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# Interactions between noroviruses, the host, and the microbiota

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In recent years, appreciation has been growing for the role that the microbiota plays in interactions between the host and various pathogens, including norovirus. Proviral and antiviral effects of the microbiota have been observed for both human and murine noroviruses, and it has become clear that direct effects of microbes and their metabolites as well as indirect effects of commensals on the host are key in modulating pathogenesis. In particular, a common thread has emerged in the ability of members of the microbiota to regulate the host interferon response, thereby modulating norovirus infection. Here, we highlight key differences between human and murine noroviruses and their interactions with the microbiota, while also underscoring shared characteristics between noroviruses and other gastrointestinal viruses.

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## Introduction

Globally, approximately 20% of cases of acute gastroenteritis are caused by norovirus (NoV) [1], making it the most common cause of viral gastroenteritis [2] and of all gastroenteritis outbreaks [3,4]. The economic burden of the disease is also high, with an estimated 4 billion USD in direct costs and 60 billion USD in societal costs each year [5]. Despite the global health and economic impacts of the disease, until recently, research into human NoV (HNoV) had been hampered by the lack of a suitable culture system or the ability to genetically modify the virus. The related murine norovirus (MNoV) has provided an extremely useful model system for the study of *in vivo* NoV pathogenesis since its discovery in 2003 [6], and recent critical advancements in the cultivation of HNoV in both standard tissue culture and organoid models have made study of HNoV more tractable *in vitro* [7,8]. Over the past several years, the gut microbiota has been identified as a major regulator of NoV virulence, based on evidence from both molecular and epidemiological observations of HNoV and *in vitro* and *in vivo* experiments with MNoV. This review focuses on the tripartite interactions between the host, the microbiota, and NoV, highlighting key similarities and differences between the human and murine viruses.

## Human and murine norovirus biology

Noroviruses are positive-sense, single-stranded RNA viruses belonging, along with other enteric pathogens such as sapoviruses, to the family Caliciviridae. The *Norovirus* genus is further divided into seven genogroups (GI–GVII), each of which includes a variety of genotypes [9–11]. Host range differs between genogroups, with human infections primarily caused by GI and GII viruses, while MNoV strains are within genogroup GV. Additionally, the prevalence of individual genotypes varies widely, with genotype GII.4 being responsible for the majority of NoV infections in humans [12,13]. Symptomatic cases of HNoV infection typically present with self-limiting diarrhea and vomiting, as well as abdominal pain and fever, before resolving within 2–4 days [14–16]. In children under two years of age and the elderly, infections can be prolonged and more severe [14,17], with NoV contributing to an age-dependent risk for mortality in older individuals [18,19]. HNoV thus represents a substantial health concern due to the high number of infections each year as well as the increased risk to individuals of high and low ages. Despite the association of NoV infection with impressive symptomatology, globally, an average of 7% of the population is asymptomatically infected with HNoV [20].

While relatively little is known about the *in vivo* replication of HNoV, insight has recently been gained into its cell tropism. HNoV proteins can be detected in T cells, dendritic cells, and intestinal epithelial cells (IECs) throughout the length of the intestine in a histopathological study of HNoV-infected intestinal biopsies from immunocompromised patients [21]. This tropism for IECs is supported by recent advancements in culturing HNoV in intestinal organoids [8], wherein GI and GII HNoV strains specifically replicate within enterocytes and not within other specialized IECs such as goblet cells or enteroendocrine cells. GII HNoV can also be successfully cultivated *in vitro* in B cells [7]. Additional

studies in infected immunocompetent individuals will be valuable to fully characterize HNoV tropism *in vivo*.

MNoV remains a useful model for NoV infections due to its robust manipulability *in vitro* and the availability of an *in vivo* infection model. MNoV was originally identified in immunodeficient mice, wherein it was found to be lethal when passaged intracranially [6]. The original strain discovered, MNV-1, has been extensively studied and has proven to be a useful model for investigating various facets of NoV pathogenesis. MNV-1, which causes an acute, systemic infection in immunocompetent mice, has been useful for identifying immune factors involved in controlling MNoV infection [6]. Mice lacking type I IFN signaling, including *Ifnar1*$^{-/-}$ and *Stat1*$^{-/-}$ mice, exhibit increased extraintestinal spread of MNV-1 to the liver and lungs, while in immunocompetent mice, the virus only spreads to spleen and mesenteric lymph nodes [6,22]. Similarly, MNV-1 causes a persistent, systemic infection in mice lacking adaptive immunity, together highlighting the importance of innate and adaptive immunity in controlling MNoV infection [6,23]. Additional strains of MNoV, such as CR6, have been found to persistently, but mostly asymptomatically, infect the healthy murine gut [24]. These strains have been widely used to investigate NoV biology *in vivo* in a small animal model, and methods have been developed to culture them *in vitro* as well, allowing for reverse genetics studies [25,26]. Significant recent discoveries made with MNoV regarding host–NoV interactions have included the elucidation of the proteinaceous receptor for the virus, CD300lf [27], and characterization of the cell tropism for different MNoV strains. The persistent strain CR6 specifically infects tuft cells, a rare chemosensory IEC subset [28••], whereas MNV-1 infects a variety of cells including T cells, B cells, macrophages, and dendritic cells [29]. These differences in tropism likely contribute to the distinct courses of infection seen with these acute and chronic viruses. In recent years, the study of MNoV has also provided many intriguing insights into interactions between the host, NoV, and the gut microbiota (Table 1).

## Effects of NoV on the microbiota

Early in the field of NoV–microbiota interactions, it was proposed that infection, which stimulates diarrheal disease, could alter the host intestinal microbiota [40]. Viral diarrhea, including that caused by NoV, decreases the diversity of the gut microbiome as a whole. In particular, Bacteroidetes, *Bifidobacterium* spp., and *Lactobacillus* spp., typically considered 'healthy' gut microbes, are decreased in children with HNoV diarrhea as compared to healthy controls. Some HNoV-infected adults have been reported to present with a similar decrease of Bacteroidetes and loss of bacterial richness and diversity, but this is found in a minority of patients [41]. Other studies have failed to detect these changes in infected individuals [42]. It is thus possible that a variety of factors including age, antibiotic usage, host or viral genetics, or starting microbial composition may govern whether the host microbiota is susceptible to alteration by HNoV infection. Continued studies accompanied by rich metadata collection may help to clarify how and when HNoV regulates the bacterial microbiota.

Similarly, there is no clear consensus on alterations to the gut microbiota following MNoV infection. One study investigating the effect of malnutrition on MNoV pathogenesis observed that malnutrition and MNV-1 infection altered the gut microbiome in similar ways to what has been seen in humans, decreasing Bacteroidetes and increasing Firmicutes [43]. However, another study exploring how MNV-1 and CR6 infection altered the microbiota at various sites along the intestine in multiple mouse strains failed to detect alterations associated with MNoV infection [44]. These discordant results may be related to differences between mouse facilities, an important consideration for microbiota analyses [45]. Because MNoV does not cause substantial diarrhea or other hallmarks of gastroenteritis in immunocompetent mice [46], alterations to the gut microbiota may be linked to diarrheal severity rather than infection [42]. While effects of NoV infection on the gut microbiota remain to be fully clarified, the ability of microbes to modulate NoV infection is better-established.

**Table 1**

**Summary of key factors related to the role of host and microbial factors in modulating NoV infection**

| | Murine | Human |
|---|---|---|
| *In vitro* cell tropism | Dendritic cells, macrophages [25], B cells [7], T cells [29], IECs expressing CD300lf [30] | B cells [7], enterocytes [8] |
| *In vivo* cell tropism | Tuft cells [28••], B cells, T cells, macrophages, dendritic cells [29] | Intestinal epithelial cells, T cells, dendritic cells [21] |
| Host attachment factors | CD300lf [27], bile acids [31••], cell surface carbohydrates [32,33] | HBGAs [34], sialic acids [35], bile acids [8] |
| Microbial-associated attachment factors | Bile acids | HBGAs, bile acids |
| Proviral effects of microbiota | Increase in tuft cell numbers [28••], induction of IgA [36] | Production of HBGAs [37] |
| Antiviral effects of microbiota | Induction of interferon responses [38,39] | |

## Antiviral effects of the microbiota on NoV

here is currently limited evidence *in vivo* of a role for the gut microbiota in regulating HNoV infection, but one recent study provides an intriguing suggestion that specific bacteria may control HNoV susceptibility [47•]. Abundance of two bacterial taxa, Ruminococcaceae and *Faecalibacterium* spp., were negatively associated with anti-NoV antibody titers in a healthy adult volunteer population. Individuals with a high abundance of these taxa may thus be protected against NoV infection, as they lack a serological history of infection. *Faecalibacterium* spp. have been investigated previously as potential anti-inflammatory agents [48], but this has not been previously correlated with HNoV. Future work will be necessary to confirm the relevance of these taxa in infection.

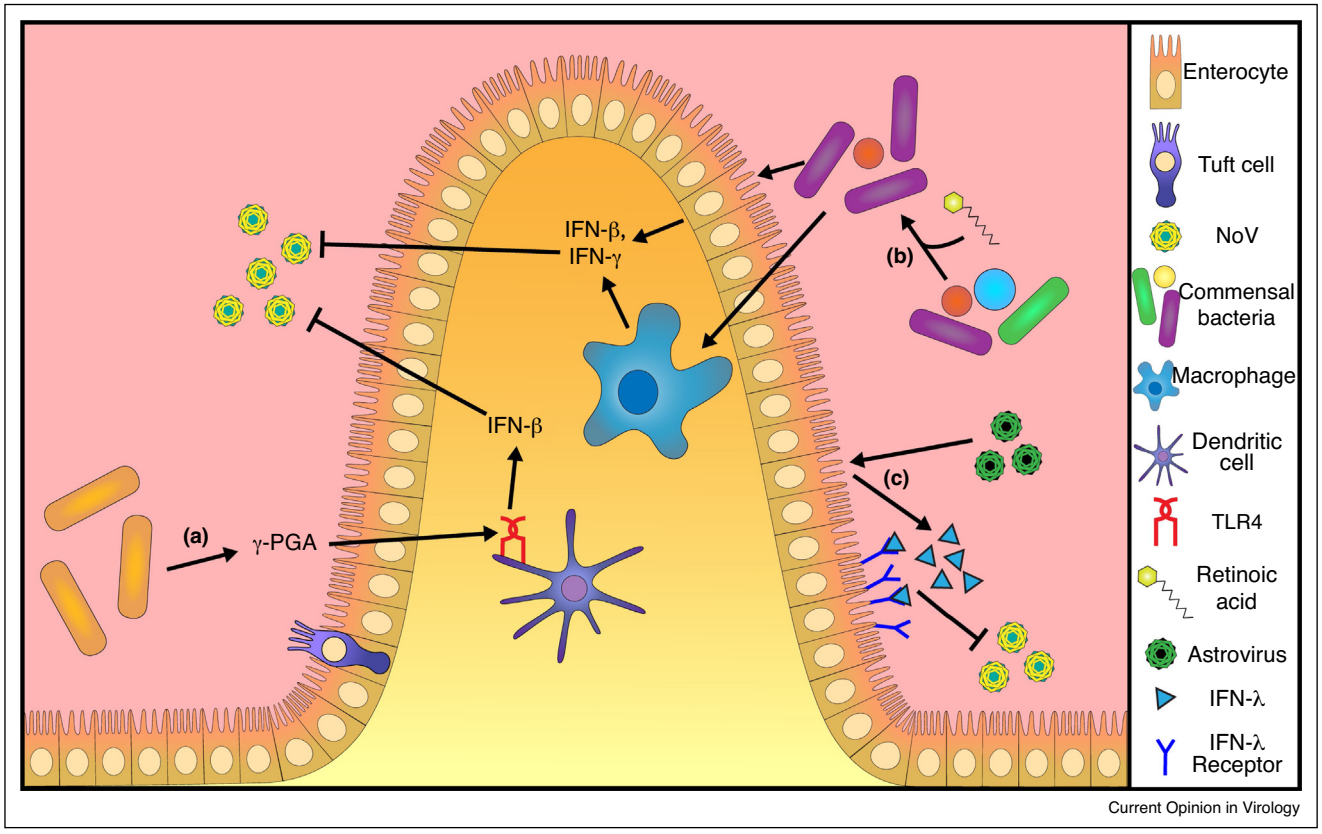
Specific microbial taxa or products which alter MNoV infection have also been identified. One study found that poly-γ-glutamic acid (γ-PGA) from *Bacillus* spp., when administered orally before MNV-1 infection, protected mice from infection [49•]. γ-PGA was also found to induce interferon-β (IFN-β) signaling, interfacing with host innate immunity to regulate MNoV infection. Another study found that *Lactobacillus* spp. induce cytokines within the gut to restrict MNoV infection [50]. Treatment of mice with retinoic acid, which can reduce the risk of HNoV infection [51], during MNV-1 infection alters the gut microbiota, resulting in an increase in *Lactobacillus* spp. and a concordant decrease in infection duration. *Lactobacillus* spp. induce IFN-β *in vitro*, which may explain how they restrict MNoV infection. Retinoic acid administration alone also restricted viral replication *in vitro*, so further work will be required to determine the relative contributions of microbiota-dependent and microbiota-independent responses to retinoic acid. It was also recently shown that the virome plays a role in restricting MNoV infection. Murine astroviruses have been found previously within the microbiota of immunodeficient mouse lines, such as *Rag1*$^{-/-}$ mice [52]. Colonization of immunodeficient mice with a murine astrovirus (STL5) induces intestinal interferon lambda (IFN-λ) [53•], a crucial restriction factor for MNoV [54,55]. This restores immunity against CR6 in otherwise susceptible mice, again highlighting the important role of interferons in NoV immunity. From these studies, it has become clear that bacterial and viral effects on IFN responses are a key component of the microbiota–host–MNoV axis. Further work will be required to investigate other mechanisms by which commensal organisms may directly influence MNoV pathogenesis or manipulate additional host pathways (Figure 1).

## Proviral effects of the microbiota on NoV

Several studies have demonstrated that commensal bacteria can increase HNoV infection *in vitro*. One microbial product associated with HNoV infection is the histo-blood group antigen (HBGA). Host HBGA status is linked to risk for HNoV infection [56], as host *FUT2* genotype is a major factor in NoV susceptibility. *FUT2* encodes a fucosyltransferase which is involved in the synthesis of HBGAs, complex terminal carbohydrates present on red blood cells and mucosal epithelial cells that can be secreted into saliva and other bodily fluids [57]. A common variant in ~20% of Europeans encodes a nonsense mutation in *FUT2*, resulting in a nonfunctional fucosyltransferase and a non-secretor phenotype when the host is homozygous for this allele [58]. Non-secretors have been shown in both epidemiological data and experimental human infections to be entirely resistant to the most common HNoV genotype, GII.4 [58,59]. Interestingly, the protective effects of non-secretor status are specific to viral genotype, as other strains of GI and GII HNoV infect non-secretors and secretors more equally, which may be explained by different HBGA binding patterns between HNoV strains [34,60]. HNoV capsids bind directly to HBGAs [34], including those produced by bacteria [37]. In the initial description of the *in vitro* B cell culture system for HNoV, it was found that commensal bacteria that produce HBGAs act as important cofactors for replication of a GII.4 strain of HNoV [7]. Administration of synthetic HBGA alone was sufficient to increase HNoV attachment to and infection of a human B cell line. The binding of HNoV particles to bacterial HBGAs has also been shown to improve viral survival following heat stress, indicating that HBGA binding may also aid in environmental survival and transmission of the virus, not just infection [61].

While HBGAs likely represent a clinically important component of microbiota interactions with HNoV, other bacterial products also alter HNoV infection. HNoV strains have been found to bind to a diverse repertoire of bacteria, including many that do not produce HBGAs [62], with a single bacterium having the capacity to bind multiple virions [37]. This is reminiscent of recent advancements in the understanding of interactions between poliovirus and the microbiota. Poliovirus binds to bacterial lipopolysaccharide (LPS), increasing its environmental stability and attachment to host cells [63], and additional unknown bacterial surface components, enhancing infectivity *in vivo* [64]. This binding groups multiple virions together, promoting co-infection and encouraging recombination between viral strains. NoV pathogenesis is similarly enhanced by grouping of virions in a single packet, as viruses including poliovirus and both HNoV and MNoV have recently been shown to be released *in vivo* within vesicle-cloaked virus clusters (VCVCs) containing several virions [65,66]. Like bacterially mediated viral clustering, these VCVCs enhance infectivity of the virus relative to free virions. Clustering virions together may be a widely used mechanism by which viruses enhance infection, including instances wherein multiple virions bind to a single bacterium,

**Figure 1**

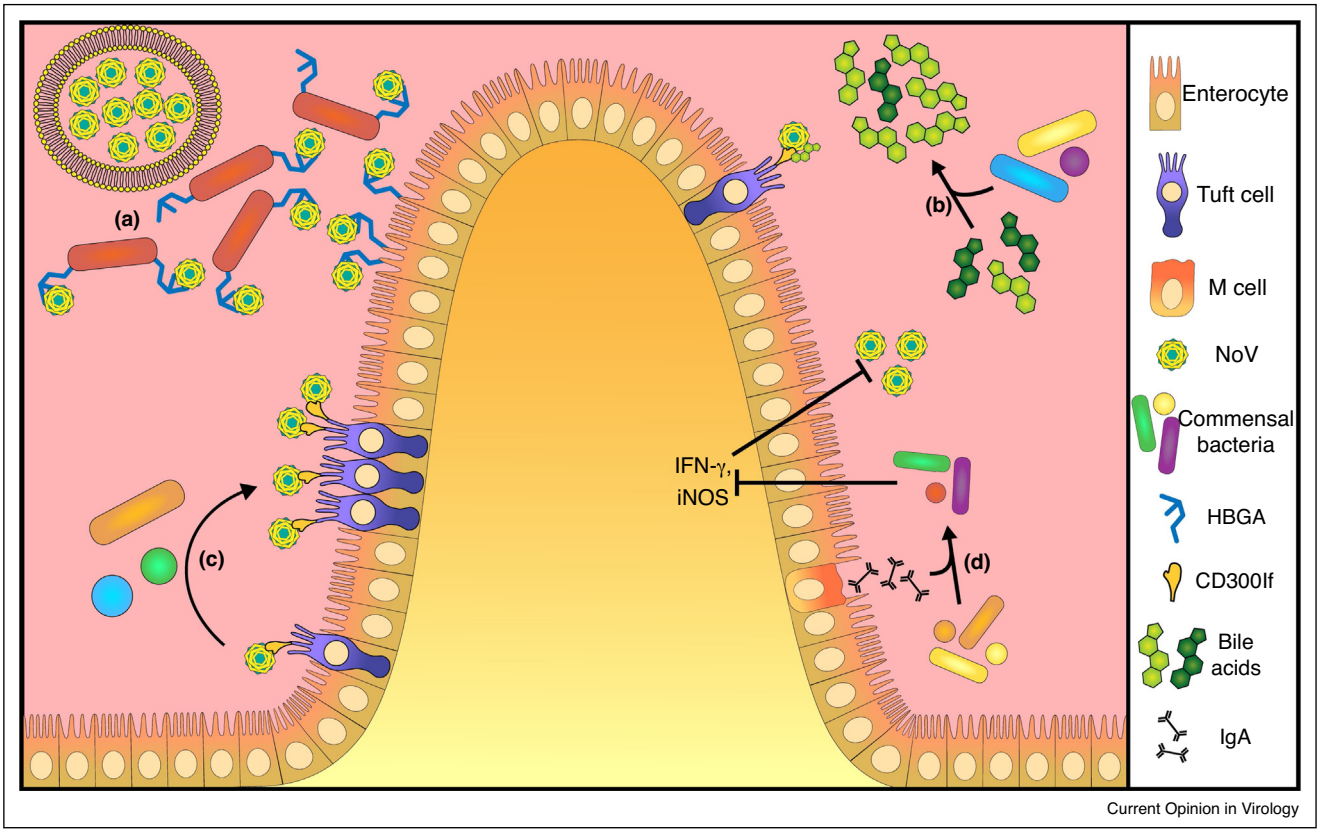



Antiviral effects of the microbiota on NoV infection. **(a)** The *Bacillus* product γ-PGA acts as a non-canonical TLR4 ligand to induce IFN-β, inhibiting MNoV infection. **(b)** Retinoic acid administration increases *Lactobacillus* spp. prevalence, inducing IFN-β and IFN-γ through unknown pathways to block MNoV infection. **(c)** A commensal murine astrovirus found in immunodeficient mice induces IFN-λ, which acts through the epithelial IFN-λ receptor to restrict MNoV infection.

though it remains to be seen whether bacterial-mediated NoV clustering increases infectivity. Bile may be an additional microbe-associated component of the intestinal milieu that modulates HNoV infection. HNoV capsids bind directly to bile acids [67••], and addition of human bile, specifically the non-proteinaceous component, to organoids greatly increases HNoV replication in a dose-dependent manner [8]. In this case, the bile was found to act not on the virus itself, but rather on the cells through an unknown mechanism. Another calicivirus, porcine enteric calicivirus, requires bile acids for replication as they facilitate escape from the lysosome following viral entry [68] and dampen STAT1 activation in response to IFN [69], and thus it is possible that bile acids affect HNoV in similar ways. Bile acids also interact with host receptors, such as TGR5 and FXR, to regulate intestinal inflammation and integrity [70,71], so similar pathways may be involved in controlling HNoV infection. Bile acids are heavily modified by the gut microbial community [72], and the gut microbiota also modulates the total amount of bile acids within the gut [73]. It is thus possible that the components of bile that act as a cofactor for HNoV infection require microbial metabolism for their activity. Intriguingly, it was recently shown that binding of bile acids by some HNoV strains facilitates their binding to HBGAs [67••], adding a further layer of complexity to the web of interactions between host, viral, and microbial components of the intestinal compartment in the context of NoV infection.

Unlike HNoV, MNoV infection is not affected by *FUT2* status *in vivo* or by the addition of HBGAs *in vitro* [27,31••]. Bile acids play an important role in engagement of the viral capsid with its proteinaceous receptor *in vitro* [31••], though an *in vivo* role for bile acids in MNoV infection has not yet been shown. It has, however, been well demonstrated that MNoV has a strong reliance on the gut microbiota for infection. Antibiotic pretreatment of mice prevents infection by the acute strain MNV-1 and persistent strains MNV-3 and CR6 [7,38]. For CR6 infection, transfer of the fecal microbiota from untreated to antibiotic-treated animals restores infectivity, supporting the dependence of this phenotype on the gut microbiota. This reliance on the gut microbiota for CR6 infection is

**Figure 2**




Proviral effects of the microbiota on NoV infection. **(a)** HBGAs present on bacterial and host cell surfaces bind to NoV capsids, increasing NoV infection, possibly by grouping together multiple NoV virions to promote coinfection as is seen with NoV in vesicle-cloaked virus clusters. HBGA binding also increases environmental stability of NoV virions. **(b)** Bile acids, which are chemically modified by the gut microbiota, bind to the MNoV capsid and enhance interactions with the MNoV receptor, CD300lf, to increase infection. **(c)** Acting through unknown pathways, the microbiota increases tuft cell numbers within the gut to provide more NoV target cells. Additionally, bacterial products like LPS may act to increase surface expression of CD300lf on tuft cells. **(d)** IgA maintains a normal microbiota, depressing levels of IFN-γ and iNOS and resulting in a permissive environment for NoV infection.

regulated by signaling through the IFN-λ pathway [38], and another immune protein, HOIL1, which is essential for IFN-λ induction [39]. Another intriguing interaction between MNoV, the microbiota, and immune factors was recently described wherein MNV-1 infection was counterintuitively decreased in *Pigr*$^{-/-}$ (polymeric immunoglobulin receptor) mice which lack intestinal IgA [36]. In germ-free mice, there was no difference in susceptibility to MNoV between the two genotypes, indicating a role for the gut microbiota in this interaction. It was demonstrated that the microbiota was changed in *Pigr*$^{-/-}$ mice relative to wild-type mice and this altered microbiota induced higher levels of IFN-γ and iNOS, controlling MNoV infection. The normal gut microbiota induces higher levels of IgA, resulting in a permissive environment for MNoV infection [36].

The reliance of persistent MNoV infection on the microbiota is also related to its cell tropism. Antibiotic treatment of mice reduces the number of tuft cells within the colon, thereby reducing the number of cells available for persistent MNoV infection [28••]. Artificially increasing tuft cell numbers in antibiotic-treated mice with recombinant IL-4 or IL-25 restores infection. A direct mechanism by which the microbiota increases tuft cell numbers has not yet been shown in the context of MNoV infection, but it has been recently found that intestinal helminths increase small intestinal tuft cell numbers via the succinate receptor sensing worm-derived succinate [74–76]. In one study, it was further shown that streptomycin treatment alone resulted in an altered gut microbiota, leading to an increase in succinate and increased tuft cell numbers [75]. This may indicate that there is a role for microbial products, such as succinate, in mediating MNoV infection by increasing target cell numbers, though this has not yet been confirmed experimentally. This proviral effect of the microbiota may also relate to the availability of the MNoV receptor, CD300lf, which is

expressed on tuft cells but not other IEC populations [77]. This receptor is induced in various hematopoietic cells by LPS stimulation [78]. While this has not been demonstrated in tuft cells, it suggests that antibiotic treatment may also limit CD300lf expression due to the loss of bacterial stimuli. Studies examining interactions between the microbiota and MNoV infection have thus elucidated the importance of trans-kingdom interactions in viral infection, and also shed light on pathways used by the host to combat these infections (Figure 2).

## Future directions

While significant advancements have been made regarding NoV–microbiota interactions, the current literature also highlights several areas where additional work is needed. The impact of both HNoV and MNoV infection on the gut microbiota remains unclear. Previous studies, particularly in humans, have been hampered by a lack of metadata and longitudinal samples. The addition of these key considerations in human cohort studies would allow for more reliable identification of how HNoV infection and additional factors, such as antibiotic treatment, contribute to alterations of the gut microbiota after infection. Controlled experimental HNoV infection studies that include monitoring of the gut microbiota would be ideal for this purpose. Such studies would also elucidate whether specific bacterial taxa modify susceptibility to HNoV infection.

Although there have been trials in recent years that show promise [79], there is currently no HNoV vaccine available. Even for those vaccines which have been tested against experimental viral challenge, there appear to be limits to protection [80]. As efforts to develop an effective vaccine continue, lessons can potentially be learned from vaccines against other enteric viruses, such as rotavirus [81] or poliovirus [82]. Vaccine efficacy is inconsistent between different countries, with multiple studies identifying links between responder status and microbiome composition [82–84]. Considering these data and the known roles of the microbiota in both permitting and preventing NoV infection, it will likely be important to evaluate the role of the microbiota in future NoV vaccine studies.

Finally, while studies have begun to identify mechanisms by which specific microbial products alter NoV infection *in vitro*, the ability of these products to modify infection in the context of a microbial community *in vivo* has not been fully assessed. Modification of the taxonomic composition or specific bacterial pathways of the murine gut microbiota will allow for better elucidation of the roles these bacteria and their products play *in vivo*. The identification of microbes and microbial components that can consistently control NoV infection may have important therapeutic applications, and further study of these factors will continue to yield insights into NoV pathogenesis.

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