Beyond antibacterials – exploring bacteriophages as antivirulence agents

Originally published in:
Current Opinion in Biotechnology 68, https://doi.org/10.1016/j.copbio.2020.11.004

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Beyond antibacterials – exploring bacteriophages as antivirulence agents

Yang Shen and Martin J Loessner

Life-threatening infections caused by multidrug-resistant bacteria are becoming increasingly difficult to treat. There is growing interest in exploiting bacteriophages (or phages) to combat bacterial infections. Phages often target bacterial surface structures that may also be important for virulence. Upon phage challenge, these molecules may be lost or modified, resulting in phage resistance and possibly phenotypical conversion. Importantly, possible trade-offs may include lower fitness, increased sensitivity to antibiotics and immune defense mechanisms, and virulence attenuation. Although evolution of phage-resistance may be difficult to prevent, the trade-off phenomenon carries potential for antibacterial therapy. Here we present some insights into the molecular principles and significance of this coincidental interplay between phages, bacteria, and immune cells, and discuss the prospect of developing phage-derived products as antivirulence agents.

Address
Institute of Food, Nutrition and Health, ETH Zurich, Schmelzbergstrasse 7, 8092 Zurich, Switzerland

Corresponding author: Shen, Yang (yang.shen@hest.ethz.ch)

Current Opinion in Biotechnology 2021, **68**:166–173

This review comes from a themed issue on **Nanobiotechnology – phage therapy**

Edited by **Martin Loessner** and **Rob Lavigne**

https://doi.org/10.1016/j.copbio.2020.11.004

Introduction

Phages are viruses that infect specific bacterial hosts. As natural predators of bacteria, they are the most abundant self-replicating organic entity in the biosphere, with an estimated 10^{30} phage particles existing at any given moment [1]. This predator-prey relationship contributes to the evolution of bacterial populations by driving microbial diversity [2]. Phages are divided into two general categories: Lytic (virulent) phages begin replication immediately after cell infection, leading shortly to the degradation of host cells through lysis from within, mediated by dedicated peptidoglycan hydrolases (endolysins) [3]; while temperate phages can integrate their genetic information into the bacterial chromosome, remaining in place until the conditions for producing progeny are met [4].

The first step of infection by a tailed phage (*Caudovirales*) is adsorbing to a susceptible host cell, mediated by receptor-binding proteins (RBPs), which are located on the distal end of the tail apparatus [5•]. Therefore, these proteins are also known as tail spikes or fibers, which are the major determinant of host range by targeting specific surface-accessible receptors distributed in a genus-specific, species-specific or even strain-specific manner. The host cell wall-associated receptors range from macromolecules (proteins and polysaccharide) to organelles (flagellum and pili) [6]. Given their extraordinary specificity and bacteriolytic activity, phages represent highly attractive antibacterial agents for biotechnological [7,8] and therapeutic applications [9,10].

Bacteria can evolve resistance to phage by modifying their surface receptors through genetic mutation in response to phage-driven selective pressure. Phage-encoded lytic enzymes (e.g. endolysins and depolymerases) are capable of degrading these binding receptors. Notably, these receptors may also serve as virulence factors contributing to pathogenicity, and modification of these structures as a result of gaining phage resistance often come at a cost and may lead to attenuation of fitness and virulence, such as defective in growth, biofilm formation, and colonization, as well as more susceptible to antibiotic treatment and immune defense. With this in mind, phages and some of their lytic enzymes may be exploited as antivirulence agents to prevent or even treat bacterial infections, adding to their bactericidal properties. In this review, we explore this idea through an overview of bacterial host receptors for phage recognition, how bacteria modify their receptors to become recalcitrant to phage infection, and the possible phenotypical consequences. We discuss recent advances and implications of developing phage-encoded enzymes as antivirulence agents. Finally, we highlight the synergistic potential of using phage-derived agents in combination with antibiotics and host defense to combat bacterial infections that are difficult to treat by conventional therapy.

Bacterial cell surface macromolecules as phage receptors

In Gram-positive bacteria, a diverse array of host cell receptors have been identified, including flagellum filament, membrane proteins, and surface-associated

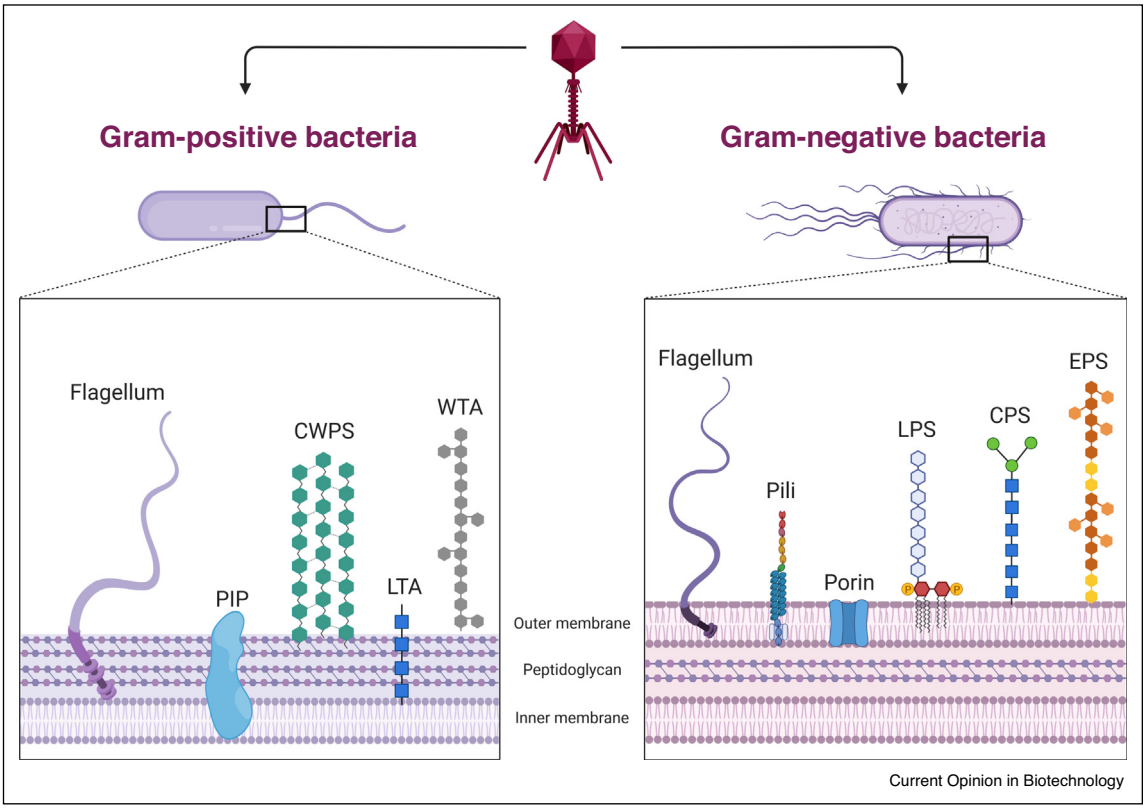
glycopolymers (Figure 1). For a comprehensive summary, we refer the reader to a recent review on this topic [5•]. Recent efforts have uncovered several types of glycopolymers-based receptors for Gram-positive infecting phages, such as lactococcal cell wall polysaccharide (CWPS) [11], streptococcal CWPS [12], enterococcal polysaccharide antigen (Epa) [13••], *Clostridium* capsular polysaccharide [14], *Listeria* peptidoglycan (PG) [15] and PG-anchored wall teichoic acid (WTA) [16].

The primary phage receptors of Gram-negative bacteria are surface-associated glycopolymers, including LPS (lipopolysaccharide), CPS (capsular polysaccharide), and EPS (exopolysaccharide). For a detailed overview of Gram-negative phage receptors, we refer the reader to the review [17••]. Phase-expression of CPS has been demonstrated to mediate the phage adsorption in *Bacteroides thetaiotaomicron* [18] and *Campylobacter jejuni* [19]. While a variety of *Salmonella* and *Escherichia* phages are known to be flagellotropic and bind to flagellin proteins upon initial adsorption, a recent study showed that the bound phages can move along the flagellum by helical movement until they reach cell wall-associated receptors (e.g. LPS) where irreversible binding occurs [20]. Other filamentous appendages, namely pili, may also represent important structures for phages to attach and infect Gram-negative pathogens, such as *Vibrio cholerae* [21] and *Stenotrophomonas maltophilia* [22]. In addition, a variety of outer membrane proteins (e.g. porins OmpC, OmpF, and TolC) have been identified as the secondary receptor for *Escherichia*, *Samonella*, and Vibrio phages [17••,23].

Receptor-mediated phage resistance often comes at a cost for fitness and virulence

Bacteria are under enormous evolutionary pressure based on natural phage-mediated predation. Therefore, they have developed numerous sophisticated defense mechanisms, such as restriction-modification, CRISPR-Cas, and other abortive infection systems to cope with viral infection [24]. One of the most common types of phage resistance in bacteria involves modification of phage receptors by mutation of genes responsible for the biosynthesis and/or assembly of these receptors, thereby preventing phage adsorption. Notably, these receptors

Figure 1



Schematic illustrations of Gram-positive (left) and Gram-negative (right) cell surface-associated virulence factors that act as phage receptors. On the left, a rod-shaped and flagellated Gram-positive bacterium is shown with surface-exposed PIP (phage infection proteins), CWPS (cell wall polysaccharide), LTA (lipoteichoic acid), and WTA (wall-teichoic acid). On the right, a rod-shaped, flagellated and piliated Gram-negative bacterium is depicted with surface-associated porin, LPS (lipopolysaccharide), CPS (capsular polysaccharide), and EPS (exopolysaccharide). Selective pressure mediated by phages can modify these receptors through genetic mutation, which results in the trade-off between fitness cost and virulence attenuation. Various colors of circles, hexagons, and squares represent different sugar moieties.

also function as virulence factors involved in various pathogenicity pathways, such as colonization in the host, evasion of the host's immune response, biofilm formation, toxic shock, and host cell invasion [25]. Hence, phage resistance may result in trade-offs to bacterial fitness and virulence, and may be exploited to improve treatment outcomes [26].

Fixed surface alteration by genetic mutations

Several phage resistance mechanisms have been described to create such trade-offs. For example, loss or modification of LPS structures has been shown to diminish phage infection and reduce virulence [27]. Targeting pili as virulence factors via phage therapy has been proposed as a potential antivirulence approach that could modify bacterial population while selecting pili-deficient strains, which often cause less severe pneumonias during acute pulmonary infection [28]. Predation by *Pseudomonas aeruginosa* phages that use type IV pilins as receptors selects for strains with glycosylated pili, to block phage infection [29••]. In a separate study, *P. aeruginosa* phage resistance was found to be associated with the reduction in efflux pump efficiency [30], a trade-off affecting antibiotic resistance. This type of directed evolution has the potential to prevent the emergence of antibiotic resistance or even possibly resensitize bacteria to antibiotics [31].

In Gram-positive bacteria, a recent study showed that enterococcal phages require Epa for adsorption, as phage predation favors mutation in nonconserved *epa* genes that are located in the gene cluster encoding enzymes for Epa production [32]. Moreover, these Epa mutants were found to be deficient in intestinal colonization, and fail to expand its population upon antibiotic treatment due to its increased susceptibility to cell wall-targeting antibiotics [13••].

We have recently demonstrated a striking trade-off between phage resistance and virulence attenuation in the pathogenic serovar 4b *Listeria monocytogenes* strains [33]. Here, challenge by phages selects for surviving clones that specifically feature a loss of galactose (Gal) from the WTA polymers, by mutations in genes involved in WTA galactosylation. Interestingly, similar mutations were also found to occur in the serovar 4d or 4e strains isolated from the environment. The loss of Gal not only prevents phage adsorption, but also features a complete loss of the surface-associated invasion protein Internalin B, the inability to form actin tails required for cell-to-cell spread, resulting in a massive virulence attenuation *in vivo*. These phage-insensitive bacteria are unable to interact with mammalian cMet and gC1q-R host cell receptors, which normally trigger bacterial uptake upon interaction with InlB. In a follow-up study, we identified the genes responsible for galactosylation of teichoic acids in the important serovar 4b strains, and demonstrated that galactosylated WTA is solely accountable for phage adsorption, InlB surface presentation, and cellular invasiveness [34••]. Collectively, these findings suggest a trade-off between phage resistance and *Listeria* virulence.

Phase-variable modification of phage receptors

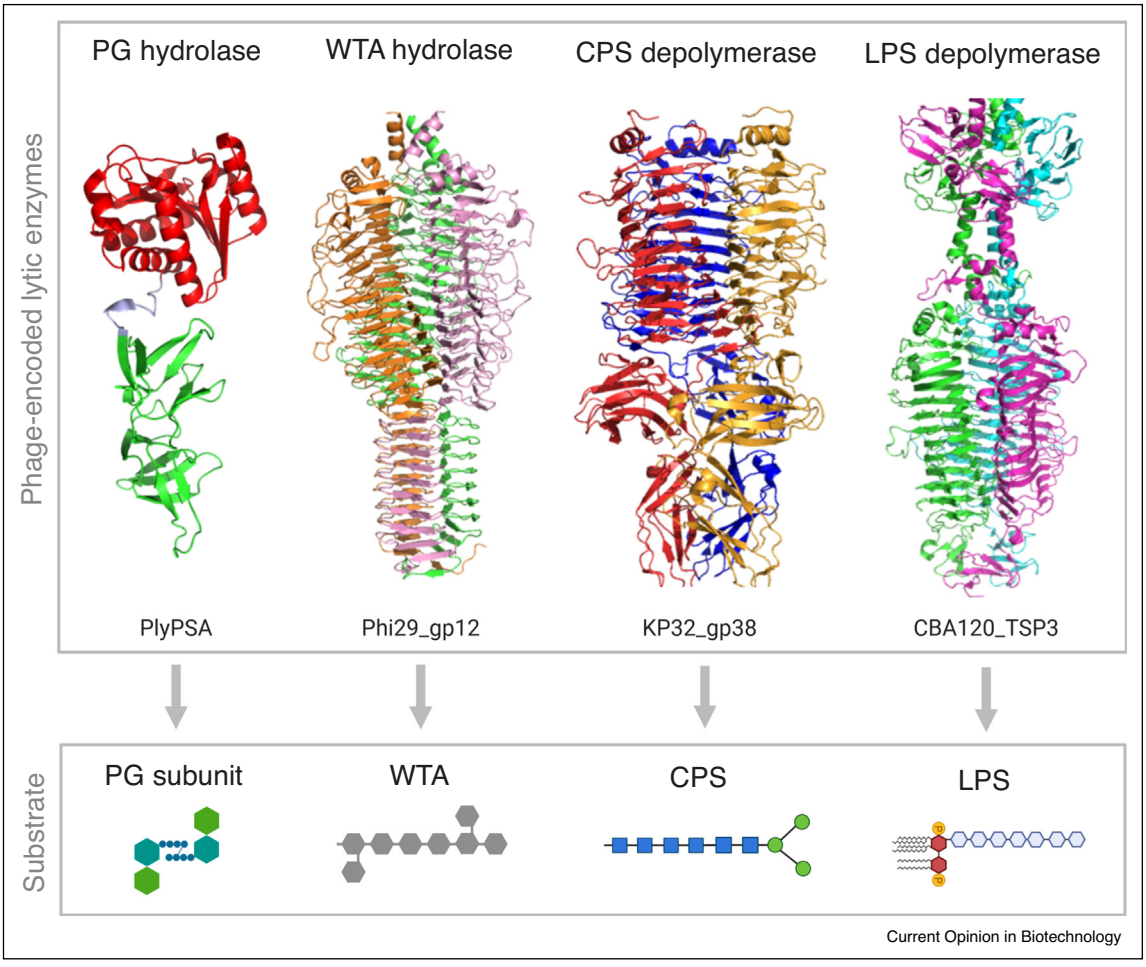
Besides genetic mutations induced by phage predation, non-genetic modification of phage receptors in a phase-variable manner has been reported to confer phage resistance and alter host-pathogen interaction. Phase-variable expression of CPS has been shown to modulate phage sensitivity in *B. thetaiotaomicron* [18] and *C. jejuni* [19]. Transient truncation of LPS in length is known to be associated with phage resistance in *P. aeruginosa* [35]. The Gram-positive pathogen *Staphylococcus aureus* exhibits decreased levels of WTAs in the cell wall, which appears to negatively affect the bacterial ability to form skin abscesses, demonstrating a novel phase-variable virulence system [36]. Altogether, such transient control systems demonstrate another very interesting type of trade-off between phage resistance and host colonization.

Phage-derived lytic enzymes as antivirulence agents

Phages possess a set of lytic enzymes that are capable of degrading bacterial surface glycopolymers that function as phage receptors and virulence factors. These enzymes are generally referred to as endolysins (peptidoglycan hydrolases) and depolymerases (including WTA hydrolases, CPS, or EPS endoglycosidases or exoglycosidases). The architectures of representative enzymes and their substrates are depicted in Figure 2.

Endolysins are peptidoglycan degrading enzymes encoded by almost all phages and are produced within the infected host bacterium at the end of the multiplication cycle [3]. The exposed cell wall of Gram-positive bacteria allows endolysins to exert bacteriolytic activity in a highly specific and efficient manner when adding externally without damaging the non-target bacteria. To digest the PG, endolysins from a Gram-positive background have evolved to utilize a modular design in which catalytic activity and cell wall recognition are separated into two types of functional domains termed enzymatically active domain (EAD) and cell wall binding domain (CBD). EADs feature 4 major categories based on their cut sites within the PG: muramidases and glucosaminidases cleave bonds within the disaccharide backbone; *N*-acetylmuramoyl-L-alanine amidases cut the amide bond between MurNAc and peptide moieties; and endopeptidases cut within the peptide stems. CBDs confer specificity (at a genus, species, or even strain level) to endolysins by targeting the carbohydrate epitope in the cell wall, which can be parts of the PG itself [37] or cell wall-associated glycopolymer [38]. Although endolysins are predominately applied for direct lysis of target bacteria,

Figure 2



Representative phage-encoded lytic enzymes that are able to degrade bacterial surface-associated virulence factors.
The cartoon representations from left to right: *Listeria* phage endolysin PlyPSA (PDB ID, 1XOV) digests the peptidoglycan (PG); *Bacillus* phage appendage protein Phi29_gp12 (PDB ID, 3GQA) acts as a wall-teichoic acid (WTA) hydrolase; *Klebsiella* tailspike protein KP32_gp38 (PDB ID, 6TKU) functions as a capsular polysaccharide (CPS) depolymerase; *E. coli* tailspike protein CBA120_TSP3 (PDB ID, 5W6F) is a lipopolysaccharide (LPS) depolymerase. The respective substrate for each enzyme is shown below the cartoon structure. Various colors of circles, hexagons, and squares represent different sugar moieties.

one should not overlook their potential to degrade the PG involved in the pathogenesis and immune evasion of different human pathogens, specifically *O*-acetylation of PG as a novel target for antivirulence therapies [39].

Depolymerases are tail spike proteins (TSPs) that degrade the highly immunogenic CPS or biofilm EPS to access the primary receptors for phage infection [40]. They normally form a stable homotrimer functioning as both receptor-binding proteins for polysaccharide recognition and glycanases. Phages that infect *Escherichia* spp. encode glycosidases (TSP1 and TSP3) [41•] to degrade LPS. In *Pseudomonas* spp. phage LKA1, the encoded depolymerase Gp49 contains an O5 serotype-specific polysaccharide lyase, which has been shown to disrupt biofilm and reduce virulence, while sensitizing bacteria to serum complement activity [42•]. *Klebsiella* phage-borne depolymerases Dep42, KP32Gp37, and KP32Gp38, have been demonstrated to degrade specific serotypes of bacterial CPS and inhibit biofilm formation [43]. Degradation of *Klebsiella* capsules renders enhanced complement-mediated serum killing and phagocytic clearance, thereby increasing the lifespan of infected animals [44]. Similar EPS depolymerases have been reported in phages infecting *Erwinia amylovora* [45] and *Providencia stuartii* [46]. These data support the development of these capsule-targeting depolymerases as promising antivirulence agents (Table 1).

To date, only scarce information is known about putative depolymerases encoded by Gram-positive phages. The pre-neck appendage protein of *Bacillus* spp. phage phi29_gp12 mediates the irreversible cell wall attachment

Table 1

Selected reports on phage-derived enzymes as antivirulence agents

Bacterial species	Phage type	Phage enzyme	Substrate specificity	Antivirulence property	Ref,
Pseudomonas aeruginosa	LKA1	Gp49	B-band LPS	Disrupt biofilm, reduce virulence, and sensitize bacteria to serum complement activity	[42•]
Klebsiella pneumoniae	SH-KP152226	Dep42	CPS	Inhibit biofilm formation and degrade formed bioflms.	[43]
Klebsiella pneumoniae	KP32	Gp37 and Gp38	Serotype K3 and K21 CPS	Enhance complement-mediated serum killing and phagocytic clearance	[44]
Providencia stuartii	Stuart	Gp52	EPS	Render drug-resistant bacteria susceptible to serum killing	[46]
Bacillus subtilis	Φ29	Gp12	Glycerol-type WTA	Depolymerize glycosylated WTA chain	[47]
Staphylococcus aureus	vB_SepiS-phiIPLA7	Dpo7	EPS	Disperse staphylococcal biofilms	[48]

and can degrade cell wall teichoic acids with phosphodiesterase activity [47]. Its homolog Dpo7 (identified in a staphylococcal phage) was shown to exhibit biofilm-degrading activity by acting on the surface EPS structures [48]. Since both WTA and EPS represent important virulence factors for many pathogenic bacteria, degradation of these matrix-like structures may facilitate the penetration of antibiotics and the clearance by immune systems, resulting in increased therapeutic efficacy.

In summary, depolymerases and endolysins feature modular structures and combine different conserved and variable modules conferring their enzymatic activities and host specificities. From a therapeutic point of view, these enzymes hold promises to be developed as tailor-made antibacterial and antivirulence agents. For a detailed examination of published endolysins and depolymerases, see previously published reviews [3,40].

Phage-derived antivirulence strategy: possible synergy with other antibacterials

With the increasing number of studies or clinical trials using phages or phage lytic enzymes as bacteriolytic therapeutics, there is growing interest in developing them as virulence-specific antibacterials for infection control. Unlike conventional drugs that directly kill bacterial cells or inhibit their growth, the antivirulence strategy aims to disarm the pathogens, rendering them less virulent and fit, or more susceptible to antimicrobials or innate host defense. A similar strategy has recently been described as 'phage steering', which could be advantageous when treating infections [31].

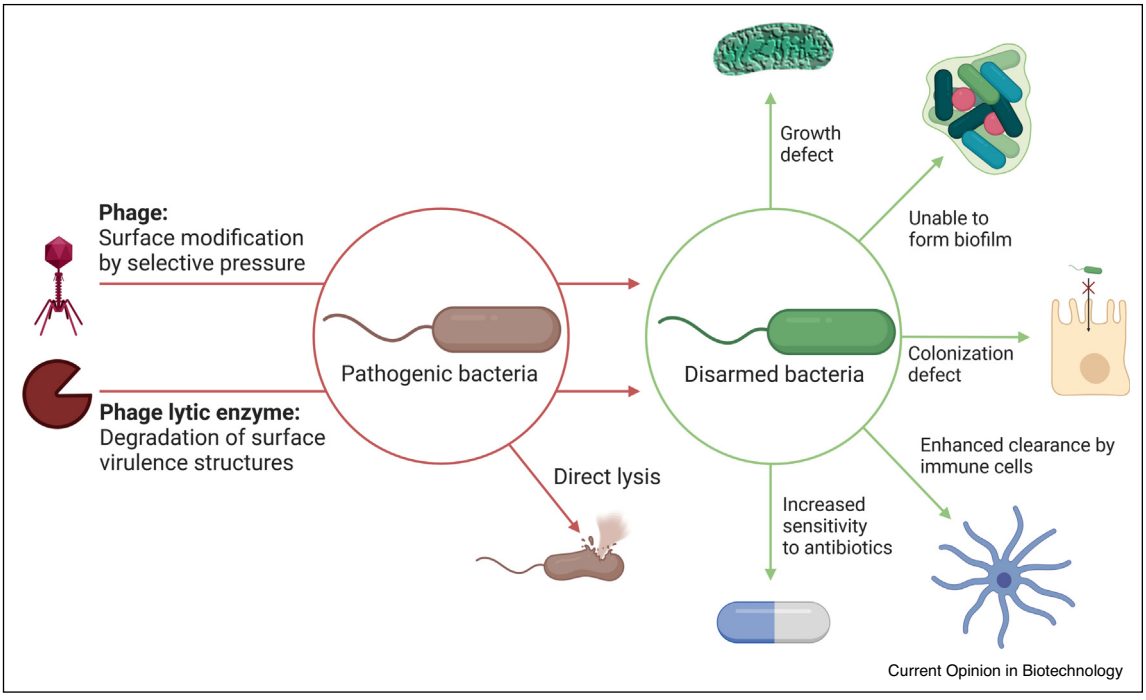
Phage-antibiotic or phage enzyme-antibiotic synergism

A combination of phage and antibiotics has been considered a preferred choice in phage therapy [49]. Yet, the mechanism drives the synergy remains unclear. Previous work has attributed the synergy to the selective pressures and resistance costs that resensitize bacteria to antibiotic treatment [13••,30,32,33], as antibiotic susceptibility and phage resistance may be based on the same phage receptor, such as the glycopolymers or the proteins of efflux pump. Recently, a patient with life-threatening *Acinetobacter baumannii* infection has been successfully treated using a personalized phage cocktail in combination with minocycline [10]. As for phage-encoded enzymes, synergy with standard-of-care antibiotics has also been demonstrated for *Klebsiella* depolymerase Dep42 [43] and staphylococcal endolysin CF301 [50]. The latter is primarily attributed to the bacteriolytic action of endolysins, whereas the former is due to the degradation of the CPS layer, permitting the increased penetration of the membrane-acting compound polymyxin. These studies substantiate that phage-mediated modification on the bacterial surface can be clinically beneficial and enable the recycling of ineffective antibiotics back into effective drugs.

Phage-host or phage enzyme-host defense interaction

Phages can modulate both innate and adaptive immunity via interaction with mammalian immune systems, thereby exhibiting profound effects on the outcome of bacterial infections [51]. The concept of 'immunophage synergy' has recently arisen in a study investigating the interplay between phage, *P. aeruginosa*, and immune cells using both *in vivo* and computational models [52]. The data indicate that successful phage therapy is supported by innate immune components, specifically neutrophils to kill phage-sensitive and emerging phage-resistant pathogens. Phage-resistant *K. pneumoniae* mutants with deficient CPS synthesis were found to be more sensitive to macrophage-mediated phagocytosis [53]. Some contrary data show phages may also mislead the immune system, thereby preventing the clearance of *P. aeruginosa* infection in an open-wound setting [54]. In this study, a filamentous temperate phage was found to be integrated into the bacterial genome and to suppress the innate immune response. Similar findings were revealed in a separate study that phages can stimulate a specific immune response, which worsened inflammatory bowel disease pathogenesis [55]. Because of this previously unrecognized complexity of host–microbe–phage

Figure 3



Approach to develop phages or phage-encoded glycan degrading enzymes as antivirulence agents for combating antibiotic-resistant infections. The graph illustrates the fitness trade-offs promoted by surface virulence factor mutation upon phage predation, and the degradation of virulence glycans by the application of phage lytic enzymes. The modes of action are: (1) direct lysis of target bacteria; (2) disarming pathogenic bacteria with surface modification by selective pressure or enzymatic treatment. The attenuated bacteria frequently bear a fitness defect, which may render them unable to form biofilm, defective in colonization, or more susceptible to antibiotics and immune cells.

interactions, further mechanistic details are needed to develop specific phage-immune combinatorial therapies for the treatment of bacterial infections. In addition, several phage-encoded depolymerases act synergistically with serum-mediated killing, complement activity, and phagocytosis [42•,44,46], as these enzymes primarily target surface glycopolymers which modulate the interaction between bacteria and immune system. These exciting synergies between depolymerases and the immune defense pave promising avenues to investigate how depolymerases enhance antibacterial therapies as antivirulence agents.

Conclusions and perspectives

Until now, phage therapy was thought to work simply by reducing bacterial numbers, in a way to known antibiotic therapies. However, treatment failure occurs when bacteria are able to develop phage resistance during phage administration [56]. Several strategies have thus been proposed that go beyond simple phage monotherapy in order to preclude resistance, such as multi-phage cocktails, phage engineering, and approaches combining phages with antibiotics or the immune defense. That being said, phage resistance may also be anticipated and used as part of a therapeutic strategy (Figure 3). This involves harnessing the evolutionary trade-off that occurs when phage-resistance is accompanied by increased sensitivity to conventional antimicrobials or the immune system or attenuated virulence. The same is true for the aforementioned phage lytic enzymes which are capable of disarming pathogens in addition to their direct killing activity.

Although phages or their lytic enzymes represent promising antivirulence biologics, several challenges exist towards possible clinical application: (1) avoid unwanted prophage-encoded virulence factors and interactions with the immune system; (2) fine-tune the selection pressure and coevolution between the specific phages and their bacterial hosts; (3) better mechanistic understanding of the synergy between phage (or phage enzymes) and antibiotic (or immune defense) to achieve the ideal outcome of combination therapies; (4) better understanding of phage-resistance mutations that pleiotropically complicates the trade-off [57••]; (5) improve stability and pharmacokinetics of recombinant phage enzymes. We have no doubt that following these new directions in phage therapy research will greatly aid the development of effective phage-based antivirulence agents.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Yang Shen: Writing - original draft, Writing - review & editing, Visualization. **Martin J Loessner:** Writing - review & editing.

Acknowledgements

We are grateful to many members of the Loessner lab for their contributions to advance our understanding of this large area of research. We thank Anja Keller for revising the figures, which were originally created with Biorender.com. We thank Eric Sumrall for critical reading of the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. Hendrix RW, Smith MC, Burns RN, Ford ME, Hatfull GF: **Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage**. *Proc Natl Acad Sci U S A* 1999, **96**:2192-2197.

2. Koskella B, Brockhurst MA: **Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities**. *FEMS Microbiol Rev* 2014, **38**:916-931.

3. Haddad Kashani H, Schmelcher M, Sabzalipoor H, Seyed Hosseini E, Moniri R: **Recombinant endolysins as potential therapeutics against antibiotic-resistant *Staphylococcus aureus*: current status of research and novel delivery strategies**. *Clin Microbiol Rev* 2018, **31**.

4. Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB: **Lysogeny in nature: mechanisms, impact and ecology of temperate phages**. *ISME J* 2017, **11**:1511-1520.

5. • Dunne M, Hupfeld M, Klumpp J, Loessner MJ: **Molecular basis of bacterial host interactions by gram-positive targeting bacteriophages**. *Viruses* 2018, **10**.

This review provided a comprehensive view of Gram-positive phage receptors in the context of their interacting receptor-binding proteins.

6. Bertozzi Silva J, Storms Z, Sauvageau D: **Host receptors for bacteriophage adsorption**. *FEMS Microbiol Lett* 2016, **363**.

7. Kilcher S, Studer P, Muessner C, Klumpp J, Loessner MJ: **Cross-genus rebooting of custom-made, synthetic bacteriophage genomes in l-form bacteria**. *Proc Natl Acad Sci U S A* 2018, **115**:567-572.

8. Sumrall ET, Rohrig C, Hupfeld M, Selvakumar L, Du J, Dunne M, Schmelcher M, Shen Y, Loessner MJ: **Glycotyping and specific separation of *Listeria monocytogenes* with a novel bacteriophage protein tool kit**. *Appl Environ Microbiol* 2020, **86**.

9. Gordillo Altamirano FL, Barr JJ: **Phage therapy in the postantibiotic era**. *Clin Microbiol Rev* 2019, **32**.

10. Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S, Segall AM *et al.*: **Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection**. *Antimicrob Agents Chemother* 2017, **61**.

11. Marcelli B, de Jong A, Karsens H, Janzen T, Kok J, Kuipers OP: **A specific sugar moiety in the *Lactococcus lactis* cell wall pellicle is required for infection by CHPC971, a member of the rare 1706 phage species**. *Appl Environ Microbiol* 2019, **85**.

12. Szymczak P, Filipe SR, Covas G, Vogensen FK, Neves AR, Janzen T: **Cell wall glycans mediate recognition of the dairy bacterium *Streptococcus thermophilus* by bacteriophages**. *Appl Environ Microbiol* 2018, **84**.

13. •• Chatterjee A, Johnson CN, Luong P, Hullahalli K, McBride SW, Schubert AM, Palmer KL, Carlson PE Jr, Duerkop BA: **Bacteriophage resistance alters antibiotic-mediated intestinal expansion of enterococci**. *Infect Immun* 2019, **87**.

This paper demonstrated that enterococcal phages require Epa for adsorption, and can drive the mutation in nonconserved *epa* genes that are located in the gene cluster encoding enzymes for Epa production. Moreover, the mutant strain is deficient in intestinal colonization, and fail to expand its population upon antibiotic treatment due to its increased susceptibility to cell wall-targeting antibiotics.

14. Ha E, Chun J, Kim M, Ryu S: **Capsular polysaccharide is a receptor of a clostridium perfringens bacteriophage CPS1**. *Viruses* 2019, **11**:11.

15. Guerrero-Ferreira RC, Hupfeld M, Nazarov S, Taylor NM, Shneider MM, Obbineni JM, Loessner MJ, Ishikawa T, Klumpp J, Leiman PG: **Structure and transformation of bacteriophage A511 baseplate and tail upon infection of *Listeria* cells**. *EMBO J* 2019, **38**.

16. Dunne M, Rupf B, Tala M, Qabrati X, Ernst P, Shen Y, Sumrall E, Heeb L, Pluckthun A, Loessner MJ, Kilcher S: **Reprogramming bacteriophage host range through structure-guided design of chimeric receptor binding proteins**. *Cell Rep* 2019, **29**:1336-1350 e1334.

17. •• Nobrega FL, Vlot M, de Jonge PA, Dreesens LL, Beaumont HJE, Lavigne R, Dutilh BE, Brouns SJJ: **Targeting mechanisms of tailed bacteriophages**. *Nat Rev Microbiol* 2018, **16**:760-773.

A comprehensive, up-to-date review focusing on the targeting mechanisms of both Gram-positive and Gram-negative phages.

18. Porter NT, Hryckowian AJ, Merrill BD, Fuentes JJ, Gardner JO, Glowacki RWP, Singh S, Crawford RD, Snitkin ES, Sonnenburg JL, Martens EC: **Phase-variable capsular polysaccharides and lipoproteins modify bacteriophage susceptibility in *Bacteroides thetaiotaomicron***. *Nat Microbiol* 2020, **5**:1170-1181.

19. Gencay YE, Sorensen MCH, Wenzel CQ, Szymanski CM, Brondsted L: **Phase variable expression of a single phage receptor in *Campylobacter jejuni* NCTC12662 influences sensitivity toward several diverse cps-dependent phages**. *Front Microbiol* 2018, **9**:82.

20. Gonzalez F, Helm RF, Broadway KM, Scharf BE: **More than rotating flagella: lipopolysaccharide as a secondary receptor for flagellotropic phage 7-7-1**. *J Bacteriol* 2018, **200**.

21. Gutierrez-Rodarte M, Kolappan S, Burrell BA, Craig L: **The *Vibrio cholerae* minor pilin TcpBb mediates uptake of the cholera toxin phage CTXΦ**. *J Biol Chem* 2019, **294**:15698-15710.

22. McCutcheon JG, Peters DL, Dennis JJ: **Identification and characterization of type IV pili as the cellular receptor of broad host range *Stenotrophomonas maltophilia* bacteriophages DLP1 and DLP2**. *Viruses* 2018, **10**.

23. Fan F, Li X, Pang B, Zhang C, Li Z, Zhang L, Li J, Zhang J, Yan M, Liang W, Kan B: **The outer-membrane protein tolc of *Vibrio cholerae* serves as a second cell-surface receptor for the VP3 phage**. *J Biol Chem* 2018, **293**:4000-4013.

24. Hampton HG, Watson BNJ, Fineran PC: **The arms race between bacteria and their phage foes**. *Nature* 2020, **577**:327-336.

25. Poole J, Day CJ, von Itzstein M, Paton JC, Jennings MP: **Glycointeractions in bacterial pathogenesis**. *Nat Rev Microbiol* 2018, **16**:440-452.

26. Mangalea MR, Duerkop BA: **Fitness trade-offs resulting from bacteriophage resistance potentiate synergistic antibacterial strategies**. *Infect Immun* 2020, **88**.

27. Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, Resch G, Que YA: **Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence**. *J Infect Dis* 2017, **215**:703-712.

28. Tang H, Kays M, Prince A: **Role of *Pseudomonas aeruginosa* pili in acute pulmonary infection**. *Infect Immun* 1995, **63**:1278-1285.

29. •• Harvey H, Bondy-Denomy J, Marquis H, Sztanko KM, Davidson AR, Burrows LL: ***Pseudomonas aeruginosa* defends against phages through type IV pilus glycosylation**. *Nat Microbiol* 2018, **3**:47-52.

This article provided a unique rationale for the prevalence of pilus glycosylation in nature, supporting an important notion that phage predation can affect host–pathogen interaction and biofilm formation.

30. Chan BK, Sistrom M, Wertz JE, Kortright KE, Narayan D, Turner PE: **Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa***. *Sci Rep* 2016, **6**:26717.

31. Gurney J, Brown SP, Kaltz O, Hochberg ME: **Steering phages to combat bacterial pathogens**. *Trends Microbiol* 2020, **28**:85-94.

32. Ho K, Huo W, Pas S, Dao R, Palmer KL: **Loss-of-function mutations in *epaR* confer resistance to ΦNPV1 infection in *Enterococcus faecalis* OG1RF**. *Antimicrob Agents Chemother* 2018, **62**.

33. Sumrall ET, Shen Y, Keller AP, Rismondo J, Pavlou M, Eugster MR, Boulos S, Disson O, Thouvenot P, Kilcher S, Wollscheid B *et al.*: **Phage resistance at the cost of virulence: *Listeria monocytogenes* serovar 4b requires galactosylated teichoic acids for InlB-mediated invasion**. *PLoS Pathog* 2019, **15**: e1008032.

34. •• Sumrall ET, Schefer CRE, Rismondo J, Schneider SR, Boulos S, Grundling A, Loessner MJ, Shen Y: **Galactosylated wall teichoic acid, but not lipoteichoic acid, retains inlb on the surface of serovar 4b *Listeria monocytogenes***. *Mol Microbiol* 2020, **113**:638-649.

This article demonstrated the trade-off between phage resistance and listerial virulence by providing a clear mechanism by which galactosylated WTA retains the invasion protein internalin B on the bacterial cell surface. Loss of the galactose residue by phage predation renders bacteria unable to perform InlB-mediated invasion and form actin tails for cell-to-cell spread.

35. Latino L, Caroff M, Pourcel C: **Fine structure analysis of lipopolysaccharides in bacteriophage-resistant *Pseudomonas aeruginosa* PAO1 mutants**. *Microbiology* 2017, **163**:848-855.

36. Wanner S, Schade J, Keinhorster D, Weller N, George SE, Kull L, Bauer J, Grau T, Winstel V, Stoy H, Kretschmer D *et al.*: **Wall teichoic acids mediate increased virulence in *Staphylococcus aureus***. *Nat Microbiol* 2017, **2**:16257.

37. Lee KO, Kong M, Kim I, Bai J, Cha S, Kim B, Ryu KS, Ryu S, Suh JY: **Structural basis for cell-wall recognition by bacteriophage PBC5 endolysin**. *Structure* 2019, **27**:1355-1365 e1354.

38. Shen Y, Boulos S, Sumrall E, Gerber B, Julian-Rodero A, Eugster MR, Fieseler L, Nystrom L, Ebert MO, Loessner MJ: **Structural and functional diversity in *Listeria* cell wall teichoic acids**. *J Biol Chem* 2017, **292**:17832-17844.

39. Brott AS, Clarke AJ: **Peptidoglycan O-acetylation as a virulence factor: its effect on Lysozyme in the innate immune system**. *Antibiotics (Basel)* 2019, **8**.

40. Knecht LE, Veljkovic M, Fieseler L: **Diversity and function of phage encoded depolymerases**. *Front Microbiol* 2019, **10**:2949.

41. • Greenfield J, Shang X, Luo H, Zhou Y, Heselpoth RD, Nelson DC, Herzberg O: **Structure and tailspike glycosidase machinery of ORF212 from *E. coli* O157:H7 phage CBA120 (TSP3)**. *Sci Rep* 2019, **9**:7349.

An extensive overview summarizing most currently known phage-derived depolymerases.

42. • Olszak T, Shneider MM, Latka A, Maciejewska B, Browning C, Sycheva LV, Cornelissen A, Danis-Wlodarczyk K, Senchenkova SN, Shashkov AS, Gula G *et al.*: **The O-specific polysaccharide lyase from the phage LKA1 tailspike reduces pseudomonas virulence**. *Sci Rep* 2017, **7**:16302.

This paper is one of the first papers describing the antivirulence property of a phage-encoded depolymerase with detailed functional analyses.

43. Wu Y, Wang R, Xu M, Liu Y, Zhu X, Qiu J, Liu Q, He P, Li Q: **A novel polysaccharide depolymerase encoded by the phage SH-KP152226 confers specific activity against multidrug-resistant *Klebsiella pneumoniae* via biofilm degradation**. *Front Microbiol* 2019, **10**:2768.

44. Majkowska-Skrobek G, Latka A, Berisio R, Squeglia F, Maciejewska B, Briers Y, Drulis-Kawa Z: **Phage-borne depolymerases decrease *Klebsiella pneumoniae* resistance to innate defense mechanisms**. *Front Microbiol* 2018, **9**:2517.

45. Born Y, Fieseler L, Klumpp J, Eugster MR, Zurfluh K, Duffy B, Loessner MJ: **The tail-associated depolymerase of *Erwinia amylovora* phage L1 mediates host cell adsorption and enzymatic capsule removal, which can enhance infection by other phage**. *Environ Microbiol* 2014, **16**:2168-2180.

46. Oliveira H, Pinto G, Mendes B, Dias O, Hendrix H, Akturk E, Noben JP, Gawor J, Lobocka M, Lavigne R, Azeredo J: **A tailspike with exopolysaccharide depolymerase activity from a new *Providencia stuartii* phage makes multidrug-resistant bacteria susceptible to serum-mediated killing**. *Appl Environ Microbiol* 2020, **86**.

47. Myers CL, Ireland RG, Garrett TA, Brown ED: **Characterization of wall teichoic acid degradation by the bacteriophage Φ29 appendage protein GP12 using synthetic substrate analogs**. *J Biol Chem* 2015, **290**:19133-19145.

48. Gutierrez D, Briers Y, Rodriguez-Rubio L, Martinez B, Rodriguez A, Lavigne R, Garcia P: **Role of the pre-neck appendage protein (Dpo7) from phage vB_SepiS-phiIPLA7 as an anti-biofilm agent in staphylococcal species**. *Front Microbiol* 2015, **6**:1315.

49. Segall AM, Roach DR, Strathdee SA: **Stronger together? Perspectives on phage-antibiotic synergy in clinical applications of phage therapy**. *Curr Opin Microbiol* 2019, **51**:46-50.

50. Asempa TE, Abdelraouf K, Carabeo T, Schuch R, Nicolau DP: **Synergistic activity of exebacase (CF-301) in addition to daptomycin against *Staphylococcus aureus* in a neutropenic murine thigh infection model**. *Antimicrob Agents Chemother* 2020, **64**.

51. Van Belleghem JD, Dabrowska K, Vaneechoutte M, Barr JJ, Bollyky PL: **Interactions between bacteriophage, bacteria, and the mammalian immune system**. *Viruses* 2018, **11**.

52. Roach DR, Leung CY, Henry M, Morello E, Singh D, Di Santo JP, Weitz JS, Debarbieux L: **Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen**. *Cell Host Microbe* 2017, **22**:38-47 e34.

53. Cai R, Wang G, Le S, Wu M, Cheng M, Guo Z, Ji Y, Xi H, Zhao C, Wang X, Xue Y *et al.*: **Three capsular polysaccharide synthesis-related glucosyltransferases, GT-1, GT-2 and WcaJ, are associated with virulence and phage sensitivity of *Klebsiella pneumoniae***. *Front Microbiol* 2019, **10**:1189.

54. Sweere JM, Van Belleghem JD, Ishak H, Bach MS, Popescu M, Sunkari V, Kaber G, Manasherob R, Suh GA, Cao X, de Vries CR *et al.*: **Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection**. *Science* 2019, **363**.

55. Gogokhia L, Buhrke K, Bell R, Hoffman B, Brown DG, Hanke-Gogokhia C, Ajami NJ, Wong MC, Ghazaryan A, Valentine JF, Porter N *et al.*: **Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis**. *Cell Host Microbe* 2019, **25**:285-299 e288.

56. Oechslin F: **Resistance development to bacteriophages occurring during bacteriophage therapy**. *Viruses* 2018, **10**.

57. •• Burmeister AR, Fortier A, Roush C, Lessing AJ, Bender RG, Barahman R, Grant R, Chan BK, Turner PE: **Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance**. *Proc Natl Acad Sci U S A* 2020, **117**:11207-11216.

This article not only demonstrated an evolutionary trade-off between phage resistance and antibiotic resistance, but also found bacterial mutations that avoid the trade-off. The findings provided insights into the co-evolution of phage and host.