

The Role of Type I Interferon in Regulating Norovirus Infections

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Abstract

Noroviruses are responsible for a large majority of non-bacterial gastroenteritis outbreaks worldwide. They are considered to be the leading cause of foodborne disease outbreaks and are classified as category B biodefense agents. Noroviruses pose huge medical and economic burdens to the global community: While they display a rapid course of infection in healthy adults, several risk groups including children, the elderly, and immunocompromised individuals are susceptible to more severe and prolonged infections. Moreover, noroviruses can affect any semi-closed community including nursing homes, schools, disaster relief situations, vacation-based and military settings, and they are a major cause of hospital closures for the purpose of outbreak control. Elucidating the host immune responses contributing to the control of norovirus infections is essential to advance our understanding of norovirus pathogenesis and eventually to the design of effective anti-norovirus therapeutic strategies. Recent work on both human and murine noroviruses has unequivocally demonstrated that type I interferon responses, and in particular STAT1-mediated signaling events, are essential for norovirus control. This regulation is mediated at least in part by direct inhibition of norovirus replication in infected cells. The pattern recognition receptor MDA5 contributes to norovirus recognition, although other as-yet-unidentified host receptors must also be involved. Host genes induced upon type I interferon signaling target norovirus translation independent of the well-characterized antiviral molecule PKR. There is suggestive indirect evidence that noroviruses can delay the induction of type I interferon, but may not encode mechanisms to antagonize interferon-mediated signaling. Future mechanistic studies aimed at elucidating the precise interferon-dependent mechanisms by which host cells target norovirus replication may contribute to the design of effective anti-norovirus therapeutic strategies modeled on this activity. Furthermore, there are ongoing efforts to determine whether exogenous interferon can be used therapeutically in the context of norovirus infections.

Keywords: Norovirus; Interferon; Antiviral response

Introduction

Noroviruses (NVs) are positive-sense RNA viruses with genomes of approximately 7.5 kilobases comprised of three open reading frames, the 5' ORF1 encoding a large nonstructural polypeptide, the internal ORF2 encoding the major capsid protein, and the 3' ORF3 encoding a small protein referred to as VP2 that may play a minor structural role [1]. Norovirus infections account for the majority of gastroenteritis outbreaks worldwide and are believed to cause significant morbidity and even mortality in specific risk groups including young children [2-4] and the elderly [5-7]. They are considered by the CDC to be the leading cause of foodborne disease outbreaks [8] and they are classified as Category B biodefense agents. Individuals infected with a NV typically develop symptoms of vomiting and diarrhea with or without nausea and abdominal cramps following a 24-48 hour incubation period, and symptoms resolve within 12-72 hours [9]. This quick course of infection implicates pre-existing or rapidly inducible host effector mechanisms of the innate immune system in the control of NV infections. Indeed, type I interferon (IFN), and in particular STAT1-dependent signaling, blocks NV replication in vitro [10-13] and is necessary to prevent severe NV-induced disease in vivo [14,15].

Type I IFN is a powerful antiviral cytokine that is produced from most virus-infected cells. The induction of type I IFN is initiated upon recognition of a pathogen-associated molecular pattern (PAMP) by a host pattern recognition receptor (PRR). Known PRRs that recognize viruses include: (i) cytoplasmic RIG-I-like helicases including RIG-I itself and MDA5; (ii) Toll-like receptors (TLRs) that are expressed at the cell surface and on membranes of endosomal compartments; and (iii) dsRNA-activated protein kinase (PKR). Once a PRR engages a viral PAMP, a signaling cascade is activated that culminates in the induction of type I IFN in addition to other proinflammatory cytokines. Type I IFN is secreted and binds the IFN- α receptor (IFNAR) in an autocrine

and paracrine fashion, activating cytoplasmic signal transducer and activator of transcription (STAT) complexes. Various combinations of STAT complexes ultimately translocate to the nucleus and bind specific regulatory elements present in the promoters of certain genes, referred to as IFN-stimulated genes (ISGs), to activate their transcription. The STAT-1 molecule is absolutely essential to the IFN-induced antiviral response [16,17]. While the protein products of several ISGs (e.g. PKR [18], the 2'-5' oligoadenylate synthase [2'-5'-OAS] system [19,20], Mx proteins [21], and ISG15 [22]) have defined antiviral activity, it is generally believed that many other ISGs encode proteins with as-yet unidentified antiviral activity. This idea is supported by a recent elegant study by Schoggins et al. which carried out a large-scale antiviral ISG screen targeting diverse virus families [23]. In this screen, multiple novel ISGs with activity against a broad set of virus families, as well as those directed against specific types of viruses, were identified. Overall, type I IFN signaling regulates the host immune response in numerous ways, including induction of an antiviral state in responding cells, activation of certain types of immune cells including macrophages (M ϕ s) and natural killer cells, and enhancement of antigen presentation. While evidence indicates that type I IFN signaling through the STAT-1 molecule directly blocks NV replication, it is currently unknown what additional role(s) this cytokine plays in clearance or memory immunity to this specific family of viruses.

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This review will focus on recent insights into the mechanisms by which type I IFN controls NV infections from the organismal to the molecular level. Historically, human NVs have been quite difficult to study because they remain non-cultivable [24,25] and until recent years there were no animal models of NV infection. Several exciting advances in the NV pathogenesis field – the discovery of murine NVs (MNVs) that share pathogenic properties with human NVs [14,26-32] and the discovery that a human NV called HS66 can infect gnotobiotic pigs and calves [33,34] – have facilitated recent progress regarding the role of type I IFN in controlling NV infection.

Type I interferon is induced in vivo upon norovirus infection

There is evidence from animal models that NV infection results in the induction of type I IFN (including IFN- α and IFN- β). First, significant levels of IFN- β are detectable in intestinal homogenates and serum of MNV-1-infected mice at 1 day post-infection (dpi) [15]. Second, IFN- α is detectable in intestinal contents and serum of gnotobiotic pigs inoculated with the human NV HS66 at 1-2 dpi [35]. Interestingly, IFN- α is also induced at later times post-infection (8 and 21 dpi in intestinal contents; 8 and 10 dpi in the serum) in these animals [35]. The basis of this late induction, subsequent to apparent viral clearance, is unknown but suggests low-level persistent infection. It is important to note that additional cytokines were significantly induced by HS66 infection of gnotobiotic pigs. In particular IL-6, IL-12, IFN- γ , IL-4, and IL-10 were detected in the serum at various times post-infection and IL-12 was detected in intestinal contents at 28 dpi [35]. Undoubtedly, a complex network of cytokine responses is induced upon NV infection that contribute to overall resolution of symptoms, clearance of virus, and induction of memory immune responses as highlighted by these observations in the gnotobiotic pig model. However, in this review we will focus solely on the role played by type I IFN because knockout studies in mice have demonstrated its essential role in preventing NV-induced lethality.

Interferon affords protection from severe pathology following norovirus infection

The MNV-1 system has clearly established the importance of type I IFN signaling and the STAT-1 molecule in regulating NV infections. While MNV-1 causes very modest intestinal pathology in immunocompetent hosts [15,26], it causes severe disease in mice lacking intact IFN signaling pathways. In fact, mouse strains deficient in the STAT-1 molecule (STAT1^{-/-}) or both type I and type II IFN receptors (IFN $\alpha\beta\gamma$ R^{-/-}) are sensitive to MNV-1-induced lethality [14]. At the clinical level, oral MNV-1 infection of STAT1^{-/-} mice causes severe gastroenteritis characterized by rapid weight loss, dramatic gastric bloating, semi-liquid fecal contents, and decreased weight of internal feces [15]. Of particular interest is the observation that MNV-1 infection causes gastric bloating – human NV infection also causes delayed gastric emptying, most likely associated with the high incidence of vomiting episodes in infected individuals [36]. This shared pathophysiological outcome of infection can now be studied in detail in the murine system, providing novel insight into NV pathogenesis that cannot easily be obtained in the human host. For example, our laboratory has recently revealed a correlation between increased MNV titers in stomach tissue and the severity of gastric bloating, suggesting for the first time that direct NV infection of the stomach may occur and may contribute to disease (submitted work, Karst laboratory). The severe gastroenteritis induced by MNV-1 infection of STAT1^{-/-} mice is associated with a marked increase in the number of apoptotic intestinal cells not observed in wild-type mice [15]. The amount of virus detected

within the intestines of STAT1^{-/-} mice, determined by measuring viral genomes or infectious virions, is also markedly higher than in wild-type mice [14,15]. Thus, IFN responses (i) control virus replication in the primary target tissue, the intestine; and (ii) prevent significant levels of infection-induced apoptosis within the intestine.

Pathology is not limited to the gastrointestinal tract in MNV-1-infected STAT1^{-/-} mice – severe pneumonia and destruction of both splenic and liver tissue are also apparent during acute infection [14,15]. Pycnotic nuclear debris indicative of apoptosis is present in the red and white pulp of the spleens of MNV-1-infected STAT1^{-/-}, but not wild-type, mice [15], demonstrating that IFN signaling also prevents apoptotic death of splenocytes. As observed in the intestine, there are significantly higher numbers of infectious virions in peripheral tissues including spleens, livers, lungs, and axillary lymph nodes of STAT1^{-/-} mice compared to wild-type mice [15]. Similarly, logs-higher numbers of viral genomes are present in lung, liver, spleen, and brain tissue as well as feces and blood of STAT1^{-/-} mice 1-7 dpi compared to their wild-type counterparts [14], indicating that IFN prevents rapid and global virus dissemination early after infection.

MDA5 is a norovirus sensor

Here, we will discuss what is known about the cellular pathways responsible for the induction of type I IFN following NV infection. Several studies have examined the role played by specific PRRs during NV infections. A definitive role for cytoplasmic MDA5 as a NV sensor in primary bone marrow-derived dendritic cells (BMDCs) has been demonstrated [37]: (i) MNV-1 replication is modestly increased in MDA5^{-/-} BMDCs compared to wild-type cells; and (ii) MNV-1-infected MDA5^{-/-} BMDCs secrete very little, if any, type I IFN, MCP-1, IL-6, or TNF- α in contrast to wild-type BMDCs. Additional studies by McCartney et al. reveal that NV replication is required for MDA5-stimulated type I IFN induction – while intact MNV-1 induces type I IFN secretion in wild-type cells, this is prevented by removal of the VPg cap analogue from the 5' end of MNV-1 genomes or by UV inactivation of the virus [37]. The most likely explanation for these findings is that MDA5 recognizes viral dsRNA produced during genome replication, as has been shown a number of other viruses [38]. While MDA5 clearly senses MNV-1 in BMDCs and induces cytokine expression upon recognition, it is surprising that increased viral replication in MDA5^{-/-} BMDCs is only modestly observed late in virus growth curves [37], suggesting that MDA5 primarily recognizes MNV-1 late in the viral replication cycle. Possible explanations include sequestration of viral replication intermediates from cytoplasmic MDA5, or antagonism of MDA5 recognition or signaling, early in infection. Understanding viral mechanisms of evading or antagonizing antiviral responses can be critical to the design of efficacious live virus vaccines so this should be an exciting area of future research in the NV field (discussed more below). MDA5 is also involved in the host antiviral response during in vivo MNV-1 infections – MDA5^{-/-} mice contain higher viral titers in the proximal intestine, MLNs, and the spleen at 3 dpi [37]. However, MDA5 is not necessary for the overall control of MNV-1 infection, indicated by the observation that MDA5^{-/-} mice clear infection with similar kinetics to wild-type mice [37]. Both in vivo and in vitro studies emphasize the importance of other as-yet-undefined PRRs in stimulating innate immune effectors upon NV infection: the increased virus loads, and viral replication in BMDCs, in the absence of MDA5 are not nearly as significant as the increased levels observed in STAT1^{-/-} or IFN $\alpha\beta\gamma$ R^{-/-} mice [15] or cells [10], respectively.

In contrast to studies of MDA5, other PRRs that have been examined to date do not appear to contribute to NV recognition. Preliminary

studies suggest that RIG-I is not a NV sensor – while the replication of a human NV replicon in Huh-7 cells is sensitive to type I IFN [13], it is not affected by the absence of RIG-I [39]. It should be pointed out that Huh-7 cells are not susceptible to de novo NV infection; no studies have yet analyzed the contribution of RIG-I to NV-stimulated IFN production in permissive cells. TLR3, a PRR that localizes to the cell surface or endosomal membranes and recognizes dsRNA, does not appear to play a major role in NV recognition –MNV-1 replicates similarly in BMDCs generated from wild-type and TLR3^{-/-} mice [37]. TLR3^{-/-} mice do have a slight increase in virus loads in MLNs, but not in intestines or spleens, compared to wild-type mice so it is possible that TLR3 plays a minor tissue-specific role in vivo. Other TLRs that would be logical candidates in playing a role in NV recognition are TLR7 and TLR8, both of which recognize ssRNA. However, no studies have yet examined whether TLR7 or TLR8 recognize NVs, or whether any TLR can recognize human NVs. Several lines of evidence indicate that PKR is not a major NV sensor. First, PKR^{-/-} mice do not display overt signs of disease following MNV-1 infection, in contrast to STAT1^{-/-} and IFNαβγR^{-/-} mice which succumb to lethal MNV-1 infection [14]. Second, MNV-1 replication is not significantly increased in PKR^{-/-} bone marrow-derived Mφs (BMMφs) compared to wild-type cells [10]. It remains possible that PKR plays a more subtle or a cell type-specific role as a sensor of NV infections. Again, no studies of PKR recognition of human NVs have been performed.

Type I interferon signaling inhibits the translation of norovirus proteins independent of PKR

To date, few studies have examined the role of individual ISGs in controlling NV infection with the exception of PKR (it should be noted that PKR is both a PRR and an ISG). Regarding its role as an ISG, PKR phosphorylates the eukaryotic translation initiation factor eIF2α which is essential to both viral and cellular translation, thereby blocking translation of viral proteins. Our laboratory has demonstrated that MNV-1 replication is blocked by type I IFN signaling at the level of viral translation [11]. Specifically, pretreatment of permissive cells with IFN-β results in an absence of MNV-1 protein synthesis even though viral genomes maintain their translatability. Similarly, IFN-α treatment of cells stably expressing a human NV replicon results in decreased viral protein and genome levels [13]. While the type I IFN-mediated block to MNV-1 replication clearly targets translation, it occurs independent of PKR [11]. Thus, other host gene products induced through type I IFN signaling must target NV translation. Schoggins et al. recently revealed that a common mechanistic theme of ISGs is translational inhibition [23], so it will be exciting to examine the role of specific effectors identified in this screen in blocking NV translation. Moreover, NV genomes appear to utilize a novel VPg-dependent (5' cap- and IRES-independent) mechanism to recruit host translation initiation factors to the 5' end of their genomes [40-42]; thus, developing antiviral therapeutics modeled on specific ISG targeting of this process represents an ideal strategy of blocking viral translation while sparing cellular translation.

Noroviruses may antagonize or evade type I interferon induction

Many, if not all, viruses encode one or more mechanisms to counteract the antiviral effects of type I IFN signaling, either through evading or antagonizing type I IFN induction [43,44], interfering with IFNAR-mediated signaling [45,46], or avoiding the inhibitory effects of ISGs [47,48]. The provided references describe examples of each type of viral antagonism strategy and are not intended to be

all-inclusive. While there is no direct evidence that NVs utilize any of these strategies, accumulating indirect evidence supports the idea that they target, or evade recognition by, the type I IFN induction pathway. First, two NV proteins are capable of inhibiting the host secretory pathway which could prevent or reduce the release of type I IFN, as well as other cytokines, from infected cells [49-51]. The amino-terminal non-structural protein, referred to as NS1 or p48, of Norwalk virus (a prototype human NV) localizes to vesicles in transfected cells, interacts with the host vesicle-associated membrane protein-associated protein VAP-A, and blocks cell surface expression of at least one protein [49]. It has also been shown to promote disassembly of the Golgi complex into discrete aggregates, which could clearly contribute to reduced host protein secretion [50]. Another Norwalk virus non-structural protein, referred to as NS4 or p22, has recently been reported to alter COPII-coated vesicle trafficking, resulting in disassembly of the Golgi and inhibition of protein secretion [51]. This activity is thought to require a novel ER export signal in the NS4 sequence. A definitive role for NS1 and/or NS4 in blocking cytokine secretion in infected cells must be confirmed through direct lines of experimentation.

Second, NV capsid expression following transfection of Huh-7 cells with human Norwalk virus genomes is modestly inhibited by pretreatment with supernatant from poly(I:C)-treated cells (a known stimulator of type I IFN), but not by pretreatment with supernatant from Norwalk virus genome-transfected cells [52]. This observation suggests that NV replication does not robustly induce host antiviral molecules such as type I IFN. In contrast, MNV-1 infection has been demonstrated to induce detectable type I IFN following in vivo [15] and in vitro [37] infections. This discrepancy could be explained by species-specific, cell type-specific, or virus strain-specific differences in the efficiency of IFN antagonism by NVs. With regard to species-specific differences, it is interesting to note that MNVs do not cause a redistribution of COPII-coated vesicles in infected cells [53], possibly because their NS4 sequences lack the ER export signal conserved in human NVs that is thought to drive this redistribution [51]. Thus, MNVs may be less efficient than human NVs at blocking host protein secretion. With regard to cell type-specific differences, human NVs are uncultivable so all in vitro studies are based on transfections of, or replicon maintenance in, nonpermissive cells whereas MNV studies monitor infection of permissive cell types. Supporting virus strain-specific differences in IFN antagonism, our laboratory has determined that MNVs display differences in the kinetics of IFN-β induction in permissive cells, with a correlation between virulence and delayed induction (submitted work, Karst laboratory). This finding leads one to speculate that the ability of NVs to antagonize IFN induction dictates their disease-causing potential.

Work by Chang et al. on a human NV replicon system suggests that such antagonism, if it exists, is not direct – the presence of Norwalk virus genomes and proteins in Huh-7 cells does not affect IFN-β gene expression in response to Sendai virus infection [13]. However, it should be noted that this system does not monitor natural NV infection of permissive cells so it may not reflect all aspects of natural NV/host cell interactions. Moreover, it is possible that NVs and Sendai virus are recognized by different PRRs in this cell type and that the NV antagonism strategy targets a signaling component not involved in Sendai virus induction of IFN-β. One possible explanation that would be compatible with results from Guix et al. (i.e. Norwalk virus does not induce significant type I IFN) [52] and Chang et al. (i.e. Norwalk replicon does not interfere with type I IFN induction) [13] is that NVs evade detection by PRRs, in contrast to directly antagonizing PRR

signaling. There is precedence for this with other virus types [43]. Another possibility is that NVs avoid early IFN responses by preventing the release of IFN protein from infected cells (the aforementioned study by Chang et al. monitored IFN- β induction at the level of gene expression so a block to protein secretion would not have been revealed). This possibility is supported by the ability of Norwalk virus NS1 and NS4 proteins to block the secretion of host proteins [49-51].

Noroviruses do not appear to antagonize type I interferon signaling

Contrary to the above-described data suggesting that NVs evade or subvert type I IFN induction, available data suggest that NVs do not block IFNAR-mediated signaling. First, pre-treatment or post-treatment of cells with type I IFN potently inhibits NV replication [11,13]. In fact, IFN- α treatment of human NV replicon-bearing Huh-7 cells reduces viral protein and genome levels to undetectable, suggesting complete virus clearance from these cells [13]. Activation of STAT-1 is apparent in these replicon-bearing cells upon IFN- α treatment [13]. Second, as described in detail above, mice with genetically deficient IFN signaling pathways are much more susceptible to MNV-1 disease than wild-type mice [14,15]. Moreover, cells deficient in IFNAR or STAT-1 support significantly higher levels of MNV-1 replication than wild-type cells [10]. Collectively, these results confirm that type I IFN signaling events significantly reduce NV replication. Based on the potency of type I IFN against NV replication, there is ongoing research testing whether type I IFN treatment is effective at treating NV infections [54].

Summary

Recent studies of human and murine NVs unequivocally demonstrate that type I IFN signaling events potently block NV replication. While the precise mechanism of this inhibition remains undefined, it has been shown to target NV translation in a PKR-independent manner. There is evidence that several PRRs contribute to NV recognition, including a major role played by MDA5 and a minor role played by TLR3. However, there is also accumulating indirect evidence that NVs may be able to evade early recognition by these innate immune sensors to delay type I IFN induction. Even so, type I IFN and specifically STAT1-mediated signaling are absolutely essential to control NV infections, at least in the murine system. Moreover, initial studies suggest that NVs do not encode robust mechanisms to interfere with STAT1-mediated signaling. Thus, current efforts to elucidate the mechanism(s) by which IFN targets NV replication, and to test whether exogenous IFN treatment will be effective to treat NV infections, are well-justified. Other important areas of future investigation include identifying the ISGs that target NVs; and determining whether type I IFN plays additional *in vivo* roles in controlling NV infections, such as activation of other immune cells and enhancement of antigen presentation.

References

- Green KY (2007) Caliciviridae: The Noroviruses: Fields Virology. (5th edn), vol. 1, Lippincott Williams & Wilkins, Philadelphia.
- Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinjé J, et al. (2008) Systematic Literature Review of Role of Noroviruses in Sporadic Gastroenteritis. *Emerg Infect Dis* 14: 1224-1231.
- Sakai Y, Nakata S, Honma S, Tatsumi M, Numata-Kinoshita K, et al. (2001) Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. *Pediatr Infect Dis J* 20: 849-853.
- Glass RI, Parashar UD, Estes MK (2009) Norovirus Gastroenteritis. *N Engl J Med* 361: 1776-1785.
- Norovirus activity—United States, 2006-2007 (2007) *MMWR. Morbidity and Mortality Weekly Report*, vol. 56, no. 33, pp. 842-846.
- Lopman BA, Adak GK, Reacher MH, Brown DW (2003) Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. *Emerg Infect Dis* 9: 71-77.
- Lopman BA, Reacher MH, Vipond IB, Sarangi J, Brown DW (2004) Clinical Manifestation of Norovirus Gastroenteritis in Health Care Settings. *Clin Infect Dis* 39: 318-324.
- Surveillance for foodborne disease outbreaks - United States (2006) *MMWR. Morbidity and Mortality Weekly Report*, vol. 58, no. 22, pp. 609-615.
- Estes MK, Prasad BV, Atmar RL (2006) Noroviruses everywhere: has something changed? *Curr Opin Infect Dis* 19: 467-474.
- Wobus CE, Karst SM, Thackray LB, Chang KO, Sosnovtsev SV, et al. (2004) Replication of Norovirus in Cell Culture Reveals a Tropism for Dendritic Cells and Macrophages. *PLoS Biol* 2: 432.
- Changotra H, Jia Y, Moore TN, Liu G, Kahan SM, et al. (2009) Type I and Type II Interferons Inhibit the Translation of Murine Norovirus Proteins. *J Virol* 83: 5683-5692.
- Chang KO, George DW (2007) Interferons and Ribavirin Effectively Inhibit Norwalk Virus Replication in Replicon-Bearing Cells. *J Virol* 81: 12111-12118.
- 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.
- Karst SM, Wobus CE, Lay M, Davidson J, Virgin HW 4th (2003) STAT1-Dependent Innate Immunity to a Norwalk-Like Virus. *Science* 299: 1575-1578.
- Mumphrey SM, Changotra H, Moore TN, Heimann-Nichols ER, Wobus CE, et al. (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.
- Durbin JE, Hackenmiller R, Simon MC, Levy DE (1996) Targeted Disruption of the Mouse Stat1 Gene Results in Compromised Innate Immunity to Viral Disease. *Cell* 84: 443-450.
- Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, et al. (1996) Targeted Disruption of the Stat1 Gene in Mice Reveals Unexpected Physiologic Specificity in the JAK-STAT Signaling Pathway. *Cell* 84: 431-442.
- Meurs E, Chong K, Galabru J, Thomas NS, Kerr IM, et al., (1990) Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. *Cell* 62: 379-390.
- Ghosh SK, Kusari J, Bandyopadhyay SK, Samanta H, Kumar R, et al. (1991) Cloning, sequencing, and expression of two murine 2'-5'-oligoadenylate synthetases. Structure-function relationships. *J Biol Chem* 266: 15293-15299.
- Zhou A, Hassel BA, Silverman RH (1993) Expression cloning of 2-5A-dependent RNAase: A uniquely regulated mediator of interferon action. *Cell* 72: 753-765.
- Haller O, Arnheiter H, Gresser I, Lindenmann J (1979) Genetically determined, interferon-dependent resistance to influenza virus in mice. *J Exp Med* 149: 601-612.
- Skaug B, Chen J (2010) Emerging Role of ISG15 in Antiviral Immunity. *Cell* 143: 87-190.
- Schoggins JW, Panis M, Murphy MY, Jones CT et al. (2011) A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 472: 481-485.
- Lay MK, Atmar RL, Guix S, Bharadwaj U, He H, et al. (2010) Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans. *Virology* 406: 1-11.
- Duizer E, Schwab KJ, Neill FH, Atmar RL, Koopmans MP, et al. Laboratory efforts to cultivate noroviruses. *J Gen Virol* 85: 79-87.
- Liu G, Kahan SM, Jia Y, Karst SM (2009) Primary High-Dose Murine Norovirus 1 Infection Fails To Protect from Secondary Challenge with Homologous. *Virus. J Virol* 83: 6963-6968.
- Hsu CC, Riley LK, Wills HM, Livingston RS (2006) Persistent Infection with and Serologic Crossreactivity of Three Novel Murine Noroviruses. *Comp Med* 56: 247-251.

28. Kim M, Lee H, Chang KO, Ko G (2009) Molecular Characterization of Murine Norovirus Isolates from South Korea. *Virus Res* 147: 1-6.
29. Kitagawa Y, Tohya Y, Ike F, Kajita A, Park SJ, et al. (2010) Indirect ELISA and indirect immunofluorescent antibody assay for detecting the antibody against murine norovirus S7 in mice. *Exp Anim* 59: 47-55.
30. Müller B, Klemm U, Mas Marques A, Schreier E (2007) Genetic diversity and recombination of murine noroviruses in immunocompromised mice. *Arch Virol* 152: 1709-1719.
31. Thackray LB, Wobus CE, Chachu KA, Liu B, Alegre ER, et al. (2007) Murine Noroviruses Comprising a Single Genogroup Exhibit Biological Diversity despite Limited Sequence Divergence. *J Virol* 81: 10460-10473.
32. Hsu CC, Wobus CE, Steffen EK, Riley LK, Livingston RS (2005) Development of a Microsphere-Based Serologic Multiplexed Fluorescent Immunoassay and a Reverse Transcriptase PCR Assay To Detect Murine Norovirus 1 Infection in Mice. *Clin Diagn Lab Immunol* 12: 1145-1151.
33. Souza M, Azevedo MS, Jung K, Cheatham S, Saif LJ (2008) Pathogenesis and Immune Responses in Gnotobiotic Calves after Infection with the Genogroup II.4-HS66 Strain of Human. *Norovirus J Virol* 82: 1777-1786.
34. Cheatham S, Souza M, Meulia T, Grimes S, Han MG, et al. (2006) Pathogenesis of a Genogroup II Human Norovirus in Gnotobiotic Pigs. *J Virol* 80: 10372-10381.
35. Souza M, Cheatham SM, Azevedo MS, Costantini V, Saif LJ (2007) Cytokine and Antibody Responses in Gnotobiotic Pigs after Infection with Human Norovirus Genogroup II.4 (HS66 Strain). *J Virol* 81: 9183-9192.
36. Meeroff JC, Schreiber DS, Trier JS, Blacklow NR (1980) Abnormal gastric motor function in viral gastroenteritis. *Ann Intern Med* 92: 370-373.
37. McCartney SA, Thackray LB, Gitlin L, Gilfillan S, Virgin HW, et al. (2008) MDA-5 Recognition of a Murine Norovirus. *PLoS Pathog* 4: 1000108.
38. Baum A, Garcia-Sastre A (2009) Induction of type I interferon by RNA viruses: cellular receptors and their substrates. *Amino Acids* 38: 1283-1299.
39. Guix S, Asanaka M, Katayama K, Crawford SE, Neill FH, et al. (2007) Norwalk Virus RNA Is Infectious in Mammalian Cells. *J Virol* 81: 12238-12248.
40. Daughenbaugh KF, Fraser CS, Hershey JW, Hardy ME (2003) The genome-linked protein VPg of the Norwalk virus binds eIF3, suggesting its role in translation initiation complex recruitment. *EMBO J* 22: 2852-2859.
41. Daughenbaugh K, Wobus CE, Hardy ME, (2006) VPg of murine norovirus binds translation initiation factors in infected cells. *Virology* 3: 33.
42. Chaudhry Y, Nayak A, Bordeleau ME, Tanaka J, Pelletier J, et al. (2006) Caliciviruses Differ in Their Functional Requirements for eIF4F Components. *J Biol Chem* 281: 25315-25325.
43. Bowie AG, Unterholzner L (2008) Viral evasion and subversion of pattern-recognition receptor signalling. *Nat Rev Immunol* 8: 911-922.
44. Wilkins C, Gale M Jr (2010) Recognition of viruses by cytoplasmic sensors. *Curr Opin Immunol* 22: 41-47.
45. Parisien JP, Lau JF, Rodriguez JJ, Ulane CM, Horvath CM (2002) Selective STAT Protein Degradation Induced by Paramyxoviruses Requires both STAT1 and STAT2 but Is Independent of Alpha/Beta Interferon Signal Transduction. *J Virol* 76: 4190-4198.
46. Young DF, Didcock L, Goodbourn S, Randall RE (2000) Paramyxoviridae Use Distinct Virus-Specific Mechanisms to Circumvent the Interferon Response. *Virology* 269: 383-390.
47. Evans DT, Serra-Moreno R, Singh RK, Guatelli JC (2010) BST-2/tetherin: a new component of the innate immune response to enveloped viruses. *Trends Microbiol* 18: 388-396.
48. George CX, Li Z, Okonski KM, Toth AM, Wang Y, et al. (2009) Tipping the balance: antagonism of PKR kinase and ADAR1 deaminase functions by virus gene products. *J Interferon & Cytokine Res* 29: 477-487.
49. Ettayebi K, Hardy ME (2003) Norwalk Virus Nonstructural Protein p48 Forms a Complex with the SNARE Regulator VAP-A and Prevents Cell Surface Expression of Vesicular Stomatitis. *Virus G Protein. J Virol* 77: 11790-11797.
50. Fernandez-Vega V, Sosnovtsev SV, Belliot G, King AD, Mitra T, et al. (2004) Norwalk Virus N-Terminal Nonstructural Protein Is Associated with Disassembly of the Golgi Complex in Transfected Cells. *J Virol* 78: 4827-4837.
51. Sharp TM, Guix S, Katayama K, Crawford SE, Estes MK (2010) Inhibition of cellular protein secretion by norwalk virus nonstructural protein p22 requires a mimic of an endoplasmic reticulum export signal. *PLoS One* 5: 13130.
52. Guix S, Estes MK (2009) Caliciviridae and Astroviridae: Cellular Signaling and Innate Immune Responses to RNA Virus Infections. Allan R. Brasier, Adolfo Garcia-Sastre, Stanley M. Lemon., ASM Press, Washington, DC.
53. Hyde JL, Sosnovtsev SV, Green KY, Wobus C, Virgin HW, et al. (2009) Mouse Norovirus Replication Is Associated with Virus-Induced Vesicle Clusters Originating from Membranes Derived from the Secretory Pathway. *J Virol* 83: 9709-9719.
54. Kim Y, Thapa M, Hua DH, Chang KO (2011) Biodegradable nanogels for oral delivery of interferon for norovirus infection. *Antiviral Res* 89: 165-173.