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Current Opinion in Virology

# Virome–host interactions in intestinal health and disease

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The enteric virome consists largely of bacteriophages and prophages related to commensal bacteria. Bacteriophages indirectly affect the host immune system by targeting their associated bacteria; however, studies suggest that bacteriophages also have distinct pathways that enable them to interact directly with the host. Eukaryotic viruses are less abundant than bacteriophages but are more efficient in the stimulation of host immune responses. Acute, permanent, and latent viral infections are detected by different types of pattern recognition receptors and induce host immune responses, including the antiviral type I interferon response. Understanding the complex interplay between commensal microorganisms and the host immune system is a prerequisite to elucidating their role in intestinal diseases.

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**Current Opinion in Virology** 2019, **37**:63–71

This review comes from a themed issue on **Viruses and the microbiome**

Edited by **Stephanie M Karst** and **Christiane E Wobus**

For a complete overview see the Issue and the Editorial

Available online 8th July 2019

**https://doi.org/10.1016/j.coviro.2019.06.003**

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## Introduction

Microorganisms can colonize any surface of the human body. Thus, a variety of archaea, eukaryotes, bacteria, viruses, and their genes can be found there [1–3]. This complex multi-kingdom ecosystem is believed to have evolved based on the mutual benefit to its inhabitants [4]. Extensive study over the last decade has established that resident bacteria shape immune development, homeostasis, and activation [5,6]. Bacteria share conserved gene sequences (e.g. 16s rRNA coding gene), which can be utilized to analyze microbial composition [7]. In contrast, viruses can have both DNA and RNA as the genetic material and lack a universal sequence platform, which makes it harder to analyze the composition of the virome [8]. However, with advances in sequencing technology and accumulating information in sequence databases, recent studies have highlighted the role of resident viruses and their genomes (the virome) in health and disease [9–11]. As the largest habitat of microbiota in the body, the intestines can be considered as the organ that is most affected by the virome; the number of viruses in feces is estimated to be up to $10^9$ per gram [12]. In this review, the direct and indirect associations of the virome with mammalian hosts and their bacterial populations are discussed with a view of their role in the pathogenesis of enteric diseases.

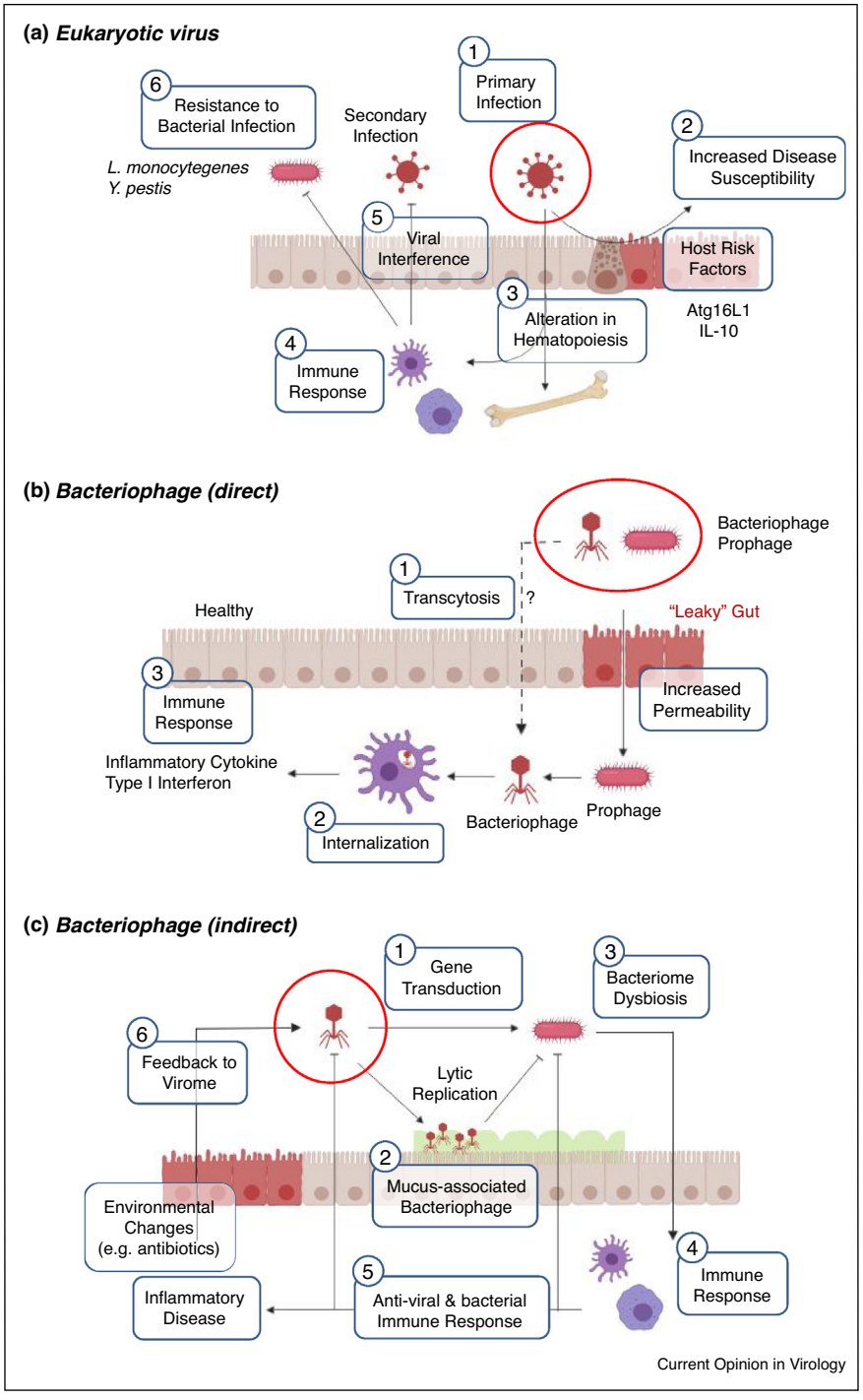
## Classification of virome–host interaction

Unlike bacteria, which are organisms independent of their host, viruses are dependent agents and need to infect cells to maintain their life cycle. Viruses in mammals, which have tropism to eukaryotic cells (eukaryotic viruses), can affect host immune responses directly, whereas viruses that infect bacteria (bacteriophages) affect the host indirectly through interaction with bacteria (Figure 1). Additionally, bacteriophages can still interact with the mammalian host, as these viruses can stimulate host cells directly (Figure 1). As a consequence of co-evolution, eukaryotic cells and bacteria developed a defense system against the virome. Anti-viral defense mechanism to counteract infection includes CRISPR-Cas9, restriction-modification, and chemicals that target double-stranded DNA (dsDNA) of bacteriophages [13–15]. Furthermore, poly-γ-glutamic acid produced by pathogenic bacteria has shown antiviral activity against norovirus infection [16]. In contrast, some studies indicate that gut bacteria can help eukaryotic enteric viruses infect host cells by providing polysaccharides [17,18]. Lipopolysaccharide (LPS) bound to surface of mouse mammary tumor virus facilitated viral transmission by Toll-like receptor (TLR)4-dependent interleukin (IL)-10 production [17]. LPS-coated poliovirus also exhibited increased infectivity and enhanced adherence to the target cells [18]. In other studies, it was shown that gut bacteria, such as *Enterobacter cloacae*, provide H type histo-blood group antigens which enable norovirus to infect B cells [19]. Gut bacteria also support persistent infection of norovirus by suppressing innate immune response through interferon (IFN)-λ signaling [20]. Antibiotic treatments in norovirus-infected mice showed alleviated viral burden in the intestine [19,20]. Thus, it is evident that resident viruses and bacteria can influence each other.

## Indirect interaction of bacteriophages with the immune system

In the same way that the bacterial community is established during birth [21], the virome is also acquired in early life and rapidly changes its composition in the first few weeks [22].

**Figure 1**




Subclassification of virome–host interactions. **(a)** Eukaryotic virus–host interactions. Primary infection with eukaryotic virus increases disease susceptibility in association with an alteration of disease risk genes (① and ②). Eukaryotic virus infection may induce alteration in hematopoiesis or immune activation (③ and ④), which interferes with secondary infection by other viruses or bacterial colonization (⑤ and ⑥). **(b)** Direct bacteriophage–host interaction. Bacteriophages can easily cross the intestinal epithelial layer in damaged 'leaky' gut (①). Bacteriophages cross the epithelium or are produced from prophages internalized by immune cells (②). Recognition of bacteriophages can induce production of inflammatory cytokines and type I interferon (③). **(c)** Indirect bacteriophage–host interaction. Bacteriophages can affect the bacteriome by transducing genes or by killing related bacteria (①–fx3). Dysbiosis in bacteria stimulates immune responses and affects the virome and bacteriome (④ and ⑤). Both changes in immune responses and commensal microorganisms contribute to inflammatory diseases. Environmental shift gives feedback to the virome to further affect host immune responses (⑥).

The adult virome is relatively stable and consists of a few dominant bacteriophages [23•]. Studies of twins have revealed that the virome is diverse among individuals, but that twins have an increased chance of harboring similar virome when they have higher overall microbiome concordance [23•,24]. These studies provide evidence that resident bacteria and bacteriophages are interconnected. When germ-free (GF) mice were infected with T7 bacteriophages, viruses were undetectable in feces at 24 hours post-infection [25]. However, bacteriophages can replicate up to $\sim 10^{10}$ pfu/g in feces and stably colonize mice upon oral introduction of *Escherichia coli* [25]. A more complex example of bacteriophage–bacteria interactions was found in gnotobiotic mice harboring a defined human bacteria consortium that exhibit linked dynamics between the virus and bacteria community [26]. Here, introduction of human originated virus-like particles resulted in a transient reduction of related bacteria. The study suggests that bacteriophages can affect the bacterial community in a target-specific manner. Lytic replication is undoubtedly the strongest way to influence the bacteria composition in the gut as it allows bacteriophages to kill the related bacteria cohort. Indeed, dense bacteriophage communities are found in the intestinal mucus layer [27,28], and a metazoan model showed that a mucus-associated bacteriophage binds to glycans in the mucus layer by utilizing its Ig-like protein, thus increasing its likelihood of killing the bacteria and prohibiting the bacteriophage from affecting the mammalian host [27].

Bacteriophages also play an important role as a reservoir of bacterial genes. They not only simply encode bacterial genes, but also transfer acquired genes to other bacteria. Gene transfer is not limited to closely related bacteria, as *Staphylococcus* phages can transfer their virulence genes to *Listeria monocytogenes* [29•]. Ecological changes induce a metagenomic shift in bacteriophages and this may contribute to bacteria adapting in new environments by acquiring beneficial genes from bacteriophages. For example, antibiotic treatment induces an expansion of bacteriophage reservoirs that encode antibiotic-resistant genes [30]. Thus, bacteriophages can also indirectly affect host responses by modifying the host–bacteriome interaction in response to ecological changes.

Considering that intestinal bacterial dysbiosis correlates with many diseases, especially gastrointestinal disorders [6,31], studies on bacteriophages, which target pathogenic bacteria, are crucial to understand the indirect contribution of bacteriophages in the pathogenesis of such diseases. So far, it has been reported that dsDNA bacteriophages, mainly of the order *Caudovirales*, comprise 76.9% of the bacteriophage and prophage population [23•]. While the dissimilarity of the virome among inflammatory bowel disease (IBD) patients was higher than seen in healthy individuals, the mucosal abundance of *Caudovirales* was increased in IBD patients [32•,33]. Increased *Caudovirales* with an alteration in functional viral gene expression were observed in ulcerative colitis (UC) patients, suggesting a potential contribution of *Caudovirales* to UC [33]. Given that a family of *Enterobacteriaceae* is associated with inflammation in the intestine [34,35], increased *Escherichia* phages and enterobacteria phages (both belonging to the order *Caudovirales*) in UC patients may reflect an indirect contribution of these viruses to pathogenesis. Recent studies also reported that a virome of a donor was transferred to a recipient during fecal microbiota transplantation (FMT) [36,37]. Of note, successful FMT was accompanied by more donor-derived *Caudovirales* in the recipient [37,38]. In a mouse model, the feeding with a bacteriophage cocktail induced bacterial dysbiosis, which was accompanied by increased intestinal permeability [39]. These mice had increased serum levels of LPS and inflammatory cytokines. This study therefore suggests that bacteriophage-mediated bacterial dysbiosis can induce pathology in the intestine.

## Direct interaction of bacteriophage to immunity

Although bacteriophages infect bacteria, not the mammalian host, there is some evidence that bacteriophages directly communicate with the host's immune cells. To achieve this, bacteriophages need to cross epithelial cell layers, which physically segregate the mucus layer from the lamina propria where most immune components reside. An *in vitro* transwell system was used to test transcytosis of T4 bacteriophages through a single layer of epithelial cell lines derived from various organs; here, bacteriophages translocated more efficiently in the apical to basal direction [40•]. Orally administered *E. coli* bacteriophages also disseminated to distal organs, including the kidney, liver, and spleen, suggesting that bacteriophages can cross the barrier in the intestine *in vivo* [41]. The fact that bacteriophages can cross the epithelial barrier supports the idea that they can make direct contact with the immune system.

Prophages may reach beyond the intestinal epithelium and produce bacteriophages to induce immune system efficiency. This occurs both in healthy guts and in leaky gut conditions, which are linked with dissemination of gut bacteria [42–44]. The effect of prophages in intestinal tissue remains obscure, but it is plausible to hypothesize that transmigration of bacteria carrying bacteriophage genomes over the epithelial layer can enable exposure of bacteriophages to the host system.

Recently, it was shown that bacteriophages produced by pathogenic bacteria are taken up by innate immune cells to induce type I IFN (IFN-I) responses, which inadvertently facilitate infection of related bacterial pathogen [45••]. Here, dendritic cells (DCs), B cells, and monocytes were able to internalize bacteriophages [45••]. In mice DCs stimulated with T4 bacteriophages in combination with tumor antigen exhibited an enhanced therapeutic effect compared with control DCs without viral

stimulation [46]. Also, RNA transcripts transcribed by T7 RNA polymerase can induce inflammatory cytokine secretion in human monocyte-derived DCs [47]. However, cytokine production by DCs in response to RNA transcripts was hampered by epigenetic modifications, which are found in transcripts of the mammalian host or eukaryotic viruses [47,48]. Of note, human and mouse RNA components failed to induce cytokine production [49]. Although much remains unknown regarding the direct interaction between bacteriophages and mammalian hosts, evidence suggests that bacteriophages may be internalized by immune cells and induce immune responses, probably via their genes and transcripts.

## Interactions between the eukaryotic virome and the host

Eukaryotic viruses can be considered as pathogens rather than symbionts from the view of the mammalian host because they infect host cells. Although eukaryotic viruses are relatively few when compared with bacteriophages [23•,50], they may have a greater potential to influence the host's immune response as they have evolved to share the host's internal mechanisms. Continuous acute, persistent, and latent virus infections make eukaryotic viruses integrated members of the commensal microorganisms.

Norovirus is widespread and is found in both healthy and asymptomatic infected humans [51,52]. Mouse norovirus (MNV) is also found in mice housed in conventional and specific pathogen-free animal facilities [53,54]. MNV infects immune cells in gut-associated lymphoid tissues and epithelial cells including tuft cells [55,56], whereas MNV-infected immunocompetent mice are typically asymptomatic [57]. A series of studies revealed that MNV can induce pathology in mice that are deficient in the risk gene for Crohn's disease (CD) [58,59••]. Hypomorphic Atg16L1 expression ($Atg16L1^{HM}$) or deletion of Atg5 in the intestinal epithelium was associated with altered autophagy responses and abnormal lysozyme expression patterns in Paneth cells [60]. Atg16L1 has been reported as a risk gene for CD [61,62], and CD patients carrying homozygous risk alleles (T300A) showed similar abnormality of Paneth cells with $Atg16L1^{HM}$ mice [60]. $Atg16L1^{HM}$ mice free of MNV did not develop pathology, suggesting that persistent eukaryotic virus infections can alter the disease susceptibility and predispose subjects to the onset of inflammatory bowel disease (IBD) [59••].

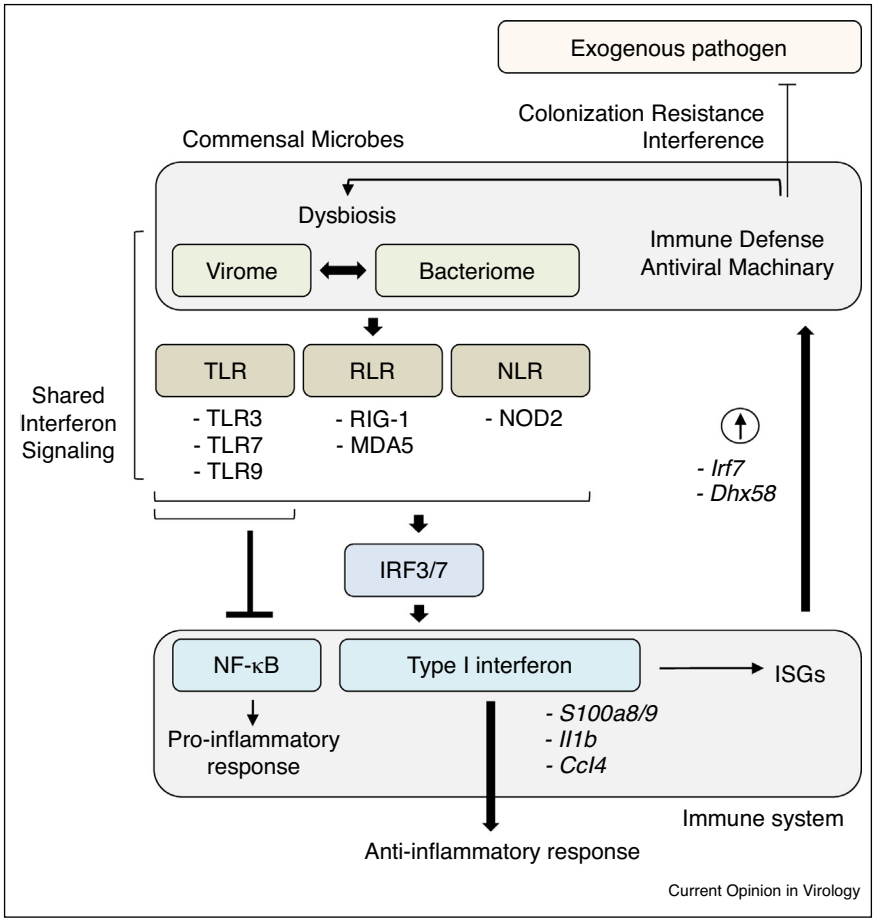
Viral interference is a phenomenon wherein primary viral infection can inhibit secondary infection of related or unrelated viruses [63,64]. Despite being the biggest reservoir of viruses, viral interference in the intestines has not yet been well studied. Recently, virome-induced interference against enteric virus infection was demonstrated *in vivo* [65]. In this study, $Rag2^{-/-}Il2rg^{-/-}$ mice infected with astrovirus STL5 strain were resistant to persistent MNV infection. Precedent STL5 infection markedly induced IFN-λ expression which is crucial to anti-MNV defense [20]. An *in vitro* study using the Caco-2 cell line showed reduced susceptibility to a live rotavirus vaccine strain when cells were simultaneously exposed to multiple enteric viruses. Here, multiple infection induced elevated cytokine production and increased TLR expression after infection [66]. Another possible mechanism of viral interference may include hematopoiesis, which influences the number and function of innate immune cell populations. Vaccinia virus infection induces the expansion of $c\text{-}Kit^{hi}Sca\text{-}1^{+}Lin\text{-}1^{-}$ hematopoietic stem cells via Myd88 signaling and accelerates myeloid cell differentiation, which in turn increases plasmacytoid dendritic cells (pDCs) which are important in antiviral responses [67].

Eukaryotic virus infection-induced IFN-γ can also lead to antibacterial immune responses. Latent infection by murine gammaherpesvirus 68 provided mice resistance against *L. monocytogenes* and *Yersinia pestis*, but not West Nile virus [68•]. This result indicates that latent viral infections can provide interference against bacterial pathogens, further suggesting possible influence on the resident bacterial community. The observed resistance against *L. monocytogenes* was dependent on IFN-γ and associated with activated macrophage phenotypes [68•]. Importantly, latent infection associated with prolonged interference as viral latency provides sustained stimulation [63,68•]. In addition to the 'colonization resistance' achieved by resident bacteria [6], viral interference may provide a second line of defense to support the host immune system against various types of pathogens. However, it is important to note that viral interference is not universal to every pathogen and can even aggravate secondary infections.

## Recognition of the virome and its role in intestinal diseases

Recognition of resident viruses is the initial step of virome–host interaction. Many extracellular and cytosolic nucleic acid sensors, including TLRs [69–75], RIG-I-like receptors (RLRs) [76,77], and NOD-like receptors (NLRs) [78–80], can detect viral genomic materials (Figure 2). Pattern recognition receptors (PRRs) have been shown to recognize viral components rather than nucleotides. For example, TLR2, TLR4, and TLR10 recognize herpes simplex virus, F protein of respiratory syncytial virus, and the ribonucleoprotein complex of influenza virus, respectively [81–83]. Recognition of eukaryotic viruses by these PRRs triggers innate immune responses to counteract infection [77–80,84]. In contrast to eukaryotic viruses, the molecular mechanisms for immune activation mediated by bacteriophages are poorly understood. However, the RNA transcripts of bacteriophages can be detected by TLR3, TLR7, and TLR8 [47]. The fact that eukaryotic viruses and bacteriophages share PRRs suggests

**Figure 2**




Role of the virome in protective immune responses. Commensal microorganisms, including bacteria and virus, and their genetic material are recognized by various types of pattern recognition receptors, including Toll-like receptor (TLR), RIG-I-like receptor (RLR), and NOD-like receptor (NLR). This recognition stimulates type I interferon (IFN-I) responses mainly through interferon regulatory factor (IRF)3 and IRF7 and contributes to an anti-inflammatory response in the intestine. IFN-I–mediated reduction in inflammatory genes (e.g. *S100a8*, *S1009*, *Il1b*, and *Ccl4*) contributes to overall anti-inflammatory response together with the inhibition of NF-κB–mediated inflammatory signaling. IFN-I also induces groups of genes called 'IFN-stimulated genes (ISGs)', such as *Irf7* and *Dhx58* which encode IRF7 and DExH-box Helicase 58, respectively, which affect host responses against commensal and invading microorganisms.

that both type of viruses can stimulate immune responses and contribute to pathology in a similar manner.

Despite clear evidence that PRRs react to specific viral pathogens, the contribution of virome-wide sensing by multiple PRRs is largely unknown. Understanding virome-immune system interactions from a broad perspective may be particularly important in intestinal disease because PRRs are probably simultaneously engaged by different viruses. We previously demonstrated that oral introduction of an antiviral cocktail induces dysbiosis of intestinal bacteria and viruses [85••]. Introduction of the antiviral cocktail resulted in reduction of both DNA and RNA viruses whereas richness and abundance of *Caudovirales* bacteriophages were increased. Furthermore, treatment of polyI:C and imiquimod, agonists for viral nucleic acid sensors TLR3 and TLR7, respectively, protected mice from dextran sulfate sodium (DSS)-induced colitis, whereas mice deficient in both TLR3 and TLR7 showed increased susceptibility to colitis [85••]. Other studies also showed effectiveness of agonists for TLR3, TLR7, and TLR9 in mouse colitis models [86–88]. Stimulation of TLR3 and TLR7 has been associated with increased IFN-I responses in pDCs [85••]. Excessive induction of IFN-I is known to increase celiac disease [89]; however, IFN-I is generally recognized to play a beneficial role and to prevent IBD. Deficiency in IFN-I receptor coding gene (*Ifnar1*) exacerbates DSS-induced colitis in mice [86]. IFNAR is a ubiquitous receptor believed to modulate various types of cells; however, the role of IFN-I in each cell type may vary. For instance, deletion of IFN-I signaling in the intestinal

epithelium resulted in hyperproliferation and increased tumor burden in an AOM-DSS mouse model [90], whereas myeloid lineage-restricted *Ifnar1* deletion resulted in abnormal monocyte characteristics and aggravated DSS-induced colitis [91]. Moreover, it was reported that *Ifnar1* is a CD-specific loci [92].

Although different nucleic acid sensors, potential virome sensors, mainly share IFN regulatory factor (IRF)3 and IRF7 to mediate IFN-I responses [78,93], this IRF3/7-IFN-I axis can also be activated by bacteria and biomolecules such as aminoglycosides [94]. Therefore, the virome may play only a partial role in IFN-I responses related to intestinal disease. Notably, a previous study showed that single association of MNV can rescue abnormal intestinal phenotypes in GF or antibiotic-treated mice by inducing IFN-I, which clearly demonstrates that virus-induced IFN-I plays a pivotal role in intestinal homeostasis and pathogenesis [95••].

## Conclusions

Huge numbers of viruses live in the gut and obviously have the potential to stimulate the host directly or indirectly. However, unlike research into the bacteriome, which utilizes a well-established broad-spectrum antibiotic cocktail or GF mice for functional studies, research into the virome mainly uses single virus infection models or metagenomic tools to analyze changes in viral composition. Further efforts are required to learn how the entire virome interacts to regulate the overall mammalian immune system. Besides the immunological influence of the virome, there is evidence that the composition and infectivity of resident viruses are affected by the host and surrounding microorganisms [10,16–18,32•,96]. Thus, interkingdom feedback exists to ensure a balance in the complete commensal ecosystem. Identifying the missing interactions between the virome and the rest of ecosystem will enhance the understanding of immune regulation and pathophysiology in the intestine.

## Funding

This work was supported by the National Research Foundation of Korea (NRF-2017R1A2B3002132).

## Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest


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