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Minireview

Rethinking phage-bacteria-eukaryotic relationships and their influence on human health

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SUMMARY

There is a rapidly growing body of research demonstrating the unique and often surprising mechanisms by which bacteriophages, specialized viruses of bacteria, can influence human health and disease states. This can occur directly by shaping their bacterial host's ecology through top-down pressure or via more indirect routes, including influencing the human body's metabolism or immune system. These microbial interactions can affect health and disease states in both the local environment or by influencing the body's distal organs or systems. Here we provide an update on the current understanding of bacteriophages' influence on human health within the context of tripartite symbioses with their bacterial and human hosts.

INTRODUCTION

The human body is host to a vast spectrum of microorganisms that primarily reside on its surfaces but are also present throughout the body. These microbes include symbiotic bacteria, which form the major constituents of our microbiomes, and the bacterial viruses, commonly referred to as bacteriophages, or simply phages for short. It is important to reiterate that these microbes do not inhabit the body in isolation. Instead, their interplay with the human host and communal microorganisms form integral and conjoined symbioses.

It is becoming increasingly apparent that these bacteria play a crucial role in our health and across a range of pathologies. During "healthy" states, bacterial species may cooperate with the human host in a pleiotropic fashion, contributing to the host's metabolism, immune cell function, and barrier protection among a range of other critical functions. Yet once this fine-tuned microbial balance is disrupted, the system can move toward a "diseased" state, more commonly referred to as a dysbiosis. Gut dysbiosis is increasingly associated with a wide range of intestinal pathologies and growing evidence demonstrates its link with colonic microbiota, their metabolic products, and the human immune system (Carding et al., 2015).

An increasing body of research demonstrates that phages, their bacterial hosts, and the human host form a critical tripartite interplay that can shape both health and disease states. In this article, we highlight recent literature demonstrating the diverse and often surprising ways that phages can influence both their bacterial and mammalian hosts, with a strong focus on tripartite mechanisms. These interactions may occur directly, via phage predation of the bacterial ecological landscape, or via more indirect routes such as influencing metabolism and immune system. Understanding these mechanisms and how they work across the different sites of the body will be necessary for future endeavors using microbial therapeutics to manage our health.

PHAGES IN THE HUMAN GUT

The gut microbiome plays an incredibly profound role in modulating human health. This is driven by a diverse microbial community whose primary function is to aid in our digestion, but which is also intimately involved with the production of diverse metabolites, the prevention of infection, and the maturation of our immune system among a variety of other roles. The next frontier for the gut microbiome is discerning its viral constituents, primarily the bacteriophages (Shkoporov and Hill 2019).

The development of the human gut virome is tightly associated with our own development and aging, moving through clear successions of viral diversity. To investigate how the gut virome is established, Liang et al. (2020) found that infant meconium showed few-to-no viral particles, yet just one-month postpartum, viral load had dramatically increased to $\sim 10^9$ viruses per gram of feces. This raises the question: from where do these phages originate? By combining *in vitro* and bioinformatics techniques, Liang et al. (2020) demonstrated that early bacterial colonizers of the infant gut establish a predominantly temperate phage community with high viral diversity. Following infancy, there was a notable restriction in gut phage richness and abundance along with increased colonization of eukaryotic viruses (albeit at a much lower abundance) (Liang et al., 2020; Gregory et al., 2020). In a complementary approach, Mathieu et al. (2020) collected 648 fecal samples from a cohort of 1-year-old children, followed by the isolation of 900 *Escherichia coli* strains, which were used to compare the infectivity of both temperate and virulent coliphages (refer to Table 1 for definitions of the bacteriophage terminology and life cycles). While

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Table 1. Definitions of bacteriophage terminology and life cycles

Term	Definition
Virulent phage	An obligate lytic bacteriophage that causes a productive infection and the destruction of the host bacterium via lysis.
Lysogenic infection	A reductive bacteriophage infection that involves the incorporation of the phage genome into the host bacterium chromosome.
Temperate phage	A bacteriophage that is capable of giving rise to either lytic or lysogenic infections.
Prophage	A bacteriophage genome as it exists during a lysogenic infection.
Lysogen	A bacterium that contains an un-induced prophage.
Lysogenic conversion	A change in the properties of a bacterial host cell as a result of it harboring a prophage.

over 60% of *E. coli* isolates spontaneously released functional temperate phage, these phages had a narrow host range, with only 8% of phages showing lysis on the tested strains (Mathieu et al., 2020). Comparatively, less than a quarter of fecal virome samples contained virulent coliphages, although those that were found exhibited a much larger host range, lysing up to 68% of the *E. coli* isolates. These studies demonstrate that the infant human gut virome is established by early bacterial colonizers harboring temperate phages with restricted host range (Mathieu et al., 2020; Liang et al., 2020), followed by a slow and steady increase in viral diversity across the rest of our lifespan.

A key colonization event is the appearance of crAssphages in the human gut (Dutilh et al., 2014). These phages are an extensive and ubiquitous family of phages found in >50% of individuals, are globally distributed, infect the human gut symbiont *Bacteroidetes*, and in some instances can constitute upward of 90% of an individual's gut virome (Edwards et al., 2019; Shkoporov et al., 2018). As crAssphages are virulent phages, the colonization of the gut by crAssphages is associated with a shift toward a more virulent-dominant gut phage community (Gregory et al., 2020; Edwards et al., 2019). This was recently highlighted by Shkoporov et al. (2019) in one of the most comprehensive gut microbiome analyses to date, where they performed longitudinal metagenomics of the microbiome and virome from ten healthy adults over a 1-year period (Shkoporov et al., 2019). The gut virome was found to be incredibly diverse, with between 10^8 and 10^{10} viral genome copies per gram of feces and over 39,000 viral genomes detected in total (Shkoporov et al., 2019). Adult gut viromes were dominated by a small number of highly individualized viruses, predominately consisting of virulent crAss-like and *Microviridae* phages that were remarkably stable over the one-year study period. The rest of the virome was much less stable and included low-abundance phages, phages infecting transient microbiota members, and other diet-associated viruses (Shkoporov et al., 2019). Taken together, the human adult gut virome shows increasing viral richness with age, with virulent crAss-like phages gaining a stronger foothold in later years against a background of highly diverse and predominantly transitory phages.

While our understanding of the succession, diversity, and lifestyle of the human gut virome has dramatically increased, much remains to be understood regarding these viral communities' interactions with their bacterial and human hosts. Yet, to understand these viruses, we must first be able to detect them. While this may seem trivial considering the vast abundance of phages in the human gut, which may exceed 10^{12} virus particles, the real challenge emerges when trying to identify viral sequences against a complex, mixed microbial-community background coupled with the lack of universal viral markers (Gregory et al., 2020). Two recent studies have made significant progress in addressing these issues by constructing two complementary gut virome databases. The Gut Virome Database (GVD) from Gregory et al. (2020) reports over 33,000 unique viral populations collated from 32 metagenomic studies, encompassing nearly 2,000 individuals from 16 countries. In a complementary approach, Camarillo-Guerrero et al., 2021 assembled the Gut Phage Database (GPD), consisting of ~142,000 non-redundant viral genomes that were mined from both metagenomes and cultured gut bacterial isolates. Notably, the GPD includes over 40,000 complete and high-quality viral genomes with an average genome length of ~47kb, compared with ~11kb average genome length reported in GVD. Both datasets revealed highly individualized virome dynamics that were tightly linked with bacterial host diversity and human lifestyles. There was clear segregation of gut viromes across urbanized versus rural lifestyles, and non-Western individuals typically contained higher gut viral diversity than their Westernized counterparts (Gregory et al., 2020; Camarillo-Guerrero et al., 2021). However, it is essential to note that there was a strong correlation between an individual's virome and their microbiome, suggesting that virome differences detected were likely driven by gut bacterial hosts. It remains to be determined to what extent phages truly shape the gut microbiome versus simply reflecting the gut's current bacterial diversity (Sutton and Hill 2019), with the reality likely lying somewhere in between.

PHAGES AND THEIR USE AS ANTIMICROBIAL AGENTS

The role of phages and their potential to influence various disease states across the body is now well appreciated (Wahida et al., 2016). Yet, the complexity of the gut environment has raised questions regarding the effectiveness of traditional "phage therapy" approaches involving the use of a single or cocktail of phages with the intent to target and eradicate an enteric pathogen.

One prototypic inflammatory bowel disease (IBD) is Crohn's disease (CD), a chronic and relapsing transmural inflammation of the gut's mucosal layers (Norman et al., 2015). Although the precise cause of CD is still unclear, inflammatory microbiome and virome components have been associated with the onset of symptoms. A recent study by Clooney et al. (2019) performed a comprehensive virome analysis of published healthy and IBD patient datasets that revealed an increased abundance of temperate phages in CD patients. While healthy subjects' viromes were dominated by a virulent core, which is consistent with the current understanding of gut virome succession and dynamics in adults (Shkoporov et al., 2019), this virulent core virome was absent in IBD patients and was replaced by an

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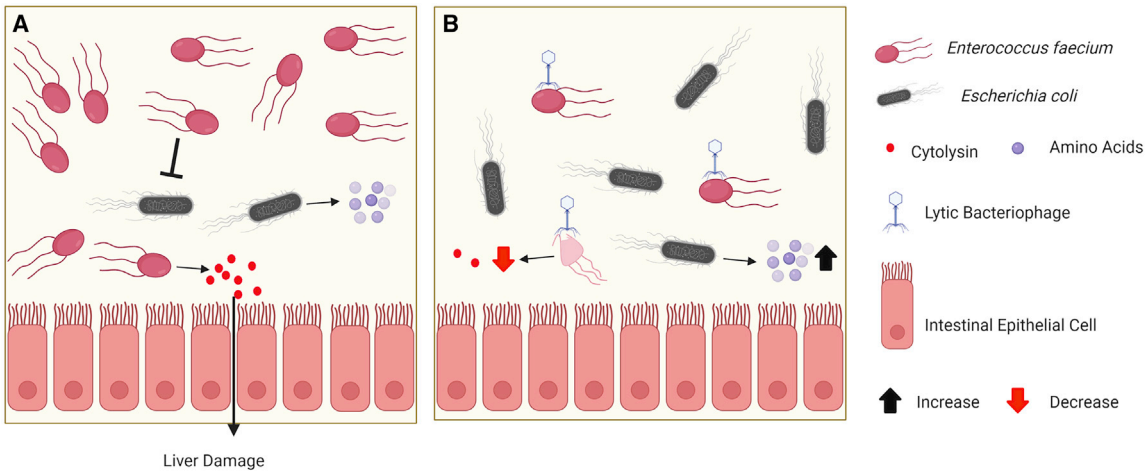

Figure 1. Phage predation in the gut has direct antimicrobial and off-target effects

The intestinal microbiota is known to be associated with various health and disease states through the production of metabolites and toxins, respectively. Phage predation in the gut has been shown not only to mediate direct antimicrobial effects on their respective bacterial hosts via top-down predation, but also to effect metabolite and toxin production in off-target bacterial hosts via inter-bacterial competition and expansion of niche space (Hsu et al., 2019; Duan et al., 2019). In this example, *Enterococcus faecalis* produces the toxin Cytolysin, which translocates to the liver, causing cell death and injury while also mediating inter-bacterial suppression of commensal *Escherichia coli*. Phage-mediated removal of *E. faecalis* both reduces Cytolysin production and alleviates inter-bacterial competition, thereby indirectly increasing *E. coli* abundance and its production of amino acids.

individual-specific shift toward a temperate virome (Clooney et al., 2019). Environmental triggers associated with an inflamed gut were hypothesized to induce temperate phages, which in turn led to a reduction in gut bacterial diversity due to activation of the lytic phage life cycle, and increased the relative abundance of temperate phage virions in the gut of IBD patients. From the bacterial perspective, one etiological group of pathogens found to be abnormally predominant on the ileal mucosa of CD patients are the adherent invasive *Escherichia coli* (AIEC) (Galtier et al., 2017; Small et al., 2013). In a targeted approach to reduce AIEC in the gut, Galtier et al. (2017) found that administration of three virulent phages targeting AIEC to transgenic mice expressing the human receptor for AIEC significantly reduced AIEC in both the feces and the adherent flora of intestinal sections in an antibiotic-induced model of dysbiosis. Importantly, a single dose of the three-phage cocktail was sufficient to both reduce AIEC colonization and prevent the progression of intestinal colitis symptoms over a two-week period (Galtier et al., 2017).

This phage-targeted removal of pathogenic bacteria within the gut is not restricted to gastrointestinal diseases and could be used to prevent or treat diseases outside of the gut. A recent example of this approach was shown by Duan et al. (2019), who found a positive correlation between alcoholic hepatitis and gastrointestinal colonization with *Enterococcus faecalis* strains that produced the compound cytolysin (Figure 1). Cytolysin production in the gut was followed by its translocation to the liver, which led to increased liver injury and hepatocyte death, leading to decreased liver function and increased mortality. Using a humanized mouse model, the authors administrated phages targeting the cytolytic *E. faecalis* strains, which led to decreased gut colonization followed by a reduction in cytolysin production and abolished ethanol-induced liver damage, steatosis, and inflammation (Duan et al., 2019). It is worth noting that in this context, phages precisely targeting gastrointestinal pathogens resulted in abolished injury to tissues outside of the gastrointestinal tract, demonstrating that phage predation of bacteria in the gut can have a profound impact on the mammalian host.

The targeted removal of specific bacterial species or strains from the gut has long been considered a pivotal milestone in biological therapeutics. A recent report by Hsu et al. (2019) investigated the use of lytic phages targeting bacterial hosts within a defined ten-member gut bacterial consortia within a humanized mouse model. Surprisingly, phage predation not only reduced target bacterial hosts' relative abundance within the gut but also caused shifts in non-target bacteria through interbacterial interactions, resulting in blooms and attritions of certain species (Hsu et al., 2019). These cascading phage-mediated changes to the microbiome also modulated the gut metabolome, with reduced production of neurotransmitters (tryptamine and tyramine) and increased yield of certain amino acids (serine and threonine). Although the results of phage-mediated modulation of gut metabolites are still preliminary, this study demonstrates that phage predation of their target species in the gut is associated with shifts in the wider microbial community linked with the production of specific metabolic products (Figure 1; Hsu et al., 2019). It remains to be determined how far-reaching and predictable these implications will be once extended beyond a ten-member microbiome community.

PHAGE NANOTECHNOLOGIES, ENZYMES, AND ATTENUATORS OF VIRULENCE

The unique specificity of phages has enabled the development of phage-guided nanotechnologies for the precise delivery of compounds and drugs with a range of therapeutic applications. Using this approach, Zheng et al. (2019) developed a phage-guided, biotic-abiotic, hybrid nanomaterial that targeted the pro-tumoral bacterium *Fusobacterium nucleatum*, allowing for

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precise delivery of chemotherapeutics against colorectal cancer (CRC). By isolating a phage targeting *F. nucleatum*, the researchers used azide modifications to covalently link dextran nanoparticles, which were loaded with a first-line anticancer drug, to the phage capsid. When administered, the modified phages were able to target and eliminate intra-tumoral *F. nucleatum* and in the process precisely deliver anticancer drugs to the CRC tumors, thereby reducing the toxic side effects of conventional chemotherapy (Zheng et al., 2019). Phage derivatives, which include lysins and depolymerases, are also of broad interest. Yang et al. (2018) reported the use of a recombinant phage-derived protein isolated from the cell-wall binding domain of phage lysin PlyV12, termed V12CBD, to attenuate virulent, methicillin-resistant *Staphylococcus aureus* (MRSA) and enhance the immune response in a mouse model. After binding to MRSA, the recombinant V12CBD protein led to a downregulation of multiple virulence factors, which subsequently led to a reduction in MRSA adhesion and invasion of epithelial cells (Yang et al., 2018). More significantly, V12CBD could directly activate macrophages through the NF-κB pathway, leading to enhanced macrophage phagocytosis of MRSA and reduced inflammatory injury in organs within a mouse model of sepsis (Yang et al., 2018). Further findings will undoubtedly provide greater insight into the diverse mechanisms by which phages and their derivatives can shape not only their bacterial hosts, but their mammalian hosts through both direct and in-direct mechanisms.

A well-recognized limitation of phage-mediated removal of bacterial species from the body is the inevitable emergence of bacterial phage resistance. In the face of phage predation, the bacterial hosts are often forced into a trade-off between becoming resistant against lytic phage predation and the fitness costs associated with maintaining this resistance. For example, phage predation of *Listeria monocytogenes*, an important intracellular pathogen, requires galactosylated teichoic acids associated with its cell walls to initiate infection (Sumrall et al., 2019). Phage resistance in this pathogen emerged through the loss of galactosylated teichoic acids, which were indirectly required for host cell invasion, thereby attenuating this pathogen. In another example, phages infecting the top-priority pathogen *Acinetobacter baumannii*, were found to mediate infection via the bacterial capsule (Gordillo Altamirano et al., 2021). Phage-resistant isolates had defective capsule production, which led to the resensitization to human complement, beta-lactam antibiotics, and alternative phages, with the capsule-deficient mutants found to be less fit in a murine model of sepsis. While these fitness trade-offs are often caused by loss-of-function mutations in phage receptors, they can also be mediated by a range of other mechanisms, including epigenetics. The gastrointestinal pathogen *Salmonella enterica* encodes an opvAB operon, which undergoes phase variation to produce two subpopulations: opvAB^{ON} and opvAB^{OFF} (Cota et al., 2015). The opvAB^{OFF} subpopulation is virulent but sensitive to phages that use the O-antigen as a receptor, whereas the opvAB^{ON} subpopulation is avirulent but phage resistant. In the presence of phages that use the O-antigen as a receptor, only the avirulent subpopulation survived (Cota et al., 2015). However, the phase variation allowed for the rapid reversion to the virulent opvAB^{OFF} subpopulation as soon as the phage pressure was removed, thereby providing only a transient fitness cost. In a lysogenic example, Li et al. (2020) compared isogenic MRSA strains from persistent endovascular infections with and without a clinically derived prophage, demonstrating that the strains harboring the prophage were associated with persistent infections (Li et al., 2020). The prophage-containing strain presented phenotypes associated with clinical persistence, including earlier activation of global gene regulators, higher purine biosynthesis, higher growth rates, lower vancomycin susceptibility, stronger hemolysis, and biofilm formation. As a result, lysogenized strains showed no clinical response to vancomycin treatments and had significantly higher bacterial densities in all target tissues within an endocarditis model (Li et al., 2020). Finally, Secor et al. (2015) found that the extracellular matrix produced by *Pseudomonas aeruginosa* isolates harboring Pf prophages were able form a liquid crystal matrix via entropic interactions between biofilm polymers and the filamentous Pf phages. These long, negatively charged, filamentous virions enhanced the biofilm viscosity, conferring increased adhesion and tolerance to desiccation and antibiotics (Secor et al., 2015). Within the lung environment, Pf phages assisted bacterial adhesion to lung mucus, further disseminating *P. aeruginosa* bacterial host across the lung environment and promoting chronic airway infections, which were particularly evident in Cystic Fibrosis (CF) patients (Secor et al., 2016). Tarafder et al. (2020) went on to demonstrate that these phage liquid crystalline biofilms formed by Pf phage not only encapsulated the *P. aeruginosa* bacterial hosts but provided increased resistance to a range of antibiotics (Tarafder et al., 2020). By understanding theses mechanisms of phage infectivity, how their bacterial hosts respond to phage pressure, and the interplay within the mammalian host, we will be better placed to develop and apply phage-based therapeutics.

PHAGES AND THE MAMMALIAN IMMUNE SYSTEM

While phages are not classical pathogens, they are able to bypass mammalian physical barriers, and it has become increasingly clear that they can influence the mammalian immune system (Van Belleghem et al., 2018; Barr 2017; Nguyen et al., 2017). Phage interactions with the mammalian immune system can occur both indirectly, through prokaryotic intermediaries, or directly by phage internalization and receptor recognition by mammalian cells.

The mammalian innate immune system is equipped with a wide variety of pathogen recognition receptors (PPRs) capable of recognizing conserved molecular epitopes found in both bacteria and viruses. Importantly, microbially-derived nucleic acids with distinct architectures are recognized as foreign and can elicit strong immune responses (Tan et al., 2018; Roers et al., 2016). Compelling evidence of phage modulation of innate immune responses was recently demonstrated by Sweere et al. (2019) using the filamentous *P. aeruginosa* phage Pf. As described above, Pf is a filamentous phage with a ssDNA genome that produces a liquid crystalline matrix surrounding its *P. aeruginosa* bacterial host cells, promoting biofilm formation and chronic airway infections (Secor et al., 2015, 2016). Extending these findings, it was demonstrated that wounds infected with Pf-positive *P. aeruginosa* strains were more commonly associated with chronic, nonhealing wounds than strains without

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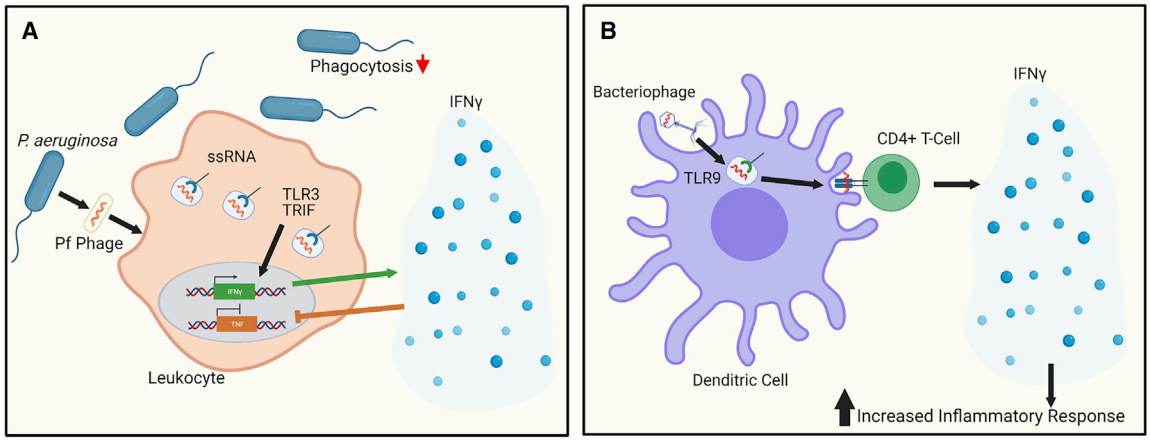

Figure 2. Phages and their components can directly stimulate the mammalian immune response

(A) *Pseudomonas aeruginosa* harboring the lysogenic Pf phage produce the filamentous phage in a wound environment. Pf phage is subsequently internalized by leukocytes with phage ssRNA triggering TLR-3 in a TRIF-dependent manner, resulting in the production of type I interferon, which subsequently inhibits TNF production. With reduced TNF in the wound environment, phagocytosis of *P. aeruginosa* is reduced, leading to persistent and chronic wound infections (Sweere et al., 2019).

(B) Increased phage abundances in the gut result in increased phage sampling and uptake by dendritic cells. Internalized phages triggered TLR-9 with phage epitopes presented to CD4+ T Cells leading to the production of IFN-γ and the activation of inflammatory responses in the gut (Gogokhia et al., 2019).

Pf phages (Sweere et al., 2019). Using both human and murine leukocytes, Sweere et al. (2019) demonstrated that Pf phages were endocytosed by immune cells leading to the synthesis of phage RNA, which in turn activated the antiviral PRR Toll-like receptor 3 (TLR-3) (Sweere et al., 2019). This activation led to TRIF-dependent type I interferon production that subsequently inhibited the production of tumor necrosis factor (TNF) in the wound environment (Figure 2A). With TNF production inhibited, phagocytosis and the subsequent clearance of Pf-positive *P. aeruginosa* from the wound environment was abrogated, leading to *P. aeruginosa* persistence and chronic wound infections (Sweere et al., 2019). A similar study by Jahn et al. (2019) investigated the presence of Ankyrin repeat domains (ANKs) within phage populations associated with marine sponges but also found within phage populations of the human body, including the oral cavity and the gut. When infecting their host bacterial cells, Ankyrin-containing phages induced the secretion of ANK proteins, which increased bacterial survival when in the presence of murine macrophages (Jahn et al., 2019). Macrophage incubation with ANK-producing *E. coli* cells led to the reduction of pro-inflammatory cytokines TNF-α, *Cxcl1*, and *Ifn1*, which subsequently resulted in decreased phagocytosis of the bacterial cells. In summary, viral innate immune responses were altered by a symbiosis between phages and their bacterial hosts, resulting in increased persistence of the bacterial and phage within the body.

The presence of whole phage particles and their components, which may include genomic DNA or RNA, proteinaceous capsids, and residual bacterial products such as lipopolysaccharides (LPS) can all directly stimulate the mammalian immune response. This was recently demonstrated by Gogokhia et al. (2019) in a study that began by investigating the use of a phage cocktail for the targeted removal of the intestinal pathogen AIEC, which is associated with colorectal cancer. The continuous oral administration of the phage cocktail to mice led to a reduction in AIEC in the intestine, the downregulation of genes associated with tumor growth and metastasis, and protection from bacteria-exacerbated colorectal cancer (Gogokhia et al., 2019). Yet upon analysis of gene expression data, a large set of immune pathways were found to be upregulated in the phage-treated versus control mice groups. It was then demonstrated that the increased abundance of phage in the gut led to the production of IFN-γ through a TLR-9 dependent pathway (Gogokhia et al., 2019), likely via dendritic cell (DC) sampling of intestinal phages; unmethylated phage DNA was detected by TLR-9 within DC endosomes, which were subsequently presented to CD4+ T cells (Figure 2B). It should be noted, however, that other IFN-γ activation pathways are possible (Gogokhia et al., 2019). Finally, extending these findings to a murine model of inflammatory bowel disease (IBD), a cocktail of *Caudovirales* phages was given to wild-type, *TLR-9*$^{-/-}$ and *IFN-γ*$^{-/-}$ mice, and inflammatory signatures were investigated. While disease severity worsened upon phage cocktail administration in wild-type mice, both the *TLR-9*$^{-/-}$ and *IFN-γ*$^{-/-}$ mice were protected from phage-enhanced colitis and had reduced inflammatory response (Gogokhia et al., 2019). These results demonstrate mechanistically how gastrointestinal phages can influence the intestinal immune response.

MAMMALIAN IMMUNE SYSTEM INFLUENCES BACTERIAL AND PHAGE SYMBIONTS

As phages and their bacterial hosts can affect the mammalian immune system, the mammalian host's immune response can in turn influence both bacterial and phage symbionts. Using a murine *Salmonella* Typhimurium diarrhea model, Diard et al. (2017), demonstrated that gut inflammation elicits the induction of prophages leading to increased lysogenic conversion and progression of enteric disease. *Salmonella* Typhimurium genomes commonly harbor prophages, some of which encode virulence genes that enhance epithelial invasion and exacerbate enteropathy of their bacterial hosts. To study the lysogenic conversion dynamics

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in vivo, two Typhimurium strains were used: a donor strain containing the prophage SpoEø, which encodes for a virulence factor, and a non-lysogen recipient strain (Diard et al., 2017). Both donor and recipient strains colonized the gut equally, yet following co-infection, the recipient lysogen frequency steadily increased; after three days post-infection, lysogens outnumbered non-lysogenic strains in more than half of the animals. *In vitro*, most prophages are activated via the stress-induced SOS response of the bacterial host; *in vivo*, much less is known about prophage activation. The authors then went on to demonstrate that *Salmonella* Typhimurium strains elicit intestinal inflammation, with the inflammatory by-products subsequently triggering the bacterial SOS response, leading to increased SpoEø induction and increased lysogenic conversion of susceptible strains in the gut (Diard et al., 2017). This demonstrated that the mammalian hosts' innate immune response was a key factor affecting phage-bacteria dynamics and the progression of disease.

In an attempt to prevent gut inflammation and the progression of disease, Diard et al. (2017) treated mice using an oral vaccine, which efficiently protected against *Salmonella* tissue invasion and inflammation without reducing total *Salmonella* Typhimurium luminal loads. Importantly, there was a significant decrease in both disease progression and lysogenic conversion following vaccination (Diard et al., 2017). This was notable, as while the mammalian innate immune response was proinflammatory, leading to increased phage dissemination and disease progression, vaccination and the subsequent Ig-A mediated adaptive immune response slowed the disease progression (Diard et al., 2017). This concept of phage immunization to prevent disease was similarly explored by Sweere et al. (2019) in their Pf phage-*P. aeruginosa* wound colonization model. Here, mice were immunized using a peptide from the major coat protein of Pf phage to generate humoral immunity, with vaccination reducing the incidence of *P. aeruginosa* wound infections by up to half (Sweere et al., 2019). These findings were then extended through the use of monoclonal antibodies (mABs), which were generated against the same Pf phage coat protein and applied to already colonized wounds, leading to a significant reduction in *P. aeruginosa* burden via enhanced phagocytosis. By uncovering the pathogenic roles phages play in bacterial diseases, it may be feasible to develop further immunization strategies, targeting conserved phage epitopes and thereby preventing disease progression.

It is important to highlight that the mammalian adaptive immune response is active against the gut microbiota, including phages, with certain microbial antigens able to stimulate memory T cells (Tanoue et al., 2019). Further, memory responses by IFN-γ-secreting CD4+ and CD8+ T cells specific for gut microbes are known to sporadically cross-react with tumor-associated antigens, contributing to anti-tumor immune response (Routy et al., 2018). A recent study by Fluckiger et al. (2020) extended this concept to demonstrate cross-reactivity between a tumor major histocompatibility complex (MHC) class-I-restricted antigen and an enterococcal phage protein. The authors found that the commensal bacterium *Enterococcus hirae* harbored a prophage that encoded an MHC class-I-restricted antigen in its tape measure protein (TMP) (Fluckiger et al., 2020). Mice colonized by *E. hirae* containing this prophage were able to mount a TMP-specific CD8+ T cell response upon treatment with a chemotherapeutic drug that induces *E. hirae* translocation from the gut to the spleen. A single, dominant epitope within the phage tape measure protein (termed TMP1) was found and subsequently shown to mimic the oncogenic peptide PSMB4. Administration of the TMP1 peptides, either directly to DCs or expressed on the surface of *E. coli*, was sufficient at restraining tumor growth and led to improved immunotherapy. Finally, returning to the gut microbial ecosystem, the authors demonstrated that the TMP1-peptide-encoding prophage was able to lysogenize the commensal microbe *Enterococcus gallinarum* within the gut, highlighting the potential broad dissemination of these phages (Fluckiger et al., 2020). Given the enormous diversity of gut microbiome and that the gut virome appears to be dominated by a small number of highly individualized yet stable phages (Shkoporov et al., 2019), there likely exist many other microbial antigens capable of stimulating an adaptive immune response and affecting human health and disease states.

CONCLUDING REMARKS

Our understanding of the diverse and often surprising ways that phages affect both their bacterial and mammalian hosts continues to multiply as we look deeper into these tripartite systems. Our current understanding of the gut virome is akin to an infant's gut viral diversity; immature, but sure to develop over time. Sequencing technologies and associated bioinformatic analyses are rapidly advancing and have led to important discoveries of crAss-like phages and identification of predominant gut viral lifestyles. Looking over the horizon, we need to marry the deep sequencing of patient microbiomes with wet-lab studies of gut phage biology to advance our understanding of and influence on this ecosystem. The once-fringe field of phage therapy has rapidly moved to the forefront in our war against antibiotic-resistant infections. However, we must be careful not to fixate only on these direct antimicrobial effects and consider the broader implications of phage predation on the microbiome, metabolome, and immune system. Finally, the tripartite interplay between phages, bacteria, and the mammalian immune system requires multidisciplinary research efforts to unravel. Recent discoveries have highlighted the complexity of innate and adaptive immune responses toward phages, triggering both pro- and anti-inflammatory responses that may contribute to health and disease states. We are arguably in one of the most exciting times to be working in the field of phage biology in its over 100-year history.

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DECLARATION OF INTERESTS

Jeremy J. Barr is a member of the Centre to Impact AMR, Monash University, Clayton, Victoria, Australia.

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