CellPress

Precision Engineers: Bacteriophages Modulate the Gut Microbiome and Metabolome

Jeremy J. Barr[1,*]
[1]Monash University, School of Biological Sciences, Clayton, VIC, Australia
*Correspondence: jeremybarr85@gmail.com
https://doi.org/10.1016/j.chom.2019.05.010

The precise and rational manipulation of the gut microbiome may enable new therapies for gut health and disease. In this issue of *Cell Host & Microbe*, Hsu et al. (2019) reveal that bacteriophage predation reduces target bacteria and results in cascading interactions that alter non-target species and the gut metabolome.

Since our explorations into the microorganisms that reside in the human gut began, researchers have been chasing the means to affect and control them. Dietary changes and antibiotics are two of the primary ways in which we influence our gut microbes. Yet these treatments are overly broad in their perturbations and lack the precision required to rationally alter specific gut bacterial populations. To this end, bacteriophages, or phages for short, are considered as top candidates for the rational adjustment of the gut microbiome due to their function as specific bacterial antagonists. The removal of gut bacterial pathogens by phages has been attempted in both *in vivo* studies and clinical trials, yet the efficacy of this approach remains inconclusive (Galtier et al., 2016; Sarker et al., 2016). Efforts to assess the capacity of phages to modulate the broader gut microbiome have shown promise, but findings are limited by the microbial complexity present. For example, one prior study colonized germ-free mice with a defined 15-member bacterial community followed by administration of an uncharacterized mixture of phages isolated from feces (Reyes et al., 2013). Phage predation did change the composition of the gut community, but the uncharacterized mixture of phages made it difficult to infer any direct or indirect effects associated with the treatment. Despite its potential, the effective modulation of the gut microbiome using phages has yet to be fully realized.

In this issue of *Cell Host & Microbe*, Hsu et al. (2019) set out to determine the effects of four lytic phages on a ten-member model microbiota comprised of commensal bacterial species known to colonize the human gut. Phages were administered in pairs into germ-free mice inoculated with the defined bacterial consortia. The subsequent effects on the bacterial community were monitored using sequencing and quantitative PCR. All phages targeted and reduced their bacterial hosts in the gut to varying extents, but none of the target bacterial species were eliminated. Surprisingly, phage predation was shown to modulate non-target bacteria through inter-bacterial interactions, resulting in blooms and attrition of certain species. Metabolomic profiling revealed functional effects of phage predation on the gut microbial community, including the reduced production of neurotransmitters. Although much work is still needed to translate these findings to the full complexity of the human gut, the results suggest that phages may allow for the rational modulation of the human gut microbiome and metabolome for therapeutic purposes.

A key question raised by Hsu and colleagues is whether phages can be effectively used to perturb key bacterial species within the gut. Current phage therapy approaches are proving to be effective in combating antibiotic resistant bacterial pathogens and the infections they cause (Gordillo Altamirano and Barr, 2019). Yet within the gut, the situation is profoundly more complex. With its high bacterial abundance and diversity, a preference for lysogeny, and concerns regarding phage persistence and replication (Shkoporov and Hill, 2019; Weiss et al., 2009), it remains unclear whether the use of lytic phages to reduce gut bacterial species is effective (Galtier et al., 2016; Sarker et al., 2016). Among their findings, Hsu et al. (2019) note that the lytic phages they administered persisted within the gut during the course of their experiment and that the target bacterial host experienced knockdown, but not eradication. Using selective media, they were able to isolate one of the bacterial hosts—*Enterococcus faecalis*—from their ten-member community and track its phage sensitivity profile. Hours after phage administration *E. faecalis* populations were completely sensitive to their lytic phages. However, within two days, 28% of the population were phage resistant, and by ten days, 68% were found to be phage resistant. These findings suggest that lytic phages may be effective for the targeted knockdown of gut bacterial species, at least within a low complexity community.

Studying the dynamic and cascading effects of phage predation within the gut is a considerable challenge. Hsu and colleagues approach this problem in an innovative, yet familiar way. In molecular biology, a general strategy to identify the role and function of a particular gene is to create a genetic knockout. Characterization of this loss-of-function mutant, followed by reintroduction of the deleted gene, allows researchers to investigate the genes' function. By analogy, Hsu et al. (2019) designed a "drop out" experiment whereby germ-free mice were colonized with a bacterial consortium which excluded each of the phage-targeted species in turn. By investigating the magnitude of colonization changes between the full consortium and the drop out experiments, a hypothesized bacterial interaction network was established. If, for instance, a bacterial drop out experiment resulted in reduced colonization of a particular species compared with the full consortium, it was proposed that the

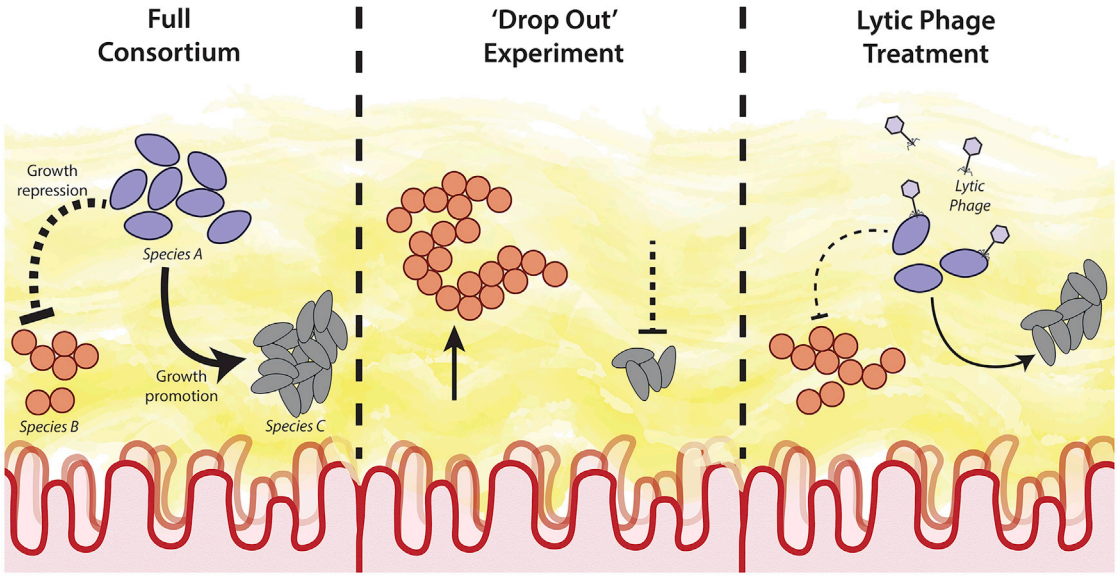

Figure 1. Bacterial "Drop Out" Experiments Establish Hypothesized Bacterial Interaction Networks that Predict Both Direct and Indirect Effects of Phage Predation on a Model Gut Microbiota

In the study from Hsu and colleagues, germ-free mice were colonized with a ten-member bacterial consortium. Colonization rates of the full consortium were quantified and compared with "drop out" experiments in which one bacterial species was omitted. By investigating the magnitude of colonization change between the full consortium and the drop out experiments, a hypothesized bacterial interaction network was established. Lytic phage treatment was shown to reduce target bacterial species, which subsequently modulates non-target bacteria through inter-bacterial interactions, resulting in blooms and attrition of certain species. Solid lines represent promotion of growth, dashed lines represent repression of growth, and linewidths correspond with magnitude of the effect.

omitted bacteria had a promoting influence on this species—and vice versa for repressive influences (Figure 1). Using this network, it was possible to hypothesize the effect phage predation had on the surrounding bacterial consortium. One interesting example was seen for the bacterium *Escherichia coli*, whose drop out experiment and subsequent interaction network suggested that it promotes *Bacteroides fragilis* growth while repressing growth of *Bacteroides vulgatus*. Following T4 phage administration, *E. coli* abundance was substantially reduced, and a subsequent contraction in *B. fragilis* and bloom in *B. vulgatus* populations were seen. Although not without their flaws, the drop out experiments reinforced some of the influences that phage predation had on both target bacterial species and the surrounding microbiota.

Previous work has explored the role microbial metabolites play in mediating interactions between bacteria and the mammalian host (Dorrestein et al., 2014). Dietary changes and antibiotics cause large shifts in gut bacterial community composition, and these changes have been shown to affect the gut metabolome (Antunes et al., 2011; Marcobal et al., 2013). Phages are also known to modulate gut bacterial community composition (Reyes et al., 2013; Shkoporov and Hill, 2019). Yet the extent by which they do so and whether this affects the gut metabolome remains unclear. To address this, Hsu and colleagues extended their *in vivo* observations with the use of untargeted metabolomics of feces before and after the introduction of phages. Overall, phage-directed modulation of gut metabolites was modest, with only 17% of metabolites significantly changing, compared with prior studies that found upward of 80% of metabolites changing upon antibiotic exposure (Antunes et al., 2011). However, the specificity of phage predation on their target species correlated with shifts in the metabolic products produced. One example was the neurotransmitter tyramine, whose production was solely associated with *E. faecalis* in the ten-member community. Administration of lytic phage VD13 caused 9- and 42-fold reductions in *E. faecalis* abundance on days 2 and 13, respectively, which corresponded with 2.7-fold and 4-fold reductions in tyramine on respective days. Due to the limited catalog of experimentally verified microbial metabolites currently available (Dorrestein et al., 2014), the authors were limited in their capacity to broadly associate phage predation with changes in metabolites. However, these preliminary results suggest that phages may allow for the targeted modulation of the gut metabolome.

This study has some limitations. The ten-member bacterial community provides a framework to study phage mediated effects within a gut environment, but its limited microbial diversity remains a far cry from that seen within the human gut. Further, the hypothesized bacterial interaction networks provide a necessary framework to begin studying both the direct and in-direct effects of phage predation, yet the mechanisms for the proposed inter-bacterial promotion and repression need to be verified. Finally, and perhaps most importantly, it remains unclear whether lytic phage predation within the full complexity of the human gut is sufficient to mediate the functional effects demonstrated here.

Overall, this work provides a deeper understanding of how phages modulate gut bacterial species, both directly and indirectly, and begins to establish the potential use of phages for the precise and

rational manipulation of the human gut microbiome.

ACKNOWLEDGMENTS

The phage research in the Barr lab is supported by the Australian Research Council (ARC) Discovery Early Career Researcher Award (DECRA) (DE170100525), the National Health and Medical Research Council (NHRMC; 1156588), and the Perpetual Trustees Australia award (2018HIG00007).

REFERENCES

Antunes, L.C.M., Han, J., Ferreira, R.B.R., Lolić, P., Borchers, C.H., and Finlay, B.B. (2011). Effect of antibiotic treatment on the intestinal metabolome. Antimicrob. Agents Chemother. *55*, 1494–1503.

Dorrestein, P.C., Mazmanian, S.K., and Knight, R. (2014). Finding the missing links among metabolites, microbes, and the host. Immunity *40*, 824–832.

Galtier, M., De Sordi, L., Maura, D., Arachchi, H., Volant, S., Dillies, M.-A., and Debarbieux, L. (2016). Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition. Environ. Microbiol. *18*, 2237–2245.

Gordillo Altamirano, F.L., and Barr, J.J. (2019). Phage therapy in the postantibiotic era. Clin. Microbiol. Rev. *32*, e00066-18.

Hsu, B.B., Gibson, T.E., Yeliseyev, V., Liu, Q., Lyon, L., Bry, L., Silver, P.A., and Gerber, G.K. (2019). Bacteriophages dynamically modulate the gut microbiota and metabolome. Cell Host Microbe *25*, this issue, 803–814.

Marcobal, A., Kashyap, P.C., Nelson, T.A., Aronov, P.A., Donia, M.S., Spormann, A., Fischbach, M.A., and Sonnenburg, J.L. (2013). A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. ISME J. *7*, 1933–1943.

Reyes, A., Wu, M., McNulty, N.P., Rohwer, F.L., and Gordon, J.I. (2013). Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. Proc. Natl. Acad. Sci. USA *110*, 20236–20241.

Sarker, S.A., Sultana, S., Reuteler, G., Moine, D., Descombes, P., Charton, F., Bourdin, G., McCallin, S., Ngom-Bru, C., Neville, T., et al. (2016). Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. EBioMedicine *4*, 124–137.

Shkoporov, A.N., and Hill, C. (2019). Bacteriophages of the human gut: the "known unknown" of the microbiome. Cell Host Microbe *25*, 195–209.

Weiss, M., Denou, E., Bruttin, A., Serra-Moreno, R., Dillmann, M.-L., and Brüssow, H. (2009). In vivo replication of T4 and T7 bacteriophages in germ-free mice colonized with Escherichia coli. Virology *393*, 16–23.

A Tale of Two Mutations: Beginning to Understand the Problems with Egg-Based Influenza Vaccines?

Kanta Subbarao[1,2,*] and Ian Barr[1,2]
[1]The WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia
[2]Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia
*Correspondence: kanta.subbarao@influenzacentre.org
https://doi.org/10.1016/j.chom.2019.05.012

The majority of influenza vaccines are produced in embryonated eggs, but mutations occur as human influenza A(H3N2) viruses adapt to grow in eggs. This can alter virus antigenicity. Wu et al. (2019) reveal that there are two mutually exclusive pathways for egg adaptation, which has potential implications for future egg-based influenza vaccines.

Seasonal influenza viruses circulate globally and influenza has been estimated to cause between 291,243 and 645,832 respiratory associated deaths (4·0-8·8 per 100 000 individuals) annually (Iuliano et al., 2018), with the highest burden seen in people over 75 years of age. Annual vaccination is the most effective measure available to reduce the mortality and morbidity caused by influenza. While there have been some improvements in the effectiveness of influenza vaccines for the elderly in recent years, including high-dose and adjuvanted vaccines, the basic production methods for these vaccines has not altered since the 1940s. The vast majority of influenza vaccines are produced by amplifying influenza viruses present in human respiratory samples in embryonated hens' eggs (EHE). After several passages in EHE and reassortment with high-growth laboratory strains of influenza, a candidate vaccine virus is obtained that has a high virus yield per egg and the desired antigenic characteristics. This adaptation of human influenza viruses to grow in EHE is a critical step in the annual production of influenza vaccine, as large amounts of virus are required in order to make the 600–700 million doses of multivalent influenza vaccines required each year (Schroeder, 2018).

However, the trade-offs for enhanced virus growth in EHE are egg-adaptation mutations that occur in key proteins of the virus, especially the haemagglutinin (HA). Some egg-adaptation mutations have deleterious effects on antigenicity of the HA (Parker et al., 2016), and vaccines made with such viruses can have