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Perspective

The dynamic interplay of bacteriophage, bacteria and the mammalian host during phage therapy

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SUMMARY

For decades, biomedically centered studies of bacteria have focused on mechanistic drivers of disease in their mammalian hosts. Likewise, molecular studies of bacteriophage have centered on understanding mechanisms by which bacteriophage exploit the intracellular environment of their bacterial hosts. These binary interactions – bacteriophage infect bacteria and bacteria infect eukaryotic hosts – have remained largely separate lines of inquiry. However, recent evidence demonstrates how tripartite interactions between bacteriophage, bacteria and the eukaryotic host shape the dynamics and fate of each component. In this perspective, we provide an overview of different ways in which bacteriophage ecology modulates bacterial infections along a spectrum of positive to negative impacts on a mammalian host. We also examine how coevolutionary processes over longer timescales may change the valence of these interactions. We argue that anticipating both ecological and evolutionary dynamics is key to understand and control tripartite interactions and ultimately to the success or failure of phage therapy.

INTRODUCTION

Bacteriophage (phage, i.e., viruses that infect bacteria) are the most abundant organisms in the biosphere. Phage abundances are 10-fold greater than bacterial abundances on average,[1] with variability in the ratio of phage to bacterial abundances ranging from 1 to 100 across different environments.[2] Phage reproduce along a continuum between a lytic to lysogenic life cycle.[3,4] Virulent phage replicate through a lytic cycle, in which phage genetic material is injected into the bacterial cell, the cellular machinery replicates viral genomes and catalyzes the self-assembly of viral particles, leading to cell lysis and the release of infectious virions into the environment. In contrast, temperate phage can initiate either lysis or lysogenic pathways. In lysogeny, phage genetic material is integrated into the bacterial chromosome, whereby the integrated phage genome is referred to as a prophage. The prophage is replicated together with the bacterial chromosome during cell division. Temperate phage can remain in this state or switch to a lytic cycle via a process called induction.[5]

In the environment, phages play a major role in shaping free-living bacterial populations: as agents of mortality, modulating the metabolic features of infected cells, and imposing evolutionary pressures on their bacterial hosts.[6] Phages affect the composition of bacterial populations, alter competition among bacterial strains, maintain bacterial diversity, and mediate horizontal gene transfer.[7–9] Furthermore, phages that initiate a lysogenic cycle can affect bacterial evolution through processes like lysogenic conversion, transduction, and host gene disruption when they integrate into the bacterial genome and are retained as prophage.[10,11]

In a therapeutic context, phages also infect bacteria which in turn can colonize and reproduce within eukaryotic organisms. Bacterial interactions with their eukaryotic hosts can be mutualistic as is the case for human gut microbiota. Shifts or disruptions in the composition of the beneficial microbiome can lead to disease or dysfunction in the eukaryotic host.[12,13] Likewise, associative studies suggest a link between human health and phage-gut community composition.[13] In contrast, some bacteria are antagonistic to eukaryotic hosts, because of cellular damage caused by microbial colonization and growth and/or via damage induced by the host immune response.[14] The trajectory of a bacterial pathogen may be limited by the host immune response, competition with microbiome and virome community members, and via treatments – including the therapeutic application of phage.

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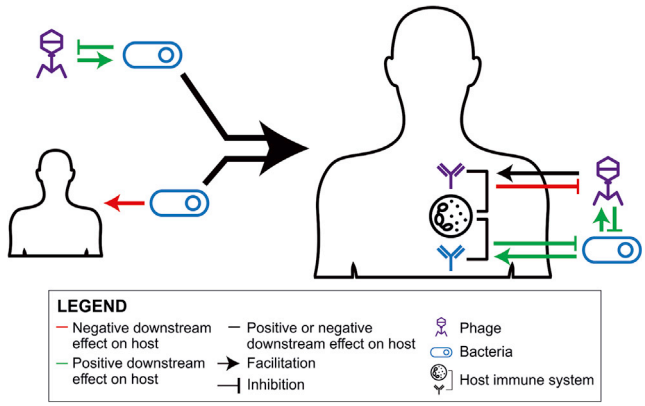

Figure 1. Schematic overview of binary and tripartite interactions between phage, bacteria and the mammalian host

For decades, studies have focused on the impact of phage onbacterial hosts and on the impact of bacterial pathogenson mammalian hosts. The presence of all the three entities together modifies their interactions, and produces unique infection dynamics in which phage can either have a positive or a negative downstream effect on the eukaryotic host.

In this perspective, we provide an overview on how phages modulate bacterial infections across cellular to population scales within a mammalian host, in ways that can be beneficial or deleterious to the host (Figure 1). Focusing primarily on phage therapy, we first address the combined role of virulent phage and the mammalian immune system in clearing bacterial infections, followed by an examination of the potential use of temperate phage to eliminate target pathogens. Finally, we present and discuss evolutionary processes that can change the nature of phage-bacteria interactions within mammalian hosts and impact the outcome of phage therapy.

VIRULENT PHAGE AND THE SYNERGISTIC TREATMENT OF BACTERIAL INFECTIONS IN EUKARYOTIC HOSTS

Phage therapeutic treatment of bacterial infections in human is approximately a century old.[15,16] Increases in the proliferation of antibiotic resistant pathogens and improvements in the availability of purified virulent phage has increased the interest in using phage as therapeutics.[17,18] The governing therapeutic principle is that self-replicating virulent phage can efficiently encounter and lyse a bacterial pathogen with minimal disruption to the beneficial components of the microbiome.[19] The potential use of phage therapy has accelerated over the past few decades, spanning both *in vitro* and *in vivo* settings.[19–22] Consequently, phage therapy is increasingly being used in compassionate treatment,[23–26] in parallel, clinical trials are set to address efficacy on larger populations of patients.[27–30]

Several studies have integrated *in vitro* and *in vivo* experiments, as well as *in silico* computational models into the design and assessment of phage therapy. These studies have advanced the quantitative understanding of the factors affecting phage therapy outcomes, such as the bacterial strain, infected tissue, and chosen delivery route for the therapeutic phage.[31–34] After delivery to the host, therapeutic phage can adsorb to, infect, and lyse bacterial pathogens and then subsequently interact – whether directly or indirectly – with resident phage, resident bacteria, and the eukaryotic host immune system. As a result, the success or failure of phage therapy should be assessed as part of a complex, tripartite system involving phage, bacteria, and immune defense (Figure 1).[35]

Eukaryotic hosts can interact directly with bacteria and phage, e.g., via immune recognition and response. Both the innate and adaptive immune systems recognize bacterial pathogens, activating signaling pathways that trigger inflammatory and immune responses, potentially clearing the pathogen.[36] The eukaryotic immune system has an important role in shaping phage therapy success.[37,38] On the one-hand, recognition and clearance of phage by the eukaryotic host may limit the effectiveness of phage therapy.[39] In contrast, when phage do not elicit a direct immune response, then the combined effect of phage and the immune response may be more potent than the impact of either phage or the mammalian immune defense system alone (Figures 2A and 2B). We explore both facets next.

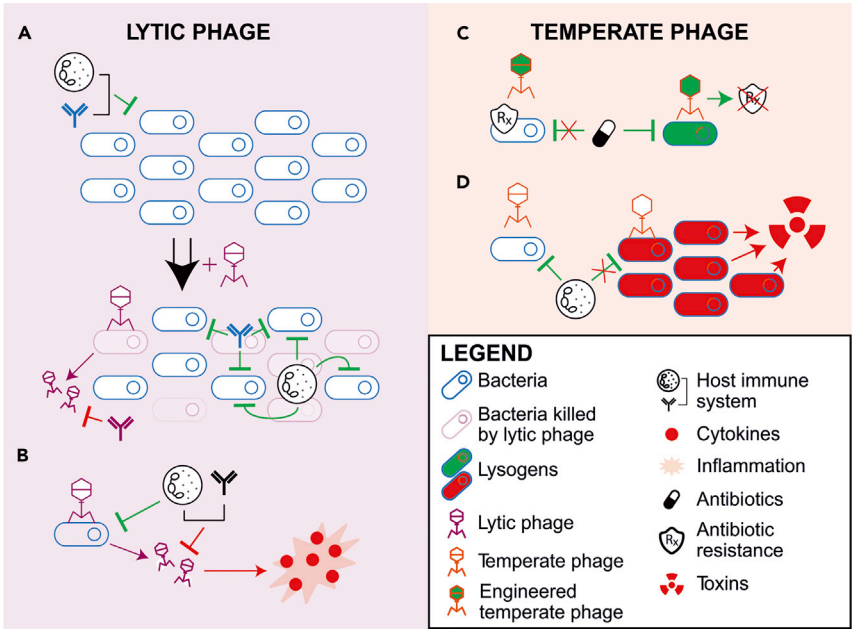

Figure 2. Positive and negative impacts of phage during bacterial infections of eukaryotic hosts

(A) At the population scale, cellular interactions between phage, bacteria and immune cells drive infection dynamics in a structured fashion depending on densities. Lytic phage, while potentially triggering immune response against themselves (red interaction, negative for the host), can progressively drive down bacterial densities so that the remaining bacteria can be killed by immune cells (green interaction, positive for the host). The infection is eventually cleared by virtue of phage-immune synergy.

(B) During an infection, phage infet and lyse bacteria, while adaptive and innate immune components are recruited to clear bacteria. Phage can enhance the innate immune system in ways that are beneficial for the host, for instance when stimulating components that help control the infection, or in ways that are negative if phage trigger a phage-specific inflammatory response.

(C) Temperate phage can be engineered to deliver CRISPR-Cas components to suppress antibiotic resistance genes of the bacteria.

(D) Temperate phage could also have negative effects when they integrate in the bacterial genome as prophage, conferring virulence traits to the bacteria, such as the ability to escape immune cells or toxin release.

First, phage interact with innate immune cells via multiple mechanisms. Accumulation of phage in the spleen and liver point to phagocytosis by immune cells and its role in phage neutralization within the eukaryotic host.[40] Phage capsid proteins can be recognized by the immune system[41] and phage DNA and RNA can be recognized by specific pattern recognition receptors (PRRs). Recognition by the innate immune system can, in some cases, elicit an immune response that is phage specific and differs from the response activated by recognition of bacteria. For example, phage of *Lactobacillus*, *Escherichia*, and *Bacteroides* found in the gut of mice may stimulate production of the pro-inflammatory cytokine IFN-γ via the nucleotide-sensing receptor TLR9. This phage-mediated inflammation has been linked to aggravation of intestinal inflammation and colitis.[42] The extent of immune activation differs depending on phage type and the extent of DNA/RNA synthesis.[43,44] In contrast, many phage appear to be well tolerated by the immune system and elicit no inflammatory response, as in the case of phage T7.[45] Furthermore, some phage might even have anti-inflammatory characteristics; co-incubation of phage with peripheral blood monocytes has been demonstrated to upregulate anti-inflammatory markers such as suppressor of cytokine signaling 3 (SOSC3), IL-1 receptor antagonist (IL1RN), and IL-6.[46] Finally, in addition to interacting with the innate immune system, processing of phage by leukocytes may promote the formation of phage-neutralizing antibodies, increasing phage clearance.[40] Increased phage clearance by the adaptive immune system can hamper therapeutic success especially if the production of antibodies increases with repeated administration of phage.[43]

Next, the potential for synergistic interactions between phage and the immune system (Figure 2A) were evaluated in an *in vivo* study of phage treatment of acute pneumonia caused by multi-drug resistant *Pseudomonas aeruginosa* (MDR Pa). In this system, MDR Pa causes acute pneumonia and the death of mice

within 24–48 h after exposure. In WT mice with competent immune systems, application of phages at ratios at least 1-fold higher leads to nearly 100% curative success.[47] Another study combining *in vivo* and *in vitro* experiments confirmed the positive outcome of phage treatment of *P. aeruginosa* lung infections in mice across different phage treatment timings. The authors found that phage act in synergy with the complement immune system.[48] But, such outcomes are not inevitable. Instead, phage therapy is unsuccessful in immune deficient mice that have neutrophil or signaling deficiencies.[49] In immune deficient mice, the proliferation of phage-resistant mutants leads to therapeutic failure – raising questions of how phage therapy works even when the immune system is competent.

Population models of phage-bacteria-immune dynamics can explain both how phage therapy is effective in immune competent mice and how phage therapy fails when the immune system is deficient.[49,50] As proposed, the eco-evolutionary model includes the impacts of phage infection and lysis, mutation of bacteria to phage-resistance, as well as the clearance of pathogens by the immune defense. In this tripartite system, phage can drive susceptible bacterial densities to low levels. A competent immune system can eliminate both the remaining phage-susceptible and phage-resistant bacterial subpopulations. In contrast, when the immune system is deficient, then phage resistant mutants can proliferate, uncontrolled by either phage or the immune defense. As a result, phage therapy works via 'immunophage synergy' (Figure 2A). This synergy is a population-level feature of the system as a whole, whose quantitative time scales reflect the spatially distributed processes occurring in the *in vivo* context.

Assessing the interactions between phage, bacteria, and the immune system will require evaluating tripartite interactions across scales. For example, the potential for phage phagocytosis by immune cells and phage-mediated anti-inflammatory processes[39,40,46] suggests ways in which population level models will need to include additional mechanistic interactions taking place at cellular scales. For example, mucus in epithelial tissues can slow down phage diffusion; this could elevate phage densities within mucus that limits bacterial colonization in the first place.[51–53] Yet in a therapeutic context, phage must diffuse through the targeted tissue to encounter infecting bacteria. Therapeutic phage binding to mucin may potentially decrease phage diffusion rates and therapeutic efficacy.[54] Evaluating the clinical relevance of immunophage synergy will require incorporating spatially extended models, as a means to account for and ultimately control pharmacokinetics and dynamics[55] – an ongoing joint challenge for experiment and theory alike.

THE DOUBLE-EDGED SWORD OF TEMPERATE PHAGE AND THEIR ROLE IN BACTERIAL INFECTIONS

Phage therapy has been predominantly focused on using virulent phage rather than temperate phage. Temperate phage have largely been avoided in therapeutic contexts because integration of a prophage into the cellular genome does not lead to the immediate death of a target bacterium (Figures 2C and 2D). Other major hindrances are the fear of spread of phage encoded virulence factors between bacterial communities via lysogenic conversion, and the potential horizontal gene transfer of bacterial virulence factors by temperate phage during packaging of viral genomes into capsids.[56] Such inadvertent packaging can occur with virulent phage as well; however, temperate phage can integrate into the infected cell's chromosome, spreading virulence factors between bacteria.[57]

Despite reservations, there is increasing interest in using temperate phage as therapeutics.[58,59] Part of this increased interest is facilitated by improvements in computational-based identification of temperate phage, e.g., by searching whole bacterial genomes for prophage hallmark genes such as integrases. Identifying phage within bacterial genomes and then trying to induce and isolate them circumvents the problem of finding wild phage that can adsorb and infect a target bacterium. Such a strategy may be particularly useful in identifying putative phage therapy candidates for pathogenic bacteria for which isolating lytic phage has proven to be difficult e.g., *Clostridium difficile*.[58,59] Another reason for the increased interest in temperate phage is the potential to leverage lysogenic integration into the bacterial chromosome – the very issue underlying earlier reservations – to disrupt cellular function or to change the bacterial genome. Re-engineering temperate phage has been accelerated by advances in next-generation sequencing and synthetic biology, potentially expanding the relevance of temperate phage-based therapy across a wide range of bacterial pathogens and systems.

As previously mentioned, lysogeny is widely distributed in nature; approximately half of all sequenced bacterial genomes contain one or more prophage.[5,60,61] Lysogeny is common in many pathogens, such

as *P. aeruginosa*, *Vibrio cholera*, *Salmonella enterica*, pathogenic strains of *Escherichia coli*.[61,62] Prophage genes are often organized in autonomous gene expression cassettes called morons, which can be expressed while the prophage remains silent or during induction to a lytic cycle.[10,63] Many of these morons can affect the pathogenicity of bacteria during infection of the eukaryotic host; this ranges from enhancing bacterial virulence to turning nonpathogenic bacteria to pathogenic variants (Figure 2D).[11] Examples of conversion to pathogenicity include *E. coli* strain O157:H7 that acquired two prophages that produce the Shiga toxin, and *V. cholerae*'s CTXφ that produces the cholera toxin.[5,7,10]

Phage enhancement of bacterial virulence is context dependent and often involves interference with the eukaryotic immune response (Figure 2D). Phage-induced interference can take the form of re-directing the immune response, like in the case of *P. aeruginosa* Pf4 phage. In this case, Pf4 phage's RNA is recognized by Toll-like receptor 3, inducing a maladaptive antiviral response, overriding the proper antibacterial response, via inhibition of tumor necrosis factor (TNF) and suppression of phagocytosis, both of which are required for bacterial clearance.[64] Interference with the immune system also occurs through reduction of the inflammatory response: for example, the *S. enterica* Fels-1 phage encodes a gene, which induces production of the anti-inflammatory cytokine IL-10.[65] Of interest, this phage also encodes for a superoxide dismutase, hypothesized to protect bacteria from oxygen radicals produced by macrophage.[66] Another anti-immune function is the direct lysing of leukocytes: *Staphylococcus aureus* phage PVL encodes for a leukocidin, made up from two gene (*lukS* and *lukF*) products. The two toxins assemble into pore-forming transmembrane complexes and lyse human polymorphonuclear leukocytes.[67,68]

Overcoming the potential for temperate phage to inadvertently enhance bacterial toxicity to a human host is central to the development of therapeutic treatment using temperate phage. The use of temperate phage as therapeutics has leveraged two main approaches, both involving genetic modifications of the phage: (1) Engineering the temperate phage to be exclusively lytic by removing the integration genes; (2) using a genetically modified phage as a delivery system by retaining the temperate nature of the phage and using phage integration into the bacterial genome to modify bacterial gene content (Figure 2C). We provide examples of both applications below.

First, de-lysogenization has been shown to be effective in converting a temperate phage into a virulent phage, e.g., removing the genomic module responsible for lysogeny in *Enterococcus faecalis* lysogenic phage φEF11[69] and in Listeria phage B025.[70] The mutant phage were unable of initiating the lysogenic cycle, they showed an increased lytic ability, and remarkably exhibited a reduced selection for phage resistance in the host.[69,70] Turning temperate phage into virulent phage can be a useful tool to extend the repertoire of phage available for therapeutic uses.[71] In principle, the host range of naturally occurring temperate phage may be different than that of virulent phage – whether because of differences in surface-associated factors on the phage particle or intracellular-associated regulation encoded in the temperate phage.

Second, the use of temperate phage to transfer genetic elements into the bacterial cell presents a broad landscape for innovation in phage therapy. For example, phage could be engineered to express proteins or non-coding RNA that will interfere with a cell's essential metabolic pathways, leading to a spectrum of outcomes including reduced growth to cell death. Such targeting by reengineered phage can lead to a bacteriostatic therapy, without inducing cell lysis, thus avoiding the potential harmful release of endotoxins.[14,59] Other options for temperate phage engineering include directly targeting programmed cell death regulators, or using the phage as delivery systems of CRISPR-Cas: deleting or replacing bacterial genes. This approach has already led to notable successes. For example, temperate phage were engineered to deliver a functional CRISPR-Cas system, which successfully removed two antibiotic resistance conferring plasmids in *E. coli* (Figure 2C)[72] and major virulence factors from the chromosome in *S. aureus*.[58] More broadly, phage delivered CRISPR-Cas antimicrobial approach has advantages compared to conventional antibiotics as phage can act on specific subsets of target bacteria without the global disruption of the microbiome.[58,59]

Genetic engineering of therapeutic phage is a developing field – engineered phage are typically developed for a specialized infection setting rather than ready-made for application across a wide range of clinical scenarios.[73] Hence, overcoming the evolution of resistance of pathogenic bacteria to engineered phage may require repeated cycles of labor-intensive (and slow) phage engineering. Reengineering phage

can be particularly challenging given that most phage genomes are not fully characterized – an issue that is also relevant for using non-engineered phage, whether temperate or virulent. Hence, caution and improved understanding of the function of phage genes is needed to avoid unpredicted (and unwanted) interactions with modified genes when using temperate phage as therapeutics.

The uncertainty in long-term outcomes, including phage-mediated spread of virulence factors, represents one of the major obstacles in the use of temperate phage in therapeutic contexts. As already noted, temperate phage can change bacterial phenotypes that are relevant during an infection, potentially making them more pathogenic.[5,74] In addition, on short timescales the presence of temperate phage in a bacterial community may have other consequences, e.g., increasing horizontal gene transfer rates that accelerate the spread of (perhaps undesirable) genes.[75] Over longer timescales, the prophage genome can mutate, lose its lytic capacity, remain integrated in the bacterial genome and continue to express pathogenic phenotypes, shaping the long-term evolution of bacteria. This may explain why many pathogenic bacteria have prophage genes integrated in their genome as phage remnants.[3,7] These facts suggest that the use of temperate phage as therapeutics must confront its impact on evolutionary dynamics and potential consequences over multiple timescales.

EVOLUTIONARY IMPACT ON PHAGE-BACTERIA-HOST INTERACTIONS: AN OPPORTUNITY FOR PHAGE THERAPY

Phage and pathogenic bacteria evolve in response to their environment, including the eukaryotic host and its resident microbes and viruses. Phage have evolved diverse mechanisms to infect and lyse bacteria,[4] likewise bacteria have evolved a myriad of surface and intracellular defense systems to reduce infection and lysis by phage.[76–78] At the population scale, phage infection pressures can select for the proliferation of spontaneous bacterial mutants in an otherwise susceptible population of bacteria.[79] In turn, phage can evolve the ability to infect bacteria that have evolved resistance to ancestral phage.[80] Such individual events can then form the nascent steps of long-term, coevolutionary dynamics.

Quantifying the coevolutionary dynamics of phage and bacteria requires an explicit link between ecological context, selection pressure, and mutational landscape. Early work posited that a sequence of mutational events by bacteria and phage could, in turn, lead to persistent coexistence between a single dominant phage and bacteria, albeit with a moving landscape of phage that infect newly evolved bacteria and bacteria that evolve defenses to newly evolved phage.[81] Yet, coevolution can also drive diversification of phage and bacteria, including increases in both phage and bacterial types.[9,77,82,83] In some instances, the selective pressure to evade bacterial resistance can also lead to the emergence of novel phage infection strategies, e.g., the evolution of virulent phage lambda to infected *E. coli* through the OmpF rather than LamB receptor.[84] Yet, despite the emergence of phage mutants (some with novel traits), *in vitro* co-culture experiments often end with the emergence of a phage-resistant bacterial mutant that leads to the local extinction of the phage population.[85]

As anticipated from *in vitro* systems, phage resistance represents one of the greatest challenges to the sustainable development of phage therapy.[32,86,87] The application of high titers of therapeutic phage can induce a strong selective pressure for the selection of phage-resistant pathogenic bacteria. The proliferation of phage resistant bacteria can, in turn, lead to therapeutic failure (Figure 3A).[25,37,49,88,89] The emergence of bacterial pathogens resistant to virulent phage drastically reduces the ability of phage to clear an infection. Likewise, the emergence of resistance would also inhibit the use of engineered temperate phage to reprogram target bacteria. Finding therapeutic strategies that are robust against phage-resistance is critical for the success of phage therapy.[90] Anticipating such evolution of resistance is paramount to understanding the consequences of phage therapy *in vivo*. However, this requires taking into account how principles of coevolution change when moving from the test tube to animal models and ultimately to clinical applications.

Bacteria face a different set of constraints when interacting with phage in a eukaryotic host environment as compared to *in vitro* environments. The infected host environment modulates the fitness landscape and evolutionary outcomes between phage and bacteria. For example, resistance *in vivo* may have pleiotropic effects, including new costs associated with changes in phenotypes relevant during infections.[32] Such pleiotropic effects can be leveraged to enhance the effectiveness of phage therapy. For instance, Chan et al. showed that the phage OMKO1, which binds to *P. aeruginosa* efflux pumps, selects for bacteria that are more sensitive to drugs from several antibiotic classes (Figure 3C).[23] As a result, joint application

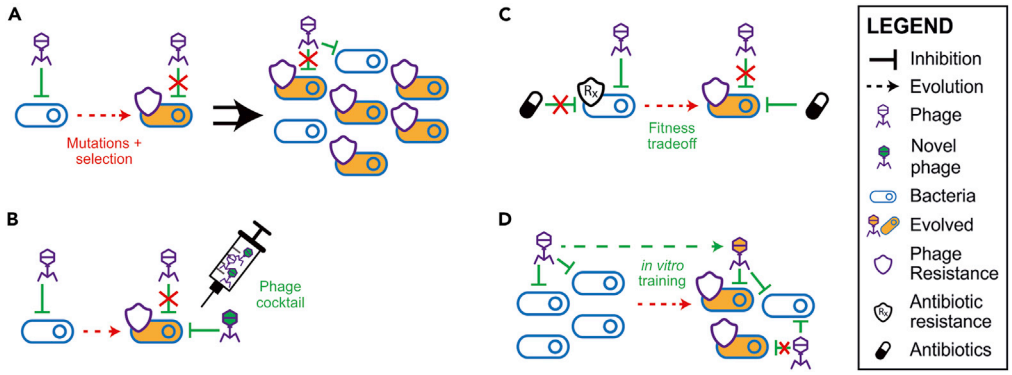

Figure 3. The impact of evolution in phage-bacteria-host interactions

(A) Bacteria can develop phage-resistance which limits phage efficacy to control infections.

(B) To restore the positive therapeutic outcome it may be possible to select combinations of phages so that no individual bacteria can develop resistance to all phages.

(C) Alternatively, tradeoffs can be exploited so that phage resistance by bacteria comes with a fitness cost.

(D) Phage can evolve new mechanisms to infect resistant bacteria. Hence, phage can be 'trained' *in vitro* against the bacteria population to be cleared, and phage sampled at the end of the coevolutionary training may be used to counter resistance mechanisms that will most likely emerge during therapy.

of phage and antibiotics can lead to curative outcomes, without selecting for either antibiotic-resistant or phage-resistant bacterial escape mutants. This is a remarkable example of how evolutionary tradeoffs can be exploited in phage therapy applications. Several other studies showed that bacteria develop phage resistance at the expenses of growth fitness, motility, virulence and susceptibility to antibiotics or to the host immune system.[32,91–95] On the other hand, such trade-offs are not inevitable, e.g., reports have shown that *E. coli* phage-resistant mutants were less sensitive to some antibiotics because of pleiotropic interactions[96] and compensatory mutations can also restore fitness benefits after phage resistance evolves. Furthermore, interactions between bacteria and phage can differ between *in vitro* and in *in vivo* settings. For example, changes in bacterial gene expression within mammalian hosts can affect bacteria-phage interactions, e.g., downregulation of a bacterial receptor in the gut of mice has decreased the susceptibility of *E. coli* to phage infection that would otherwise be vulnerable *in vitro*.[97]

Multiple strategies have been proposed to prepare for and potentially overcome the evolution of phage resistant bacterial mutants. First, characterizing the molecular mechanisms (and receptors) of phage infections can help select for therapeutic phage against which it is likely harder for bacteria to develop resistance.[98] Inevitably the challenge of such approaches is that identification of phage receptors is arduous and may not be timely to clinical interventions. Second, the joint use of multiple phages (i.e., a phage 'cocktail') that infect distinct receptors[22,99] may make it harder for bacteria to acquire resistance in a single (or few) mutation-selection steps (Figure 3B).[32,100–102] Such principles have been applied in practice: cocktails with phages targeting different cell receptors have higher efficacy against *P. aeruginosa*.[103] To the extent that a phage cocktail is identified, then additional improvements may be possible by optimizing the timing and distribution of relative abundances given a constrain on phage titer.[104] For example, mixed cocktails of phages may outperform sequential phage administration, because bacteria are prevented from acquiring sequential mutations leading to multi-resistance.[102,105]

Finally, overcoming the evolution of phage resistance amongst bacteria *in vivo* may benefit from leveraging the evolution of resistance *in vitro*. This principle is called 'evolutionary training' – in which bacteria mutants selected for resistance against a phage are used as the target for selecting phage mutants *in vitro*. Multiple rounds of such coculturing *in vitro* can generate a repertoire of phages that can infect bacteria that are likely to emerge during therapeutic treatment (Figure 3D). Training a phage in a coevolutionary experiment against its bacterial target leads to greater bacterial suppression and delayed resistance emergence *in vitro*.[106] Combining phages that have evolved counter resistance mechanisms to infect resistant bacteria has been hypothesized to serve as the basis for developing evolutionary-informed cocktails. Despite some early evidence in favor of such an approach,[107] additional theoretical and experimental work is needed to assess the extent to which one can look toward the 'future' to restrict mutational escape paths of pathogenic bacteria *in vivo*.[108]

CONCLUSIONS

This review highlighted different ways by which phage modulate bacteria dynamics within a eukaryotic host as a result of tripartite interactions, spanning both direct mechanisms (at the cellular scale) to indirect/system mechanisms (at the population scale). In therapeutic applications, phage can have a positive effect on the host. Positive effects arise when lytic phage act in synergy with the immune system to clear bacterial infections and when temperate phage are engineered to suppress bacterial antibiotic resistance genes. On the other hand, phage may have also negative effects, as they can elicit inflammatory responses from the innate immune system and their induction from lysogens could trigger the transfer of virulence factors in bacterial populations.

When facing the selective pressure imposed by therapeutic phage, bacteria are likely to evolve phage-resistance, potentially hindering the success of phage therapy.[109] We argue that exploiting evolutionary aware strategies against phage-resistance has the potential to yield new breakthroughs in improving the efficacy of phage therapy. For instance, evolutionary trade-offs can be leveraged to steer bacteria to evolve resistance at the cost of susceptibility to antibiotics or of some other quantitative trait. Alternatively, phage can be trained *in vitro* before delivery so they are effective against anticipated resistant mutants, or phage cocktails can be devised to reach the same goal of preventing the proliferation of resistant mutants or mitigating resistance when it does arise.

Moving forward, it is critical to examine these evolutionary processes in the context of therapeutic applications, where the outcome is driven by the tripartite system of phage, bacteria and eukaryotic host. We expect that collecting as much direct data as possible on the emergence, nature and impact of phage resistance during human clinical studies and compassionate treatments will help identify key drivers of interactions of this tripartite system.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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