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Editorial overview: Viruses and the microbiome

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The microbiome is a collection of microorganisms (e.g. bacteria, viruses, fungi, protozoa) that reside together in a given environment and their combined activities [1]. The microbiome that resides in and on the host is an important factor in determining health or disease. Examples are the beneficial roles of the microbiome in host metabolism, the development of the host immune system, and protection from opportunistic infections. However, detrimental roles for the microbiome are observed following dysbiosis (i.e. disruption in the normal state of the microbiome), for example during inflammatory bowel disease, cancer, and obesity. Studies estimate that there are roughly as many bacterial cells and 10 times more viruses as human cells in our bodies [2,3]. The polymicrobial interactions in the intestine associated with this diverse and rich ecology have fascinated scientists and spurred tremendous activity in basic and translational research.

In the 1960s, researchers first started probing whether the intestinal microbiome influences virus infections using germ-free mouse models. Although these early studies provided clear evidence that the intestinal microbiome can have both proviral [4] and antiviral [5–7] effects, progress in understanding the basis for these effects was quite limited over the next 50 years. However, two seminal studies published in *Science* in 2011 sparked renewed interest in this topic. First, Kuss *et al.* demonstrated that the intestinal microbiome enhances poliovirus and reovirus infections in mice and that poliovirus bound to bacterial lipopolysaccharide (LPS) displays increased infectivity [8]. Second, Kane *et al.* revealed that transmission of mouse mammary tumor virus (MMTV) from an infected dam to a newborn mouse through maternal milk requires MMTV-bound LPS and consequent tolerance induction [9]. Substantial progress has been made over the past eight years in elucidating mechanisms underlying commensal bacterial regulation of viral infections. In this issue of *Current Opinion in Virology*, six outstanding reviews by Robinson, Baldridge, Schultz-Cherry, Thackray, Dimopoulos, Vlasova and colleagues summarize the current state of knowledge about the complex and varied influence of the intestinal microbiome on eukaryotic viral infections in the mammalian host.

While the bacterial component of the intestinal microbiome has been extensively studied, the viral component (i.e. the gut virome) is less well-studied. The array of virus types (e.g. DNA versus RNA genomes) and extreme genetic diversity across virus families and even within related viruses represent just a few of the challenges researchers face when attempting to profile virome constituents. The gut virome comprised a vast number and type of bacteriophages in addition to an array of eukaryotic viruses that can infect mammalian cells, fungi and parasites present in the intestinal microbiota, and even plant cells that are ingested by the mammalian host as part of our diet. The bacteriophages in the gut microbiome

generally adopt a temperate lifestyle in healthy individuals. They serve as a reservoir of bacterial genes that can be transferred between microbes [10]; and they can adhere to the mucus layer lining the intestinal tract, protecting the host from bacterial infections [11]. Three fascinating reviews by Matthijnssens, Kweon, and Saxena and colleagues highlight the impact of viral component of the microbiome on shaping human health and disease; while one by Roossinck expands the view of the microbiome to include plants by discussing the viral component of the phytobiome.

How does the intestinal microbiome influence virus infections?

The intestinal microbiome enhances infections of a variety of enteric mammalian viruses. The mechanisms underlying bacterial enhancement of picornaviruses, reoviruses, and MMTV are summarized by Robinson; and mechanisms promoting noroviruses are summarized by Baldridge et al. Five general strategies have been described. First, bacterial glycans can enhance the stability of certain viruses. Virions incubated with specific bacterial glycans display increased thermostability and resistance to dilute chlorine bleach. This stabilizing property confers a fitness advantage to poliovirus in transmission to new hosts. Second, bacterial glycans and bacterial metabolites can promote virus attachment to entry receptors on the surface of target cells. Third, recognition of commensal bacteria during an enteric virus infection can skew the host immune response in a manner that promotes virus infection. For example, MMTV bound to LPS induces a tolerogenic immune response to viral antigens that is conducive to the establishment of MMTV persistence. Furthermore, microbial suppression of the type III interferon (IFN) response is critical to murine norovirus persistence. Fourth, commensal bacteria can enhance the numbers of host cell targets for particular viruses. For example, the persistent reservoir for a murine norovirus is the intestinal tuft cell and commensal bacteria increase tuft cell numbers. Fifth, the intestinal microbiota can facilitate viral coinfections and viral genetic recombination. This is thought to result from multiple virions binding to a single bacterium that comes into contact with a host cellular target. Less is known about the role of the intestinal microbiome in astrovirus infections but Schultz-Cherry et al. highlight early intriguing studies and the case for additional investigations.

Just as the distinction between pathogenic and nonpathogenic bacteria is often blurry and dependent on environmental conditions, the same appears to be true of viruses. For example, noroviruses and astroviruses are major global causes of gastroenteritis, yet a persistent avirulent norovirus infection can replace the benefits of commensal bacteria in germ-free mice through induction of type I interferon (IFN) and interleukin (IL)-22 [12,13]. Moreover, a murine astrovirus can provide protection from other enteric virus infections in a type III IFN-dependent manner under certain circumstances [14]. These intriguing observations are also discussed in this series of reviews.

Numerous mammalian viruses that infect at sites other than the intestinal tract are also impacted by the intestinal microbiome, as highlighted by Thackray et al. The intestinal microbiome plays a protective role in these infections, as evidenced by increased virus titers and disease in antibiotic-treated and germ-free mice. Calibration of the type I IFN response by commensal bacteria plays a crucial role in mediating this protection to systemic virus infections. Commensal bacterial stimulation of the inflammasome and induction of proinflammatory cytokines at distal sites have also been implicated in controlling certain extraintestinal viruses. Importantly, adaptive immune responses to systemic viruses are enhanced by the intestinal microbiome, which could contribute to diminished viral control in bacteria-deplete hosts. The underlying mechanism(s) by which intestinal bacteria provide immune-mediated protection against systemic viruses are being pursued by many research groups. A major contributing factor is host pattern recognition receptor (PRR) engagement of commensal bacterial molecular-associated microbial patterns (MAMPs), which then orchestrate innate and adaptive immunity. Moreover, bacterial metabolites produced within the intestinal tract can enter circulation and prime immune responses to viruses at extraintestinal sites.

Arthropod-borne viruses, or arboviruses, are ingested by insects when the insect takes a blood meal from an infected vertebrate host. Arboviruses then encounter the intestinal microbiome of the insect. Dimopoulos et al. provide a review of this interplay between mosquitos, their microbiome, and arboviruses. The intestinal microbiome can be proviral or antiviral in mosquitoes. One proviral mechanism involves bacterial production of a mucus-digesting protein which is thought to enable virus contact with the intestinal tissue. One instance of the intestinal microbiome playing an antiviral role in mosquitoes is the priming of host immune responses to provide protection from certain arboviruses. Direct mechanisms of viral inhibition have also been reported. For example, one commensal bacteria produces an aminopeptidase that degrades the Dengue virus envelope protein. There has been intense interest in the antiviral property of one specific commensal bacteria that naturally colonizes many insects called *Wolbachia pipientis*. When artificially infected into mosquitoes at high doses, *Wolbachia* strongly inhibits infection by a variety of clinically relevant arboviruses and restricts viral transmission, prompting investigation of using *Wolbachia* for biocontrol of mosquito-borne diseases.

The collective body of work described in the aforementioned reviews clearly establishes that the intestinal microbiome can modulate antiviral immune responses, leading one to question whether they also impact immune

responses to viral vaccines. It has long been appreciated that oral vaccines (e.g. poliovirus, rotavirus, and cholera vaccines) are less effective in children from developing nations than in children from industrialized nations. While multiple factors underlie poor vaccine efficacy in these children, evidence has accumulated that microbiome composition and intestinal dysbiosis play significant roles. Vlasova et al. describe how intestinal dysbiosis associated with undernourishment and repetitive intestinal infections shape immune responses and negatively impact vaccine efficacy. They also summarize recent efforts to counter this by testing the effect of probiotics on vaccine immunogenicity and effectiveness.

While the main focus of these reviews was the impact of the intestinal microbiome on viral infections, it is also of interest to consider how viral infections in turn influence the intestinal microbiome. For example, astrovirus, norovirus, and HIV infections have all been associated with decreased bacterial diversity. Likewise, the microbiome composition in mosquitoes is influenced by arbovirus infections. However, these changes are not uniformly observed across infected cohorts or in every study, so it is likely that factors such as viral genetics, host genetics and age, and environmental conditions influence the outcome of viral infection on the microbiome.

How does the virome influence health and disease?

Although the gut virome remains poorly understood in terms of total composition and impact on human health and disease, progress in addressing these knowledge gaps is advancing rapidly. Matthijnssens et al. highlight virome dynamics as revealed by longitudinal interpersonal and intrapersonal studies. Although there is substantial diversity in virome composition between people, there is also evidence for a core virome, or a set of shared gut viruses in the human population. Factors correlating with virome diversity across populations include diet, birth mode, and disease. In terms of intrapersonal virome profiling, the virome changes drastically during the first weeks of life but it is relatively stable in adults. It is important to recognize the limitations of current methods to study the gut virome where 60–99% of sequences are in essence 'dark matter' that cannot be annotated. This portends a vast amount of discovery yet to be undertaken and underscores how much remains to be learned about the importance of the gut virome in human health.

Kweon et al. discuss the consequences of the gut virome on host immunity. Eukaryotic viral constituents of the gut virome can infect host cells so it is more straightforward to envision how they modulate host immunity. Indeed, they can activate immune responses that protect from other pathogenic viral or bacterial infections, and contribute pathologically to disease in individuals carrying risk genes for inflammatory conditions. Although bacteriophages do not infect mammalian cells, there are several plausible scenarios, in which they could be sensed by the host immune system. They embed in the intestinal mucosa and can cross the epithelial barrier. Furthermore, bacteria that invade the host intestine may carry prophages and/or produce bacteriophages. Thus, there is ample opportunity for bacteriophages to be sensed by cell surface and endocytic PRRs. Indeed, a variety of immune cells have been shown to internalize bacteriophages and respond by expressing inflammatory cytokines. Underscoring the importance of the gut virome to maintaining intestinal health, reducing virome richness and abundance induces intestinal dysbiosis whereas activating viral PRRs protects mice from developing colitis.

Saxena et al. focus on the effects of bacterial and viral dysbiosis in the specific disease states of cancer and HIV infection. Bacterial dysbiosis can induce carcinogenesis through direct and indirect mechanisms. For example, certain bacteria are directly oncogenic, while others are highly inflammatory and may be indirectly oncogenic by causing epithelial cell injury or formation of reactive oxygen species and damage to nucleic acids. Certain viruses are also associated with cancers, in particular herpesviruses such as EBV and KSHV, human papillomaviruses, and hepatitis B and C viruses. All of these viruses are capable of establishing chronic or latent lifelong infections in the human host and are maintained in the absence of disease for variable lengths of time before initiating tumorigenesis. During HIV infection, the intestinal bacteriome is altered as is the microbiome at other body sites, which can leave hosts more vulnerable to other pathologies. HIV-infected individuals are also more susceptible to pathogenic outcomes of virome constituents like KSHV-induced Kaposi's sarcoma.

The final review in this issue by Marilyn Roossinck describes the viral component of the phytobiome, focusing on viruses of terrestrial plants. Certain viruses provide heat, cold, or drought tolerance to plants, either by infecting the plant directly or infecting a fungus that colonizes plants. In the latter instance, the plant fungus provides a fitness advantage to the plant so it is considered an endophyte. Many endophytes in fact carry viruses which could influence the plant host but these interactions have been largely unexplored. Other plant fungi are pathogenic and when these carry viruses, they can reduce fungal virulence. For example, chestnut blight is caused by the fungus *Cryphonectria parasitica* but the plant host can survive if the fungus is infected by Cryphonectria hypovirus 1. Plant-associated bacteria can be infected by bacteriophages in the same way that mammalian-associated bacteria are, and there has been a long-standing interest in the possibility to use lytic bacteriophages in the control of bacterial plant diseases. Finally, thousands of viruses that directly infect plants. These viruses cause

acute or persistent infections, and asymptomatic or pathogenic disease similar to animal viruses. In contrast though, the majority of acute plant viruses are transmitted by insects without infecting its vector. While progress has been made at characterizing the vast ecology of the phytobiome including the viruses that affect plants, there are substantial gaps in knowledge and undoubtedly many mysteries left to unravel.

In summary, this collection of articles highlights the various ways in which the host, viruses, other microorganisms, and the environment interface to shape the outcome of this interaction. The reviews also highlight the many remaining open questions. It is our hope that this collection of reviews will spur further investigations to uncover new facets of this intricate interaction between multiple kingdoms of life.

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