. It has been reported that human noroviruses are capable of replicating in human B cells [26, 27]. Attempts to obtain sustained norovirus replication in B cells using unfiltered, unprocessed stool as inoculum showed that bacterial surface expressed human blood group antigens (HBGA) are important factors for successful virus replication [26]. Additionally, the addition of HBGA-expressing *Enterobacter cloacae* to the cell culture could restore the infectivity of filtered human norovirus positive stool filtrate, whereas a non-HBGA-expressing bacterium could not [26]. BJAB and Raji B cell lines initially showed promise for replicating human norovirus however these studies have been shown to be difficult to reproduce [26, 27].

Several replicon models that stably express human norovirus RNA have been developed as tools to facilitate studying the immune response to norovirus infection [28–30]. A number of animal models have also been utilized to study human norovirus including non-human primates [31, 32], gnotobiotic pigs [33, 34] and humanized mice [35]. Each of these models had limitations including low levels of virus replication. Recently, zebrafish larvae have been reported as a robust model for human norovirus infection [36]. However, this model does not necessarily represent cells in the human gut and the ensuing innate immune responses after a norovirus infection. Despite the above-mentioned efforts, what remains unclear are the antiviral proteins that specifically restrict virus replication as well as the molecules that recognize norovirus to initiate the antiviral signal pathways. Until recently, much of our understanding of the molecular mechanisms involved in norovirus pathogenesis and immune response has been derived from transformed cell lines and infection with human norovirus surrogate viruses such as feline calicivirus, porcine calicivirus, MNV and Tulane virus [37–39] (Fig. 1).

In recent years, technical advances in the culture of primary human intestinal epithelial cells using intestinal 3D organoid cultures [40] along with availability of advanced molecular technologies, have revolutionized approaches to study the immune response in norovirus infection. Techniques such as gene manipulation, bulk and single-cell RNA sequencing (scRNA-seq), proteomics [41–43], as well as gut-on-a-chip technology, which mimic the

intestinal physiological environment [40–42], have the potential to be applied to human norovirus studies in order to move the field forward (Fig. 2).

Human intestinal enteroid/organoid cultures and norovirus tropism

HIEs and organoid cultures [40, 44–47] have created platforms to study the cellular processes and signalling pathways involved in restricting replication of enteric viruses including human norovirus [48, 49]. Human intestinal organoids (HIO) and HIE are three-dimensional (3D) cultures containing multiple intestinal cell types that are derived from Lgr5+ intestinal stem cells [44] (Fig. 2). HIO contain a mesenchymal niche and are derived from embryonic or pluripotent stem cells (iPSCs) [50], whereas HIE are derived from adult stem cells isolated from intestinal biopsies [40, 51, 52]. These stem cells are propagated in a 3D format supported by Matrigel which allows assembly of the cells into organoid structures that retain cellular composition and physiological functions of the intestinal epithelium [53]. Additionally, adult stem cells are intrinsically programmed with their location-specific function [54], and the differentiated cells that are derived from these stem cells retain an immune profile akin to that of the cells in the corresponding intestinal segment [55]. For replication of human norovirus infection *in vitro,* these 3D HIE cultures are dissociated and plated as monolayers which are then utilized in a wide variety of studies [56] (Fig. 2).

Successful norovirus replication *in vitro* using monolayers of HIEs was first reported in 2016 [57] and later confirmed by several other laboratories [58–62]. The cell types found in enteroid cultures include enterocytes, goblet, enteroendocrine, and Paneth cells [53]. Human norovirus potentially replicates in multiple cells types including enterocytes and enteroendocrine cells (EECs) [63, 64]. Presence of human norovirus in enterocytes was first discovered by histological comparison of tissue biopsies from infected and uninfected immunocompromised transplant patients which showed presence of the major capsid protein VP1 in enterocytes from infected individuals [63]. Interestingly, the VP1 expression was also detected in other cells types including, macrophages, T cells and dendritic cells, however, non-structural proteins RdRp and VPg were detected along with VP1 only in enterocytes [63]. Recently human norovirus has been shown to replicate in enteroendocrine epithelial cells (EEC) [64]. Immunohistochemical staining of tissue from the jejunum and ileum of a paediatric intestinal transplant recipient with severe gastroenteritis showed the presence of human norovirus VP1 protein in EEC. Confocal fluorescence microscopy showing colocalization of positive and negative sense human norovirus RNA with the EEC marker (chromogranin A -CgA), confirmed active norovirus replication in this cell type *in vivo* [64, 65].

The use of commercial media has further optimized human norovirus replication in HIE yielding higher levels of virus replication compared to home-made conditioned media [61]. However, not all norovirus strains can replicate in HIEs [57, 58] with success rate of samples with high viral load as low as 20% [58]. GII.4 viruses demonstrate higher replication levels compared to other genotypes such as GII.3 [57, 58]. A potential explanation for this difference could be that norovirus strains respond differently to the antiviral mechanisms employed by the host cell to restrict virus replication. Other components of the complex intestinal environment, such as the intestinal microbiome and M cells, may also play a role

in the strain-specific differences in virus replication [61]. The absence of these components potentially represent major drawbacks of the HIE system.

Human norovirus replication is enhanced or depends on the inclusion of bile acids in the cell culture media [57, 66]. However, the requirement for bile is strain dependent as inclusion is critical for replication of GI.1, GII.1, GII.3, GII.6, and GII.17 strains whereas GII.4 virus replication occurs without supplementation, but is enhanced by bile [61]. This breakthrough has cleared the way for other lines of research including investigation of the antiviral mechanisms that restrict virus replication.

Genetic manipulation of enteroids

Several research groups have begun exploring whether cells derived from HIE and HIO are amendable to genetic manipulation. Using CRISPR/Cas9 technology, a knockout cell line for the FUT2 gene was created [16], FUT2 encodes an enzyme that affects HBGA expression in intestinal epithelial cells and susceptibility to human norovirus infection [17]. This FUT2 knockout cell line demonstrated diminished replication of GII.4, GII.17, and GI.1 viruses. Further, norovirus replication was shown in secretor-negative J4 cells by knocking in the FUT2 gene, thereby demonstrating that FUT2 expression is necessary and sufficient for norovirus replication in HIEs. While the role of FUT2 has been established epidemiologically, these knockout HIE cell lines provided the genetic basis for this observation [16].

In another study which explored the role of interferon signalling in norovirus infection, lentiviral vectors were used to express proteins that antagonize interferon signalling thereby generating intestinal organoid lines incapable of interferon signalling [59]. Specifically, lentiviral vectors were used to express bovine viral diarrhoea virus NPro or parainfluenza virus type 5 (PIV5) V proteins. BVDF Npro blocks IFN production by degrading interferon regulatory factor 3 (IRF3) whereas the PIV5 V protein compromises IFN production and signalling by targeting key molecules such as STAT1, melanoma differentiation-associated protein 5 (MDA5), and LGP2 for degradation [59]. This study demonstrated that enteroid cells are robust enough for transfection and have the potential to be genetically modified to create cells that reliably sustain norovirus replication. Importantly, it showed that the enteroid cells can be modified to increase virus yield by disrupting the interferon signalling pathway, as demonstrated by a 33-fold increase in norovirus GII.3 replication in BVDF Npro-expressing cells compared to control (nontransduced) cells, and a six-fold increase in PIV5 V protein-expressing cells compared to control cells.

What is currently known about innate immune response to norovirus infection?

While the receptor for human norovirus is yet to be found, CD300lf (Fig. 1a) has been identified as the primary receptor for MNV [19, 67–69]. Following viral entry, virus particles are recognized by molecular sensors on the plasma membrane, in endosomes, and in the cytosol which trigger induction of an antiviral or inflammatory response [70] (Fig. 1b). The specific sensors that recognize human norovirus have yet to be identified; however, a recent study showed that Toll-like receptors (TLR) 2 and 5 are activated by norovirus virus-like particles (VLPs) [71] (Fig. 1b). Using a TLR2-transfected HEK293 responder

cell line, the authors demonstrated that norovirus VLPs can attach to TLR2. Using a TLR5 expressing cell line with an NF-kB-luciferase cassette, they also demonstrated that norovirus VLPs attachment to TLR5 can induce NF-KB driven inflammatory signalling (Fig. 1b, c). These results suggest that TLR 2 and 5 may be involved in recognition of human norovirus leading to induction of an inflammatory response upon infection. Whether this observation can be recapitulated in a physiologically relevant system such as enterocytes derived from HIE requires additional studies.

An early study utilizing 293FT cells transfected with stool-isolated human norovirus RNA showed that while these cells were capable of replicating norovirus RNA, a robust type I interferon response is not induced [72], which was in contrast with the prominent role of type I interferon in the restriction of MNV replication in macrophages and dendritic cells [73–75]. It has now been demonstrated that human noroviruses indeed induce a robust innate immune response chiefly orchestrated by type I and type III interferon [59, 76, 77] (Fig. 1d). Studies with MNV have further dissected this pathway to reveal pivotal roles for transcription factors STAT1 and 2 as well as interferon regulatory factors (IRF) 1, 3 and 7 [59, 78, 79] (Fig. 1b, e). MDA5, another molecular sensor that recognizes single stranded RNA, is also thought to be involved [80, 81]. Using the Norwalk replicon system, it was shown that MDA5 activation by human norovirus RNA results in activation of the JAK-STAT pathway which leads to production of interferon, a cytokine response that induces an antiviral state in infected cells and surrounding uninfected cells [81]. Fig. 1 summarizes what is currently known regarding the antiviral response to norovirus infection. Although some factors involved in the antiviral response to MNV are included, the immune response to MNV infection was out of the scope of this review and has been covered extensively elsewhere [76, 82].

HIE and the innate immune response to human norovirus infection

Interferon (IFN) is a major component of the antiviral response that is induced upon norovirus infection [75]. To investigate this, HIE cells that had been treated with exogenous IFN (type I IFN [IFNα1 and IFNβ1] or type III IFN [IFNλ1, IFNλ2, and IFNλ3]) were infected with norovirus GII.3 or GII.4. Both strains showed reduction in replication suggesting that GII.4 and GII.3 norovirus strains are sensitive to IFN [77]. Consistent with this finding, when enteroid-derived IFN-receptor-knockout cell lines were infected with GII.3 and GII.4 strains, both strains showed higher levels of replication compared to infection in wild-type cells [77]. However, GII.3 virus replication was rescued to a greater extent than GII.4, suggesting that GII.3 infected cells are more susceptible to IFN restriction [77]. This was further confirmed using transcriptome analysis which demonstrated that human norovirus elicits a predominantly type III IFN response, and that GII.3 strains induced a more robust IFN-stimulated gene response compared to GII.4 strains [77].

Using a specific Janus kinase 1 (JAK1)/JAK2 inhibitor Ruxolitinib (Rux) to disrupt IFN signalling downstream of the IFN-receptor prior to infection of duodenal IECs with GII.3 or GII.4 strains of norovirus resulted in an increase in GII.4 virus replication. This further demonstrated the importance of IFN signalling in restricting virus replication in HIE [59].

Altogether, these studies clearly highlight the benefit of using HIE in understanding the role of IFN in the antiviral response against human norovirus.

Interferon stimulated genes that restrict human norovirus infection

The IFN signalling pathway is a cytokine-based response that results in restriction of virus growth in infected cells and upregulation of antiviral genes in surrounding uninfected cells. IFN secreted from virus-infected cells functions in an autocrine and paracrine manner to engage the IFN-receptors on the cell surface and activate JAK kinases and phosphorylation of STAT1/2 which facilitate upregulation of hundreds of interferon stimulated genes (ISG) (Fig. 1e, f). These ISGs encode effectors of the antiviral response, which antagonize virus replication [83, 84](Fig. 1f).

Little is known about specific antiviral genes that restrict norovirus replication in enteroid/organoid-derived cells. To investigate this, monolayers from two organoid-derived cell lines (terminal ileum organoids) were infected with GII.4 viruses [59] and using RNA-sequencing, 162 genes were found to be differentially regulated in one cell line, and 70 genes were differentially regulated in another cell line [59]. A majority of these were ISGs, demonstrating that human norovirus induces a robust ISG response. The highly upregulated genes included IFI44L, OAS2, OASL, MX-1 and ISG15 which have shown antiviral activity against several viruses including, Zika virus, respiratory syncytial virus, and influenza [85–89].

In another study, transcriptome analysis of two enteroid cell lines using RNA-sequencing also demonstrated a robust transcriptional response 72 h after infection. Additionally, this study found that diverse type I (IFN β) and type III (IFN λ) IFN-driven responses were induced [90]. The use of HIE/HIO has significantly advanced the identification of potential antiviral genes that control norovirus infection. However, much work remains to be done to fully understand the molecular mechanisms by which the antiviral proteins restrict virus replication.

FUTURE PERSPECTIVES

A major caveat of using this enteroid/organoid system is that the gene expression changes that have been found using transcriptomic analysis represent changes in a bulk population of cells. Norovirus replicates in enterocytes, however the proportion of enterocytes that are infected and sustain virus replication is unclear. Furthermore, the extent to which other cellular types in these culture systems can sustain norovirus replication is not known. Other important information for the development of targeted therapies that could mitigate norovirus replication such as the contribution of each cell type to the immune response during infection, remains unclear. Several recent technologies, such as single cell transcriptomics [41, 42, 91], viral proteomics [92, 93] and gut on a chip system [94–96] are promising approaches to further dissect the innate immune response to norovirus.

Single-cell RNA-sequencing has also led to the discovery of rare intestinal cell types [97], and the capability of norovirus to infect these rare cell types is yet to be clarified.

A crucial component of the innate immune response to enteric pathogens are Microfold cells (M cells) [98]. M cells are unique as they function as a first line of defence in an innate immune capacity but also bridge the innate immune response and adaptive immune response by functioning as antigen presenting cells that facilitate the production of antibodies to protect from subsequent infection. Because of their important role in the intestine during the immune response against murine norovirus infection [99, 100], it may be important to develop culture conditions that support differentiation of M cells from enteroid monolayers [101] in order to ascertain their role in human norovirus infection. By using single cell approaches, the transcriptional response in each cell type can be determined to understand the individual contribution of each cell type to the response [102].

Along with single cell transcriptomics, advancements in mass spectrometry and high-throughput cell imaging allow large-scale surveys at protein level [103]. Mass spectrometry proteomics approaches are frequently employed to study cell-viral interactions, how viruses affect cellular signalling pathways, and which cellular proteins are crucial for viral persistence [92, 104–107]. Every virus encodes proteins that manipulate key cellular pathways to promote viral replication and evade the host immune response [92]. Data from a proteomics study on HIE has confirmed that Paneth and goblet cells generated from intestinal stem cells *in vitro* share features typical of these cell types observed *in vivo* further confirming that HIE are useful models to investigate normal and disease processes in the intestine [108]. Applying viral proteomics to norovirus infections in enterocytes/HIE will help understand cellular responses during viral pathogenesis as well as in identifying diagnostic and therapeutic targets against human norovirus. Combining data generated by transcriptomics and viral proteomics methods [93] will allow a more comprehensive understanding of the regulatory network driving the human host response to norovirus infection (Fig. 2).

The knowledge derived from using the HIE/HIO system to understand the innate immune response to norovirus infection can be applied to other systems such as the recently developed gut-on-a-chip system which is an innovative *in vitro* platform for studying gut physiology [94–96]. This technology attempts to mimic the complexity and physiology of native tissues *in vitro* using cells grown in a series of chambers and maintained in culture medium under conditions that maintain physiological function of the tissue from which the cells were derived [94]. Compared to static cell culture, gut-on-a-chip technology allows the cells to be maintained under mechanically active conditions, and small amounts of the media can be continuously sampled for metabolites, cytokines, or even virus production [94, 95, 109].

Recent work in which this technology was used to study Coxsackie B virus 1 (CVB1) replication in CaCo2 cells showed that gut-on-a-chip has the potential to be applied to norovirus studies [110]. In this study, CaCo2 cells were seeded in a gut-on-a-chip device containing two hollow microchannels separated by a porous membrane. Six days after seeding the cells were polarized and CVB1 was injected into the device allowing for infection on the apical side of the cell monolayers. Media was collected and virus replication and cytokine production (IP-10 and IL-8) were detectable at 24 h post-infection [110]. Differentiated organoid cells also have the potential to be maintained in this

microenvironment [111], which could expand the scope of the studies done with organoid cells in norovirus infection.

CONCLUSIONS

HIE/HIO are non-transformed cell culture models that contain multiple intestinal epithelial cell types that comprise the intestinal epithelium. HIEs provide an excellent platform to study human norovirus replication, which until recently has been a major hurdle in advancing human norovirus research. Fundamental questions and challenges can now be addressed to further our understanding of norovirus infection. Remaining questions in the field include identifying the human norovirus receptor that facilitates virus entry, the molecular sensors that trigger an antiviral response once infection has been established as well as the antiviral genes most relevant for restricting norovirus infection.

Another major challenge in studying norovirus replication and innate immune response in differentiated HIEs is the relatively low success for virus replication and difficulty in passaging the viruses. To overcome this, detailed understanding of the complexity of virus host interactions is required. Use of recently described molecular approaches such as RNAseq analysis and CRISPR/Cas9 modification of HIEs have identified many potential immune targets involved in norovirus replication opening opportunities to study the innate immune response after human norovirus infection.

Going forward, advanced technologies such as single cell transcriptomics, viral proteomics and gut on a chip technology may help to better understand the molecular mechanisms that regulate norovirus replication allowing the development of effective prophylactic and therapeutic interventions.

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Abbreviations:

EEC	enteroendocrine epithelial cell
FUT2	α-1,2-fucosyltransferase
HBGA	human blood group antigen
HIE	human intestinal enteroids
HIO	human intestinal organoids
IFN	interferon
ISG	interferon stimulated gene
JAK	Janus kinase
MDA5	melanoma differentiation-associated protein 5

MNV	murine norovirus
PBMC	peripheral blood mononuclear cell
PIV5	parainfluenza virus type 5
RIG-I	retinoic acid-inducible gene 1
STAT	signal transducer and activator of transcription
TLR	toll-like receptor
VLP	virus-like particle
VP1	virus protein 1
VPg	viral protein genome-linked

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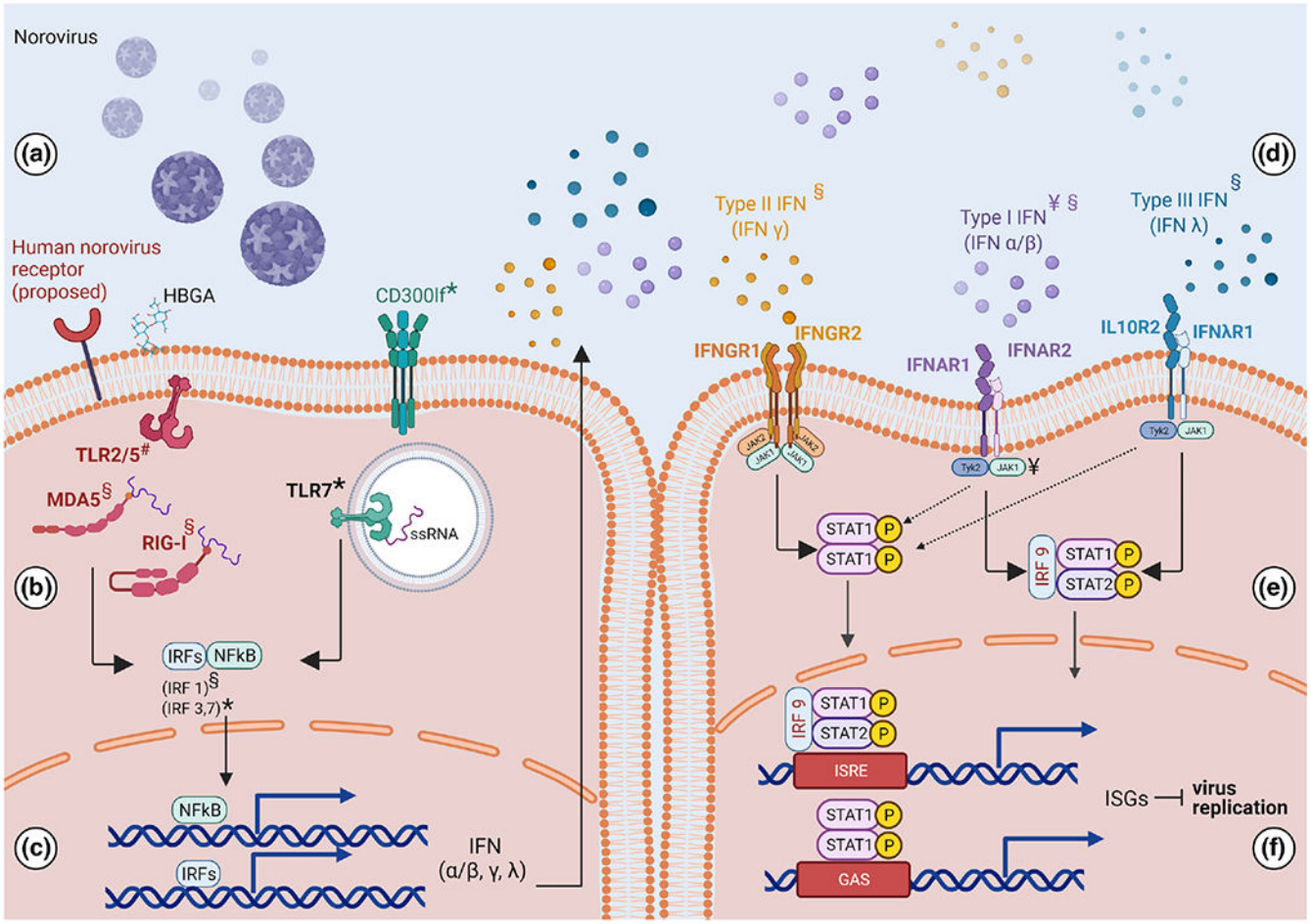

Fig. 1.

The antiviral response in norovirus-infected cells. Norovirus infection induces an antiviral response that restricts virus replication. a. Human norovirus attachment is facilitated by human blood group antigens (HBGA) and a receptor that is yet to be identified. In mice the receptor is CD300lf [66]. b. Viral entry results in sensing of the virus and viral components by molecular sensors. The molecular sensors that are triggered during human norovirus infection have not been identified, however human norovirus virus-like particles (VLPs) have been shown to trigger TLR2 and TLR5 (70). MDA5 and RIG-I are also involved in the response against human norovirus [102] and TLR7 has been shown to trigger an antiviral response that protects against murine norovirus (MNV) [103]. c. Virus sensing results in activation of transcription factors including NFkB [103] and interferon regulatory factors IRF1(102), IRF3 and IRF7(3) which facilitate transcription of genes encoding type I, type II and type III interferon. d. Interferon is secreted and engages corresponding receptors on the cell surface, resulting in phosphorylation and activation of STAT1/2 through the JAK/STAT pathway [55]. e. STAT1/2 translocate to the nucleus and facilitate upregulation of hundreds of antiviral interferon stimulated genes (ISGs). f. The proteins encoded by antiviral ISGs restrict virus replication. This figure summarizes the immune components involved in the antiviral response to norovirus infection and includes findings from human norovirus infection as well as its surrogates: ¥human norovirus infection in human intestinal

enteroids, *murine norovirus, # human norovirus virus like particles (VLPs), §human norovirus replicon. Created with BioRender.com (accessed on 12 October 2021).

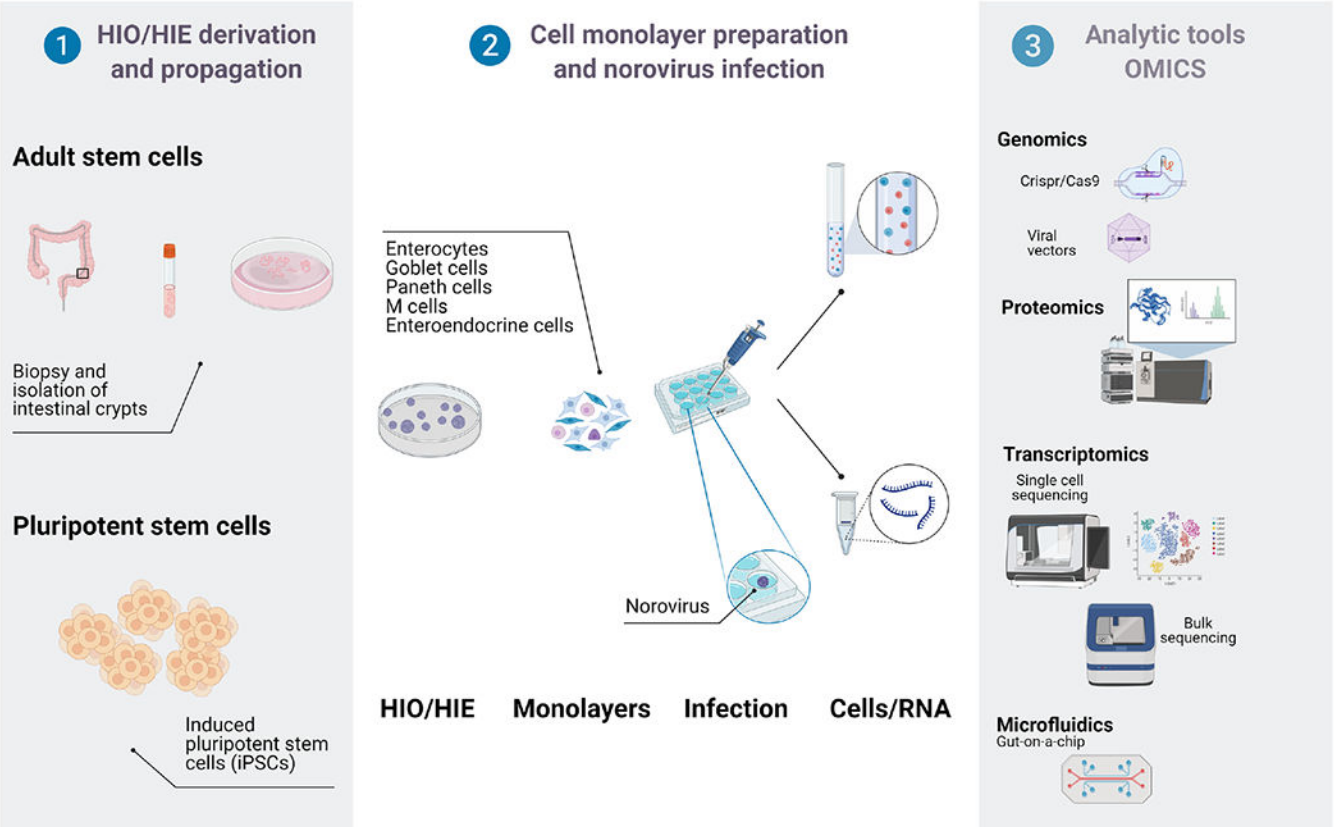

Fig. 2.

Application of human intestinal enteroids/organoids (HIE/HIO) in understanding the immune response to human norovirus infection. HIE/HIO can be applied in multiple ways to investigate the immune response to norovirus infection and determine host antiviral factors that restrict norovirus replication. HIE and HIO are derived either from adult intestinal biopsies, or adult induced pluripotent cells (iPSCs). Once HIE/HIO are propagated and differentiated, they can be infected with human norovirus. The cells can also be genetically manipulated using CRISPR/Cas9 or viral vectors to create mutant cell lines that can also be used in norovirus studies. Cells and RNA derived from infected HIE/HIO can be analysed for transcriptional changes using RNA sequencing. Similarly, the cells can also be subjected to proteomics-based analyses to investigate effects of norovirus infection on expression. Another potential application of HIE/HIO is measuring metabolites during norovirus infection using gut-on a chip technology. Created with BioRender.com (accessed on 12 October 2021).