The secret garden's gardeners

Research increasingly appreciates the crucial role of gut viruses for human health and disease

Philip Hunter

Research has shown that the microbiota—the population of micro-organisms inhabiting the gut—has a profound influence on health in both humans and animals. However, most studies have largely ignored the viral population of the gut—the virome—although it is much larger, both in number of organisms and in genetic diversity. This is because the virome was thought to be less important for health and immunity, as it mainly comprises bacteriophages that only affect bacteria. However, researchers are beginning to realize that the viruses present might well be important in human health, as they manipulate the microbiota, swapping genetic virulence factors among bacteria, and through interaction with the host immune system.

There are two distinct categories of virus in the gut: phages, which infect bacteria, and viruses that target host cells. Although these two categories are apparently independent of each other, there is a relationship between them, as indicated by growing evidence that the microbiota as a whole, including phages, has a crucial role in protecting against bacterial and viral infections [1].

The phage and bacteria populations of the gut have an intricate relationship, which raises the potential therapeutic use of phages to treat a variety of conditions caused by bacteria in the gut, especially those involving chronic inflammation. The first step, however, is to explore and analyse the phage populations in the gut in terms of diversity and number, along with their interactions with their bacterial targets. This has proven to be a major challenge, given the enormous difficulties in identifying, isolating and amplifying genetic material from the phage population.

Nevertheless, researchers from the Weizmann Institute of Science and Tel Aviv University in Israel have made substantial progress by indirectly identifying phages through clustered regularly interspaced short palindromic repeats (CRISPRs) in their bacterial hosts [2]. CRISPRs function as a prokaryotic adaptive immune system against genetic invaders such as phages by recognizing foreign DNA and then silencing its expression in a manner analogous to RNA interference (RNAi) in eukaryotes. Short segments of the foreign DNA, known as spacers, are incorporated into the bacterial genome to provide the memory of past exposures to enable recognition of phage DNA.

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The Israeli study reconstructed the CRISPR bacterial immune system in the human gut microbiomes of 124 European individuals, and from that identified 991 phages targeted in at least one of the individuals. Of these phages, 78% were present in at least two individuals and some turned out to be the same ones that had already been identified in Japanese and American people. This global distribution of particular phages was a surprise, given that in other ecological niches, notably seawater, where phages are highly abundant, there is great genetic diversity among the populations, even over short distances.

The Israeli team further succeeded in deducing the bacterial hosts of 130 of the phages, which allowed them to study the associated phage–bacteria interactions. It turned out that a subset of the phages had developed closer associations with their host bacteria as lysogenized prophages after fusing their DNA with the bacterial chromosome or as plasmids. Rotem Sorek, a specialist in microbial warfare at the Weizmann Institute of Science and co-author of the Israel study, commented that this behaviour allows bacteria to take advantage of the phage by incorporating and transmitting genes that provide vital functions and occasionally aid pathogenesis. “There are clear instances of phages ‘helping’ pathogenic bacteria to attack humans,” Sorek said. “The toxins of the Cholera, Diphtheria and Shigella (dysentery) are all carried by phages that are integrated into the bacterial genome.”

Horizontal gene transfer among bacteria has long been known to increase the adaptability of several potentially virulent bacterial species, but it is only recently that the mechanisms involving prophages have begun to be elucidated. A significant advance was made in a Japanese study from the University of Miyazaki, inspired by the observation that many sequenced bacterial genomes contain multiple prophages carrying a wide range of genes involved in virulence, and that these often seem to contain genetic defects [3]. The team analysed a virulent strain of *Escherichia coli*, known as O157, which contains 18 prophages that encode various genes involved in the production of virulence factors, including two potent cytotoxins: Shiga toxins 1 and 2. Most of the prophages they identified contained multiple genetic defects, yet they seemed to be

capable of transporting virulence elements between not only members of the same strain but also different *E. coli* strains.

The conclusion from their study was that defective prophages in close proximity within *E. coli* cells were still capable of recombining to yield a new phage that was released from the cell and could infect other cells nearby in the gut. It seems that these defective prophages were not just evolutionary leftovers, but were important components of the bacterial genome, conferring additional adaptive flexibility through horizontal gene transfer. Many other bacteria contain multiple prophages with genetic defects, so it is probable that this mechanism is not confined to *E. coli*.

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Other studies have focused on the composition of phage virus populations outside bacteria in the gut, as part of initiatives to compare and contrast the virome and bacteriome in response to individual genetic variation and environmental factors such as diet. One might imagine that phage and bacteria populations should be closely correlated, but it turns out that there are significant

differences in the level of variation between individuals, as well as over time within the same individual. A study at the Washington University School of Medicine, USA, on monozygotic twins, found that in contrast to bacteriomes, viromes tended to be unique to individuals and less varied over time in response to changes in diet or other factors. By contrast, the bacterial population changed much more with diet and was also quite similar between twins.

Given that there is a direct relationship between the bacteria of the gut and the immune system of the individual—which is not the case for phage viruses—these findings make a degree of sense. Furthermore, as noted by Jeffrey Gordon, co-author of the Washington University study, there is not a one-to-one relationship between bacteria and the phages they host: “It’s been shown in other environments that you can have several different viruses capable of infecting the same bacterial host, while the specificity of each virus is usually quite narrow, typically extending only to a few strains within a species-level phylotype,” he explained. “This leads to a greater genetic diversity in the virome. Furthermore, a viral genome is enriched for genes involved in genome replication and virion assembly. Thus, the functional composition of the virome and the microbiome are quite different.”

The situation is different for non-phage viruses in the gut that have a direct relationship with the human or animal host. The main research interest here is the three-way relationship between the virus, the bacteriome and the host’s immune system. Research on this front has already led to a new understanding of the role played by the entire microbiome in immunity. Often, the microbiome provides protection against viruses, but in some cases it can encourage their propagation. This is relevant in the context of human immunodeficiency virus (HIV), for example, given that the virus infects immune cells such as helper T cells, macrophages and dendritic cells, the activity and production of which in turn are related to the microbiota. An important question is whether the course of HIV and its possible development into symptomatic acquired immunodeficiency syndrome (AIDS) might be encouraged by the microbiota, if it stimulates production of such immune cells. Whilst this has yet to be established for AIDS, there is evidence that it is the case in monkeys carrying the related simian immunodeficiency virus (SIV), which infects at least 45 species of African primates. Unlike HIV in humans, SIV is usually non-pathogenic, as many primates evolved to coexist with the virus; but it does cause AIDS in rhesus macaque monkeys.

Another Washington University School of Medicine study, this time looking at the link between viruses, the bacteriome and host immunity, began with the insight that animals developing AIDS experience immune hyperactivation, including higher levels of inflammatory chemokines, cytokines and activated T cells. This observation suggested that excessive inflammation is an important factor in progression to AIDS, presumably because it increases the number of cells vulnerable to HIV and SIV infection. The US team investigated whether there were any corresponding changes in the virome, finding that whilst it remains unchanged in uninfected animals, including rhesus macaque monkeys that had not succumbed to AIDS, its diversity increases significantly in infected macaques with full-blown AIDS [4].

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The team is following up by probing the relationship between enteric viruses and AIDS and how the immune system is stimulated. The animals suffer from progressive damage to their intestinal walls, which could increase the absorption of viruses that in turn stimulate the immune system, promoting replication of SIV and possibly encouraging more opportunistic viral infection, thereby creating a vicious circle. "This is one of several possible scenarios that we are actively investigating," commented Scott Handley, lead author of the study. "What is uncertain is what is causing the damage to the intestinal wall. It could be the viruses which expand in the enteric virome during SIV infection, or some other factor, which could be immune-mediated or some other microbe or microbial product in the enteric microbiome."

Handley's team has identified some of the viruses involved, including both common ones and some previously undiscovered.

"Many of the viruses we identified have been associated with [gastrointestinal] disease in one form or another," Handley said. "It is true that many of the viruses we identified are common; however, we identified at least 32 novel subtypes of these viruses never seen before." He added that it remains to be established whether SIV infection encourages opportunistic infection by these viruses or not: "We don't really have a good handle on what viruses would be considered 'commensal' viruses in any animal, including research primates. So whether they are already there when infection with SIV occurs, or are just more susceptible to opportunistic infection, is unclear."

Handley further commented that this work could lead to new therapeutic targets for treating HIV infection and preventing AIDS, and he is investigating whether the same expansion of the enteric virome occurs in humans infected with HIV. "In addition, we are interested to see if vaccination can reverse the enteric virome expansion," he explained. "We would also like to better understand if the viruses we have identified were already circulating in these primates, or are succumbing to opportunistic infections."

Handley argued that his team's work and that of others provides a compelling case for devoting more resources to studying the role of viruses in the gut, which would require further advances in laboratory and analytical techniques. "One challenge with studying the viral members of a microbiome is that there are no well-defined marker sequences," he said. "Therefore, we are largely dependent on random shotgun sequencing approaches, which are less efficient and more expensive. Not only do you have to gather much more data, the computational analysis required is much more complex than the well-established techniques developed for studying the bacterial microbiome. While we know there is a great deal of interest in studying the virome, current techniques and technology tend to

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limit the number of labs that can participate in these efforts. We are very interested in developing new tools and techniques to help alleviate this issue."

Work undertaken so far has already shown that probiotic or prebiotic treatments that provide or encourage beneficial gut bacteria can benefit patients infected with HIV. Improvements in intestinal health could reduce the leakage of all antigens, including viral ones, through the intestinal wall. More generally, a better understanding of how phages, viruses and bacteria in the gut interact could lead to new therapies that manipulate the microbiome to restore intestinal health in sufferers of a variety of conditions, including those involving chronic inflammation.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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