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Enteric viruses exploit the microbiota to promote infection

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Enteric viruses infect the mammalian gastrointestinal tract which is home to a diverse community of intestinal bacteria. Accumulating evidence suggests that certain enteric viruses utilize these bacteria to promote infection. While this is not surprising considering their proximity, multiple viruses from different viral families have been shown to bind directly to bacteria or bacterial components to aid in viral replication, pathogenesis, and transmission. These data suggest that the concept of a single virus infecting a single cell, independent of the environment, needs to be reevaluated. In this review, I will discuss the current knowledge of enteric virus-bacterial interactions and discuss the implications for viral pathogenesis and transmission.

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Introduction

The microbiota is now widely recognized for being vital to human health [1]. Consisting of bacteria, fungus, and viruses, the microbiota inhabit multiple sites on the human body where they have developed an ecological niche that is a benefit to both the host and colonizing organisms. Among these sites, the gastrointestinal tract (GI) is home to the most extensive collection of the microbiota, including a dense community of commensal bacteria. This diverse community is made up of approximately 10^{13} bacteria from 500–100 different species [2–4]. A majority of these bacteria reside in the lower gastrointestinal tract; however, due to pH, oxygen, and nutrient availability, distinct bacterial populations inhabit both the small and large intestine [5]. Further, intestinal bacteria vary among individuals and can be influenced by genetics, diet, and lifestyle [6–8]. Additionally, imbalances in the microbial composition have been linked to many human diseases including inflammatory bowel diseases, type 2 diabetes, and obesity [9–14].

Enteric viruses initiate infection in the mammalian GI tract where they encounter these microbiota. Previous data demonstrate that multiple enteric viruses utilize the microbiota to promote replication, pathogenesis, transmission. Many of these viruses have been shown to interact directly with intestinal bacteria, and in this review, I will highlight our latest understanding of the interaction between enteric viruses and intestinal bacteria and the consequences. Specifically, I will discuss the influence of bacteria on members of the picornavirus, reovirus, and retrovirus families. Additional enteric viruses also exploit the microbiota; however, these viruses will be discussed separately in this special volume. Overall these studies suggest that the conventional view of a single virus infecting a cell is too limited. Other factors, including the microbiota, need to be considered when investigating the outcome of viral infections *in vivo*.

Intestinal bacteria impact enteric viral infections

It comes to no surprise that, given the proximity of enteric viruses and intestinal bacteria, recent research indicates that bacteria can impact enteric viral infections. While these bacteria could impede viral infection by acting as a physical barrier in the intestine, recent compelling evidence has shown that some enteric viruses utilize bacteria to promote infection. Poliovirus, a non-enveloped enteric virus in the *Picornaviridae* family that initiates infection in the intestine, can disseminate to the central nervous system to cause paralytic poliomyelitis. In mice depleted of intestinal bacteria by antibiotics, poliovirus replication and lethality is significantly reduced compared to untreated mice [15••]. These data indicate that bacteria promote poliovirus intestinal replication and pathogenesis. Further, data reveal that poliovirus can bind to bacteria through interactions with bacterial surface components, lipopolysaccharide (LPS) and peptidoglycan, to enhance infectivity [16,17•].

Rotavirus, a non-enveloped RNA virus from the *Reoviridae* family, is a leading cause of diarrheal disease in children worldwide [18]. Depletion of intestinal bacteria by antibiotics reduced diarrhea duration and rotavirus fecal shedding in mice [19]. Further, germ-free mice also exhibit a delay in rotavirus fecal shedding, implicating a role for bacteria in rotavirus infection. Reovirus, another member of the *Reoviridae* family, also

had reduced replication and pathogenesis when intestinal bacteria are depleted [15••]. Similar to poliovirus, both Gram-positive and Gram-negative bacteria can bind and enhance reovirus infectivity [20]. This interaction is likely facilitated by viral binding to LPS and peptidoglycan, suggesting that reovirus, like poliovirus, may utilize a broad array of different intestinal bacterial isolates to facilitate infection in the intestine.

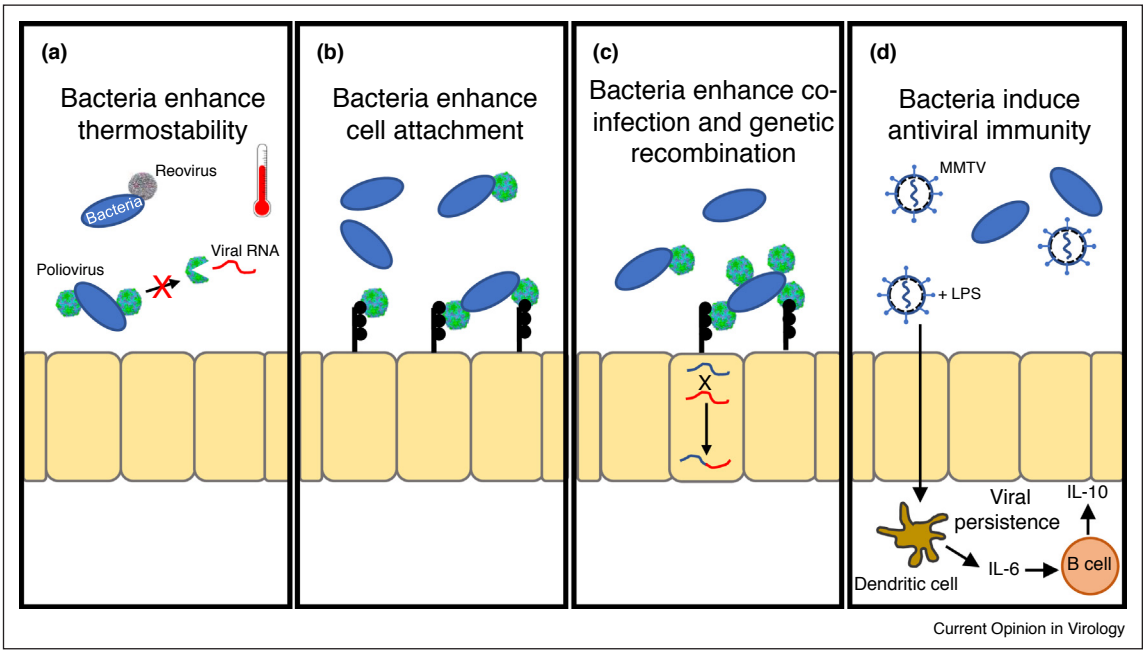
Finally, mouse mammary tumor virus (MMTV) is an enveloped retrovirus that infects lymphoid cells in Peyer's patches of the intestine [21]. MMTV can establish persistence in mice and is transmitted from infected females to suckling pups in milk. Commensal bacteria are required to maintain viral persistence as the virus can be abolished in germ-free mice [22••] Specifically, MMTV can bind to LPS via LPS-binding proteins that become integrated into the viral envelope during egress [23•]. Overall these data provide compelling evidence that some enteric viruses have evolved to utilize intestinal bacteria to promote infection.

Bacteria enhance viral thermostability

Recent advances have shed light on the mechanistic outcome for these virus-bacterial interactions. Data suggest that enteric viruses bind to bacteria to stabilize the virion to retain infectivity. For example, both Gram-positive and Gram-negative bacteria enhance the thermostability of poliovirus (Figure 1a) [15••]. Binding of poliovirus to LPS retain viral particles in their infectious state, limiting inactivation and premature release of viral RNA. [16]. Further a mutation in the poliovirus VP1 capsid protein, T99K, reduces LPS binding and environmental fitness, suggesting that bacterial enhancement of thermostability may also play an essential role in viral transmission from host-to-host. These effects are also seen in other members of the *Picornaviridae* family as well. LPS and peptidoglycan enhance the thermostability of Coxsackievirus A21, Coxsackievirus B5, and Echovirus 30 [24], and bacteria improve the stability of Coxsackievirus B3, Aichi, and mengovirus during bleach treatment [25]. Overall, these data suggest that bacterial-mediated enhancement of thermostability may be a shared mechanism for picornaviruses.

Similar to poliovirus, recent evidence suggests that bacteria also enhance the stability of reovirus. Both Gram-positive and Gram-negative bacteria improve reovirus thermostability *in vitro* through interactions with LPS and peptidoglycan [20]. Furthermore, intermediate infectious subvirion particles (ISVPs) also are stabilized by LPS and peptidoglycan. These ISVPs are formed by proteolytic processing of the virion and required for attachment and entry of reovirus into host cells and contribute to intestinal infection [26–28]. These data suggest that the binding site of these bacterial

Figure 1



Bacteria promote enteric virus infection. **(a)** The binding of bacteria to poliovirus and reovirus enhance thermostability. For poliovirus, enhanced thermostability limits premature release of viral RNA. Poliovirus and reovirus adapted from PBD 1HXS and 2CSE, respectively. **(b)** Bacteria increase poliovirus binding to the poliovirus receptor on permissive cells. **(c)** Poliovirus bound to bacteria increase co-infection and genetic recombination between progeny virions. **(d)** MMTV bound to LPS induce an antiviral response to allow for viral persistence.

components to reovirus does not involve capsid proteins, μ1 or σ3, since they are either cleaved or absent on ISVPs. Interestingly, individual bacterial components have different effects on specific strains of reovirus. While LPS and peptidoglycan enhance have both the Type 1 Lang (T1L) and Type 3 Dearing (T3D) strains of reovirus, lipoteichoic acid and chitin only enhanced T3D thermostability, but not the T1L strain. Therefore, specific reovirus strains may have different affinities for distinct bacterial components; however, if these affinities affect strain-specific replication and pathogenesis differences remains unknown.

Bacteria enhance viral attachment to host cells

Another mechanism for bacterial–viral interactions is through enhancement of viral attachment to the host cell. In addition to enhancing poliovirus thermostability, incubation of poliovirus with bacteria increase attachment to poliovirus receptor (PVR)-expressing cells (Figure 1b) [15••]. Evidence suggests that this enhancement is through direct interaction with the receptor since poliovirus, incubated with LPS, bound to significantly more purified PVR than virus incubated in PBS alone [16]. Interestingly, the VP1-T99K poliovirus mutant, which reduces LPS binding, still retained LPS-mediated enhancement of cell attachment. These data suggest that the affinity and concentration of LPS molecules may influence poliovirus cell attachment and thermostability.

Bacteria can facilitate viral co-infection and promote viral genetic recombination

In addition to the virion thermostability and cell attachment, Erickson *et al.* demonstrated that binding of poliovirus to specific bacterial strains increased viral co-infection of host cells (Figure 1c) [17•]. Using DsRed-expressing and GFP-expressing poliovirus, the authors found that poliovirus mixed with certain bacterial isolates increased the number of dual-infected HeLa cells. These data are not surprising since electron microscopy studies revealed that multiple poliovirus virions bound to a single bacterium. Interestingly, the increase in co-infection rates correlated with viral binding to bacterial isolates that had increased adherence to HeLa cells. Further, using drug-sensitive and temperature-sensitive poliovirus mutants, the authors found that bacterial enhancement of co-infection also increased the likelihood of genetic recombination among poliovirus virions. Overall, these data suggest that poliovirus may utilize bacteria to improve viral fitness in the mammalian GI tract through increased genetic recombination.

Bacteria promote viral immune evasion

Commensal bacteria provide vital stimulation to develop a mature host immune system [29,30]. While these immune signals from bacteria promote colonization resistance to pathogenic organisms, evidence suggests that some enteric viruses have exploited these pathways to evade the immune system. For example, antibiotics enhance the antibody response to a rotavirus infection, suggesting that bacteria can modulate the immune response to rotavirus [19]. The mechanism behind this response, however, is unclear. Similarly, MMTV can subvert the host immune response to establish persistence in mice. Immune evasion for MMTV requires functional Toll-like receptor 4 (TLR-4) signaling and the immunosuppressive cytokine IL-10; however, the mechanism was largely unclear [31]. Kane *et al.* shed light on this mechanism when they demonstrated that MMTV uses commensal bacteria to facilitate persistence by binding to bacterial LPS (Figure 1d) [22••]. To enable binding to LPS, MMTV incorporates LPS-binding factors, such as MD-2 and CD14, into the viral envelope during egress from infected cells [23•]. These LPS-binding factors play a critical role in the transfer of the LPS molecule to TLR-4 and can help trigger LPS-induced signaling pathways [32]. Upon binding of LPS to the viral membrane, MMTV can stimulate the TLR-4 pathway leading to IL-6 production of the immunosuppressive IL-10 cytokine. This cascade activates an immune evasion pathway, facilitating persistent MMTV infections.

Negative or unclear effects of bacteria on enteric viruses

While this review focuses on the beneficial outcome of bacterial and viral interactions, these effects may not be conserved. Mouse adenovirus 1 does not require intestinal bacteria for intestinal infection [33]. Additionally, defensins, host antimicrobial peptides induced in response to intestinal bacteria, can neutralize some adenoviruses types [34]. Interestingly, recent data suggest that α-defensin may enhance mouse adenovirus type 2, a mouse enteric pathogen, suggesting that further work is necessary to determine the role of intestinal bacteria on enteric adenoviruses [35]. Further, even though some experimental data suggest that intestinal bacteria may promote rotavirus infection, other data contradict this. For example, probiotics have been shown to reduce the duration of viral diarrhea and administration of *Lactobacillus rhamnosus GG* reduces rotavirus shedding [36–38]. Additionally, soluble factors from commensal bacteria can block rotavirus infection *in vitro*, and bacterial flagellin can eliminate chronic rotavirus infection through induction of IL-22 and IL-18 *in vivo* [39]. Overall, these data suggest that some enteric viruses may utilize intestinal bacteria to promote infection, while others may not.

Conclusions

Traditionally, viral infections have been viewed in the context of a single virus infecting a host cell. This straightforward view has allowed a reductionist approach to understanding the fundamental processes of the viral life cycle. This reductionist approach, however, does not always paint a full picture of the complex interactions that arise during a viral infection *in vivo*. In light of this

potential complexity, recent evidence indicates that additional extrinsic factors, such the microbiota, may play a significant role in establishing a viral infection within a host. Overall, these new factors have established a new field of study in virology where viral infections are understood in the context of the environment for which they initiate infection. Future experiments identifying these complex interactions and their mechanisms need to be elucidated to provide a clear picture of the viral cycle, not only within the cell, but within the entire host.

In light of all these recent findings, there is clearly a need for more studies to understand how intestinal bacteria and the microbiota impact viral infections. Many questions remain as to the precise mechanisms of bacterial-mediated viral enhancement. First, which specific bacteria are required to enhance viral infection? Specific bacteria required to enhance infection for one enteric virus likely not enhance another. Data suggest that poliovirus and reovirus have different binding affinities to particular bacteria and bacterial components, and identifying the mechanism behind these binding affinities may provide potential therapeutic targets. Second, does biological sex influence these interactions? Microbiota differences between males and females exist and have been shown to influence type 1 diabetes in a mouse model [40]. Recently, Coxsackievirus B3 replication in the intestine was shown to be sex-dependent [41]. Since bacteria interact with Coxsackievirus B3 [25], perhaps sex-dependent microbiota may influence viral replication through direct or indirect mechanisms. Third, do bacteria affect other non-enteric viruses? Data suggest that bacteria can affect the immune response to influenza virus [42–45]; however, the impact of bacteria on other viruses is unclear. Since the bacteria colonize multiple sites on the human body, likely other viruses have interactions with bacteria. Additionally, viruses may play a complementary role in promoting bacterial infections [46]; however, additional data are required to determine if this impacts infections in the gastrointestinal tract. Finally, what is the role of other components of the microbiota? Much of the current data have focused on the effect of the bacterial component of the microbiota on enteric viruses, but fungal inhabitants of the intestine and other viruses may also influence enteric viral infections as well. Additional studies are needed to investigate these interactions in the intestine to determine their influence on viral infection.

Finally, it is important to note that while current data suggest that antibiotics may be an effective antiviral therapy, the side effects of disruption of commensal bacteria would largely outweigh any potential gain. Antiviral effects in mice require a substantial depletion of intestinal bacteria, with multiple antibiotics given prophylactically, which would likely disrupt the health benefits that these bacteria provide. Future experiments identifying specific interactions between enteric viruses and specific bacteria or bacterial components may offer a better opportunity for therapeutics. For example, the precise modulation of these virus-bacteria interactions may provide benefits in controlling outbreaks by disrupting viral transmission.

In conclusion, the role of the microbiota on enteric viruses represents a promising area of future study. It is imperative that this field moves forward so that we can truly understand the overall environmental factors that underlie a viral infection within a host. While we are only beginning to uncover the mechanisms behind interactions between enteric viruses and bacteria, I anticipate that future studies will continue to shed light on other factors of the microbiota that influence enteric viral infections. This knowledge will be vital as we continue to understand the complex interactions and environments within the human body that influence viral infections.

Conflict of interest statement

Nothing declared.

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