Bacteriophages: Molecular and Virologic Review Study

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DOI: https://doi.org/10.63939/7zbcct09

Abstract

Earth's most common viruses, bacteriophages, infect bacteria and archaea. Protein capsids and sometimes lipid envelopes encase phage genomes, which vary in kind and structure. Most are double-stranded DNA phages, whereas *Cystoviridae* are RNA. Phages control bacterial populations, transfer horizontal genes, lyse molecules, and alter metabolism in different situations. Based on their structural morphotypes, which are largely determined by tail architecture, phages are classified as *Podoviridae* (short tails), *Siphoviridae* (long, flexible tails), and *Myoviridae* (long, contractile tails). They build genome transport and host recognition virions with portal proteins, tail fibers, and baseplates. DNA is transferred into capsids by ATP-dependent terminases during genome packing. The lysogenic cycle comprises temperate phages integrating into the host genome as prophages and replicating passively until stimulation activates the lytic phase. Phages with non-lytic chronic infections or carriers exist. Phages limit their host range by recognizing lipopolysaccharides, outer membrane proteins, teichoic acids, and flagella. They can make anti-CRISPR proteins to attack bacteria. Phages indirectly affect human health by influencing microbiota and immune systems. Immunoglobulin-like capsid domains connect them to mucosal surfaces for bacterial clearance and barrier protection. They can also enter tissues and circulation, where they are immunologically tolerated. Phages activate innate and adaptive immunity. Innate sensing and cytokine production are enabled by TLR3, TLR7, and TLR9. However, adaptive immunity produces neutralizing IgM, IgG, and IgA antibodies. Recurrent phage therapy can be reduced by phage-neutralizing antibodies. Phage biology is characterized by the discovery of "huge phages" with genomes that rival small bacterial genomes and encode complex functions like

tRNAs, translation factors, CRISPR-Cas systems, and nucleus-like compartments that protect phage genomes from host defenses. Phage-encoded CRISPR-Cas systems often lack spacer acquisition or interference genes, resulting in host apparatus repurposing or gene transcriptional silence. Targeting competing phages and host regulatory mechanisms is possible. Phages treat antibiotic-resistant microorganisms. Phage treatment has potential despite bacterial resistance and host immune neutralization. They also carry toxin genes (*cholera, diphtheria*) through lysogenic conversion, boosting bacterial pathogenicity. Dynamic evolutionary arms races affect microbial ecology through phage-host and phage-phage interactions. Recent metagenomics and meta transcriptomics advances have increased the variety of dsRNA and tailless dsDNA phages. This showed unique viral families and the prevalence of phages in human microbiomes, particularly crAss-like phages in the intestine. The molecular details of phage interactions with eukaryotic cells are still emerging, despite the vast knowledge of bacterial receptors for phage attachment. Mammalian cells can use endocytosis mechanisms to internalize phages for immunological regulation and therapeutic delivery. expanding the taxonomy of dsRNA phages, understanding non-lytic infection mechanisms, characterizing phage-host range and interactions, and using phages for biocontrol in agriculture and medicine. It is required to overcome immune clearance, understand phage immunogenicity, and understand the tri-kingdom interactions between phages, bacteria, and human hosts that maintain microbial and immunological homeostasis to maximize phage therapy.

A class of viruses, bacteriophages are complex, diverse and critical to the environment. This organization covers everything from bacteria to immune system contact, horizontal gene transfer, and the quickly developed field of biotechnology and phage therapy. In addition to their role as predators of bacteria, researchers are beginning to uncover fascinating stories about phages with novel life cycles, structural biology and interactions with both human and bacterial hosts. Provide an overview of bacteriophages (the infectious agents that infect both bacteria and archaea), their diversity, double-stranded DNA and double-stranded RNA phages, their structure and methods of replication, the ramifications for the environment, the role of immunity in mammals, and new directions in medicine such as phage therapy. Proteins with complex structural functions in phage virus assembly. These well-studied phages with tails/capsids suitable for genome transfer and host recognition include T7 (like Autographviridae/Podoviridae), SPP1 (like Siphoviridae) and T4 (like Myoviridae). Baseplate and tail fibers bind biomolecules (e.g. lipopolysaccharides and

proteins) from the outer membrane. ATPases that package the genome assist phage DNA in capsid insertion. T4 phages utilize the lytic cycle to rapidly replicate and lyse their hosts, while temperate phages integrate into the host chromosome and remain in a lysogenic state until induction leads to lytic replication. Phages that infect pseudolysogenically or enter into persistent states of infection do not lyse quickly. These choices influence the ecology and utility of phages in treating bacterial pathogens. Phages are able to identify their hosts by detecting polysaccharides, proteins and flagella. The host range of the virus is restricted by phage specificity. Phages escape bacterial defense by genomic adaptation and encoding anti-CRISPR protein. Phages arm bacteria with virulence factors (e.g., toxin genes) through lysogenic conversion. When they aren't infecting humans, phages can still influence immune systems and microbiomes. the lysogenic cycle to inoculate

Phages can infect tissues and cells via endocytosis, altering immune responses. Phages destroy bacteria to fight antibiotic-resistant illnesses, but immunological responses including neutralizing antibodies can hinder phage treatment. Recent discoveries include "huge phages" with microscopic bacteria genomes. Complex machinery is provided by tRNA, translation factor, and CRISPR-Cas phages. Metagenomic studies have found tailless dsDNA phages from new viral orders and families, enhancing our understanding of phage diversity. CRISPR-mediated phage-host and phage-phage interactions show dynamic evolutionary arms races. Biomedical applications, gene transfer, microbial community dynamics, and bacterial population regulation depend on bacteriophages. Research on their structural biology, trplicative cycles, and host immunological interactions determines their therapeutic and ecological potential. Bacteriophages are everywhere in the biosphere and influence bacterial ecology and evolution. DNA-containing bacteriophages in ecosystems are well-studied, whereas RNA-containing ones are often overlooked.

Keywords: Bacteriophages, phages, Lytic cycle, Lysogenic cycle, Phage therapy, Phage immunogenicity, Microbiome, Anti-CRISPR proteins

Classification and Diversity:

The ICTV has classified more than 50 virus families of double-stranded DNA bacteriophages. The *Cystoviridae* is the sole family of dsRNA bacteriophages, consisting of seven species. In contrast to dsDNA phages, classified dsRNA phage isolates exhibit a restricted host range. Most phages are double-stranded DNA tailed (order *Caudovirales*), but double-stranded RNA (family *Cystoviridae*) and tailless phages also contribute to viral variety. New metagenomic research have revealed a great diversity of phages, including giant phages with massive genomes that encode complicated functionalities including tRNAs, translation factors, and CRISPR-Cas systems. Phylogenetic investigations of signature genes like RNA-dependent RNA polymerase and capsid protein structures are currently used more than morphology or host specificity to progress taxonomy.

In the present taxonomy, dsRNA viruses are classified as either *Pisuviricota* or *Duplornaviricota*. The majority of animal dsRNA viruses (e.g., rotavirus and bluetongue virus) and several significant microbial and plant viruses are members of the *Cystoviridae* virus family (class: *Vidaverviricetes*, order: *Mindivirales*) of the phylum *Duplornaviricota*. The phylum *Pisuviricota* comprises dsRNA viruses, including amalga-, curvula-, partiti-, and *picobirnaviruses*. This initial taxonomy classification of RNA viruses is based on a comprehensive sequence-based comparison of viral RdRp sequences [1]. Nevertheless, a recent phylogenetic analysis of RdRp sequences based on sequences revealed that cystoviral and dsRNA virus RdRps are classified in *Pisuviricota* [2]. Additionally, higher-order groupings were identified through a structure-based phylogenetic analysis of viral RdRps [3].

The *Cystoviridae* family was recognized by the ICTV in 1978 [4], and the phi6 group and phage phi6 were recognized in 1976 [5]. At present, the Cystoviridae virus family comprises a single genus, Cystovirus (formerly known as the phi6 group), which comprises seven virus species: phi6, phi8, phi12, phi13, phiNN, phi2954, and phiYY. Due to their unique similarities in virion structures and genomes, these phages were classified together, despite their low nucleotide sequence identity (<50%, with the exception of phi6 and phiNN; [6]. The current criteria for identifying *Cystoviridae* species are 95% nucleotide sequence identity. The Cystoviridae family comprises six viruses that remain unidentified: phi7, phi9–phi11, phi14, and phiNY. phiZ98 and CAP3-7 may also be included in the *Cystoviridae* family due to their genetic and structural similarities [7,8]. The ICTV has recently introduced supplementary higher-order ranks for virus taxonomic classification and is

in the process of transitioning to a system that more accurately reflects virus phylogenetic relationships than host specificity, morphological features, or disease symptoms [9]. The polymerase gene, which encodes RdRp or reverse transcriptase, is a critical locus for the classification of RNA viruses and phylogenetic analysis. RdRp-encoding RNA viruses are found in the *Orthornavirae* kingdom of the Riboviria domain. *Duplornaviricota*, *Kitrinoviricota*, *Lenarviricota*, *Negarnaviricota*, and *Pisuviricota* are the five official phyla of *Orthornavirae* [10].

In the recently adopted megataxonomy of viruses, double-stranded DNA phages are divided into two huge realms: *Duplodnaviria* and *Varidnaviria* [11]. Viral structures in these realms include MCP and packing ATPases. Archaeal, tailed, and herpesviruses are *duplodnaviria*. The phylum *Nucleocytoviricota*, which comprises mimiviruses, tailless bacteriophages, and related archaeal viruses, is included in *Varidnaviria*.

Tailed *Duplodnaviria* bacteriophages are the most prevalent. The upper oceans are dominated by non-tailed varidnaviruses, which account for 50% to 90% of viral particles, according to metagenomic investigations [121,13]. Varidnaviria's icosahedral capsids are composed of the double jelly-roll (DJR) MCP [14,15]. The DJR MCP core structure is conserved across the entire spectrum of *Varidnaviria* members that infect hosts from all three domains of life, despite the fact that some viruses are difficult to recognize and the sequences are highly varied [16,17]. By employing sensitive profile-based methods to search metagenomic sequence data for DJR MCP contigs, an unexpectedly extensive array of varidnaviruses was identified, which are likely to infect bacterial and archaeal hosts. This diversity surpassed that of prokaryote viruses in the families *Turriviridae*, *Tectiviridae*, *Corticoviridae*, and *Autolykiviridae* in the class *Tectiliviricetes* [18]. In the 'PM2-like' category, which included several prophages, tailless phages were most prevalent. This group includes two *Corticoviridae* phages, PM2 [19] and Cr39582 [20], eleven Vibrio phages of the Autolykiviridae family, and the unassigned f No16 phage [21], which infect *Pseudoalteromonas* species. *Vinavirales* includes *Corticoviridae* and *Autolykiviridae* [22]. Most phages in this group have 9-12 kb genomes that are circular (*Corticoviridae*) or have inverted terminal repeats.

Vinavirales viruses encode structural components and genes that govern transcription, genome replication, and cell lysis. Proviruses from Vinavirales members are extensively incorporated into aquatic bacteria genomes. Their occurrence in other habitats is unknown. DJR MCP and packing ATPase are the only Vinavirales-shared proteins [23]. Metagenomics has been used to study the human intestine virome, a major microbiome factor. Most identified viruses are

Duplodnaviria domain tailed bacteriophages [24,25,26]. The most common human-associated viruses are crAss-like phages, which the ICTV recently categorized into the Crassvirales order. These phages were discovered by metagenomics investigation and have a wide variety of very varied members. Gut metagenomics has found many new abundant-tailed bacteriophage families [27,28] *Varidnaviria* members are likely a minor component of the gastrointestinal virome and are not well-known. Study conducted a search of gut metagenomes for DJR MCP and discovered that a significant number of the sequences encoding this hallmark protein of *Varidnaviria* are associated with prophages that are distantly related to the families *Corticoviridae* and *Autolykiviridae*. These prophages suggest a new Vinavirales order with at least three families. It found an extended, conserved gene core of Vinavirales with 12 genes, one of which encodes a hitherto undiscovered lysin protein, using sensitive protein structure prediction and sequence analysis methods. [29,30.31]

Structure and composition:

Unlike cells, phages viruses that infect bacteria cannot perform most biological processes needed for reproduction. They modify ecosystems by preying on certain bacterial populations, mediating lateral gene transfer, altering host metabolism, and redistributing bacterial chemicals by cell lysis [23,33,34,35,26]. They spread antibiotic resistance and pathogenic agents that infect humans and animals [37,38]. Our knowledge of phages comes from laboratory studies, most of which had genomes a few tens of kb. Many isolation methods remove big phage particles from phage concentrations obtained by 100-nm or 200-nm filters. Only 93 isolated phages with genomes over 200 kb were published in 2017 [32].

Community-wide DNA sequencing is capable of detecting phage-derived fragments; however, fragmentation can obscure large genomes. A novel clade of megaphages associated with humans and animals was identified in the genomes of metagenomic datasets that were manually curated [39]. In order to ascertain the prevalence, diversity, and environmental distribution of large-genome phages, we implemented a more comprehensive investigation of microbial communities. In the past, phages with genomes over 200 kb were called "jumbophages" or megaphages [40]. These phages have evolved a unique "life" strategy that entails substantial host biology interception and enhancement while replicating their massive genomes. [41,42,43].[44] were able to reconstruct 351 phages, 6 plasmid-like, and 4 unclassified sequences. It exclusively retained CRISPR–Cas loci and excluded plasmids. We incorporated three phage sequences that were ≤200 kb in length as a result

of the CRISPR-Cas loci. As anticipated, we identified numerous phage-relevant genes, such as those that are involved in the encoding of structural proteins and lysis, as well as other genomic properties of phages. It was anticipated that certain structural proteins would be as long as 7,694 amino acids. 175 phage sequences were circularized, and 35 were manually curated to completion, occasionally by resolving complex repetition sections to disclose encoded proteins. Although the majority of genomes are incomplete, a few may be complete but linear. Bidirectional replication is indicated by the GC asymmetry of 30% of genomes, while unidirectional replication is indicated by 30% of genomes [45]. The largest phage genomes known are our 4 largest complete, carefully curated, and circularized genomes, 634, 636, 642, and 735 kb. The largest circularized phage genome known was 596 kb [46]. The prior work reported a 630-kb circularized genome, but it was an assembly artifact. Concatenation artefacts were so severe in IMG/VR [47] that we excluded these data from future analyses. We revised our phage genome size distribution using full and circularized genomes from our study and published genomes. Without the enormous phages mentioned here, entire phages had median genomic sizes of 52 kb. Thus, these sequences greatly increase the number of phages with extremely large genomes. [44]

Nine of our genomes have coding densities that are less than 78%, which may be attributed to a genetic code that deviates from the standard code. This effect is uncommon in phages; however, it has been observed in Lak phage. The UAG stop codon appears to have been reassigned to encode an amino acid in numerous genomes, primarily human and animal. By transitioning into a flanking bacterial genome sequence, only one region exceeding 200 kb was identified as a prophage. Nevertheless, prophage integration is feasible, as half of the genomes were not circularized. Genomes containing integrases suggest a temperate lifestyle in certain circumstances. [48] There are significant structural differences within these groupings, especially in tail tips and capsid diameters. Since the tail tube is isolated, this knowledge is enough to place the main tail protein in its structural context. Capsid, fiber, and baseplate proteins will also be skipped.

SPP1 is a phage of the tail-morphotype that is similar to *Siphoviridae*. The dodecameric portal protein gp6 is located on one vertex of the icosahedral procapsid of the SPP1 phage. The maturation of procapsid into capsid is facilitated by the translocation of viral DNA into the head and the release of Gp6 [49,50]. ATP is utilized by the packaging terminase to facilitate the passage of DNA through the portal complex. The hexameric ring–shaped adaptor protein complex gp15 and stopper protein complex gp16 attach to the portal complex after capsid formation to limit DNA

leakage, resulting in the formation of the head-to-tail connector [51]. The tail of SPP1 is dependent on the hexameric ring-shaped distal tail protein (Dit) gp19.1. The distal tail adsorption function and the tail tube [52] are connected by this junction. The length of the tail tube [53] is determined by 40 stacked hexameric rings of MTP gp17.1 and its C-terminally extended form gp17.1∗, which are constructed around a tape measure protein gp8, which may be trimeric. A ribosomal frameshift leads to the C-terminal expansion of gp17.1∗, which contains an immunoglobulin (Ig) fold and a fibronectin type III (FN3) domain. The tail tube is composed of gp17.1 and gp17.1∗ in a 3:1 ratio. Nevertheless, virions that contain only gp17.1 are infectious [54]. The FN3 domain may transiently interact with *Bacillus subtilis* cell wall carbohydrates, thereby increasing infection, during two-dimensional diffusion of virions on the outer bacterial surface. The tail tube top is tapered by a hexameric ring of the tail completion protein gp17, which binds to the stopper protein gp16 and connects the tail to the head-to-tail connector. The trimeric tail tip protein gp21, as well as potentially gp22, gp23, gp23.1, and gp24, are involved in the distal tail adsorption function [55]. The positions of these proteins are ambiguous. The YueB receptor ectodomain of Bacillus subtilis is irreversibly bound by gp21 or a protein, resulting in infection. The formation of the tail in λ phages commences with an initiator complex that includes tape measure protein, assembly chaperones, distal tail protein, baseplate proteins, and tail tip proteins. This combination enables MTP to polymerize onto the distal tail protein ring and tape measure protein, resulting in the formation of a helical tube that replaces assembly chaperones. When the tail tube completely engulfs the tape measure protein, MTP oligomerization concludes. The tail completion protein will truncate the tail and connect it to the head-to-tail connector to complete the virion [56].

In contrast, SPP1 phage MTP (gp17.1) can self-polymerize without protein [57]. The tail creation process may differ. Dissociating the tail tip after binding to the YueB receptor primes the release of the metastable tape measure protein from the tail tube. Next, the stopper protein gp16 opens diaphragm-like and ejects DNA into the tail tube to start infection [58]. Tape measure proteins may produce host membrane pores for DNA translocation [59]. T4 phage is a *Myoviridae*-like tail-morphotype phage. This phage has a more complicated head assembly than the others: The membrane-spanning initiation complex of the dodecameric portal protein gp20 and gp40 binds 11 scaffolding proteins to start procapsid assembly [60]. The prohead's fivefold vertices in the capsid shell are produced by the major capsid protein gp23 and the vertex protein gp24. Procapsids are released from the membrane and space is created for DNA by proteolytic cleavage of the

scaffolding and capsid proteins [61]. The ATP-dependent terminase translocates DNA through the portal complex and expands the procapsid into the final capsid. This process creates binding sites for the small outer capsid protein (gp soc) and the highly antigenic outer capsid protein (gp hoc) that decorate the capsid [62]. Icosahedral extremities and cylindrical equatorial central regions are features of the T4 phage capsid. The head is completed and the portal complex is sealed by the binding of the Gp13–gp14 neck complex. The six segments of the baseplate are arranged around a tube [63] to initiate the tail assembly.

The junction for tail tube polymerization [64] at the proximal end of the baseplate is composed of hexameric rings of gp48 and gp54, while the tail tip at the distal end is composed of gp27, gp5, and gp5.4. The trimeric proteins gp5 and gp5.4 puncture host membranes and hydrolyze host peptidoglycan, while gp27 is capable of passing DNA. To create the tail tube, the MTP gp19 polymerizes into 24 hexameric rings around the baseplate-anchored tape measure protein gp29 [65]. The tail tube terminator protein gp3 [66] taper the proximal end of the tail tube. Gp18, the sheath protein, undergoes helical polymerization around the tail tube [67]. Gp15 completes the tail by binding to gp3 and the terminal ring of the tail sheath. The virion is completed by the interaction between gp15 and (gp13–gp14), which connects the tail and head-to-tail connector. T4 is equipped with a head whisker at the head-to-tail connector and short and long tail fibers at the baseplate. The short tail fibers unwind from beneath the baseplate and irreversibly bond to LPS, anchoring the baseplate to the outer membrane, when E. coli's long tail fibers bind to LPSs and OmpC of the outer membrane [68].

The tail sheath is constricted by the baseplate's reorganizations, which compel the tail tip to pass through the outer membrane. This process digests the peptidoglycan layer and translocates the tape measure protein gp29 and viral DNA through the tail tube. An inner membrane pore may be generated by the tape measure protein gp29 and/or gp27, which enables DNA to enter the host cytoplasm [69]. Phage virions have complicated designs for host recognition, genome packaging, and genome delivery, with tail morphologies resembling *Podoviridae*, *Siphoviridae*, and *Myoviridae* families. Mechanisms of phage assembly and infection reveal coordinated protein interactions and ATP-driven DNA packaging motors. Unique RNA bacteriophages with lipid envelopes and multilayered capsids, enveloped dsRNA phages (*Cystoviridae*) share structural similarities with eukaryotic dsRNA viruses, suggesting evolutionary linkages.

Currently, phi6 and phi12 are the best-characterized dsRNA phages, their component proteins, and assembly intermediates. However, similar gene sets in known dsRNA phage isolates suggest a similar virion organization. However, new studies show structural variance, especially in host recognition and entrance components. Phi6, phi8, phi12, phi2954, phiNN, phiYY, phiNY, phiZ98, and CAP3 have enveloped spherical virions in negative-stain transmission electron microscopy (TEM) [70,71], cryo-EM, and cryo-ET [72]. Detergent or organic solvent sensitivity studies have shown that dsRNA phage virion lipids exist.

These are the sole membrane-enveloped RNA bacteriophages that are currently known. phi6 and phi8 virions exhibited envelope spikes of 2 and 7 nm following cryo-EM characterization. These structures are host attachment spikes produced by multimeric P3 complexes, as indicated by prior research on P3-deficient phi6 virions [73]. Subsequently, cryo-ET and three-dimensional reconstruction revealed toroidal or elongated structures on phi12 and phi2954 virions. By employing the S and L segments from phi12 and the M segment from phi2954, a recombinant phi12 phage was generated through reverse genetic technique. The recombinant phage exhibited host specificity and envelope surface features similar to those of phi2954 [74].

By dsRNA phage envelopes, icosahedrally symmetric nucleocapsids are enclosed. The nucleocapsid surface shell and polymerase complex were identified in these particles in the initial EM investigation on phi6 virions [75]. The protein P8 trimer nucleocapsid shell is arranged into an incomplete icosahedral T = 13 lattice, which is interrupted at the five-fold symmetry positions by P4 complexes protruding from the polymerase complex layer, as demonstrated by high-resolution cryo-EM imaging and three-dimensional reconstruction analyses of phages phi6 and phi12 [76]. The cystovirus phi8 deviates from this fundamental structure by lacking a nucleocapsid shell. Phi6 bonds to and penetrates the host plasma membrane through the P8 layer [77]. This suggests that polymerase complex components are responsible for these critical responsibilities, as purified phi8 core particles have the capacity to infect spheroplasts of host cells [78]. Additional research is necessary to ascertain the presence of a nucleocapsid surface shell that enables plasma membrane penetration in other dsRNA phages that are similar. The phi6 polymerase complex, which consists of the proteins P1, P2, P4, and P7, replicates and transcribes the viral dsRNA. Cryo-EM has identified dimers of the primary inner capsid protein (MCP) P1 on an icosahedral T = 1 lattice in the empty and/or genome-containing polymerase complex particles of phi6, phi8, and phi12 [79]. I find it intriguing that the capsid organization of picobirnaviruses, *Reovirales* order animal and plant

dsRNA viruses, and members of the families *Partitiviridae*, *Totiviridae*, *Chrysoviridae*, *Quadriviridae*, and *Megabirnaviridae* is similar (referred to as "T = 2") [80,81]. This organization is not present in any other viruses. This suggests that dsRNA viral capsids have a shared ancestry.

The P2 structure of the phi6 polymerase subunit was described by Butcher et al. in 2001. The first high-resolution structure of RNA-dependent RNA polymerase (RdRp) from dsRNA virus. The phi6 P2 structure was found to be remarkably similar to the RdRp of the hepatitis C virus, indicating that the polymerase subunit of a dsRNA phage and a eukaryotic positive-sense single-stranded RNA virus may have shared a common evolutionary origin. Several high-resolution structures for additional viral RdRps have been deposited in the protein data bank over the past two decades. A structure-based computational comparison has demonstrated that the phi6 and phi12 RdRps [82] are structurally similar to all currently structurally characterized viral RdRps [83].

Protein P4 is one of the most extensively studied cystovirus proteins in terms of its physical and functional characteristics. P4, a molecular motor that organizes viral single-stranded genomic precursor molecules into empty polymerase complexes, is powered by NTP hydrolysis. The structures of the P4 proteins of phi6, phi8, phi12, phi13, and phiYY are of high resolution [84]. Despite structural differences, these proteins form hexameric complexes that resemble RecA-type ATPases. A genome with three dsRNA segments: L (large, 6.3–7.1 kb), M (medium, 3.6–4.7 kb), and S is present in each *Cystoviridae* family member and related dsRNA phage isolate. The genome is in the range of 12.7–15.0 kb (phi2954–phi8). Comparative genomic analyses indicate that certain dsRNA phage isolates are closely associated with *Pseudomonas* phage phi6, while others are distant. The L, M, and S segments of *Pseudomonas* phages phiNN and phi6 exhibit 80%, 55%, and 84% nucleotide sequence identity, respectively. However, phiNY does not appear to share any similarities with other cystoviruses or phages.

The genome organizations of the six-dsRNA phage isolate CAP3–7 and phiZ98, as well as the currently recognized cystoviruses, are comparable. The L segment encodes proteins for the polymerase complex (MCP P1, RdRp P2, packaging NTPase P4, and assembly factor P7), the M segment encodes proteins for host recognition and outer membrane penetration (P3 and P6), and the S segment encodes the nucleocapsid shell protein (P8), major membrane protein (P9), putative membrane morphogenetic factor (non-structural protein P12), and the RNA polymerase. In contrast, the majority of the open reading frames that are expected to be generated by phiNY contain proteins of unknown function, and only the RdRp and MCP genes in the L segment and

the glycoside hydrolase gene in the S segment could be predicted, necessitating further investigation. Nevertheless, the coding regions are flanked by non-coding sections in all dsRNA phage genome segments. According to phi6 research, these non-coding regions are essential for genome packaging and replication. [85,86]. Despite their high gene synteny, certain cystoviruses possess open reading frames with ambiguous functions. The P3 host recognition spike complex contains a single P3 protein or its multimer in the spike complex of cystoviruses phi6, phi2954, and phiNN [87]. In contrast, the heteromeric spike complex of phi8, phi12, phi13, and phiYY contains two or three viral proteins [88]. Recent metatranscriptomic surveys indicate that dsRNA phages have developed a variety of lytic enzymes. Cystoviridae members encode lytic transglycosylases from the lysozyme superfamily. However, some cystovirus-like contigs encode putative N-acetylmuramoyl-L-alanine amidase, metallopeptidase (families M15 and M23), lipase, and L-alanyl-D-glutamate endopeptidase genes. The variety of lysis genes in these potential dsRNA phages may suggest a broader host range. [89]

Phage Replication Cycles and Infection Strategies:

Bacteriophages undergo lytic or lysogenous cycles. Furthermore, certain phages are pseudolysogenic. Proteins that are anticipated to locate the bacterial membrane or cell surface are encoded by the phage genomes. These factors may influence the susceptibility of the host to phage infection. Almost all of the host metabolism-enhancing genes that were previously identified were discovered. Numerous phages possess genes that synthesize purines and pyrimidines de novo and convert nucleic and ribonucleic acids and nucleotide phosphorylation states. These gene sets are reminiscent of microorganisms that are remarkably minuscule and likely have symbiotic relationships. Numerous phages contain transcription and translation genes. The unique sequences of up to 67 tRNAs are encoded by complete phage genomes. In general, the number of tRNAs increases as the genome length increases (Spearman's $\rho = 0.61$, $P = 4.5 \times 10{-}22$, $n = 201$). Each genome of large phages contains up to 15 tRNA synthetases that are distinct but related to those of their hosts. Via these proteins, phages can charge their tRNA variations with host amino acids. Genes for tRNA modification and ligation of host defense-cleaved tRNAs are present in certain genomes.

Genes that intercept and redirect host translation are present in numerous phages. Among these genes are the ribosomal proteins S4, S1, S21, and L7/L12, as well as the initiation factors IF1

and IF3. Ribosomes were recently discovered in phages. rpS1 and rpS21 are indispensable for the initiation of translation in bacteria, which renders them advantageous for the hijacking of host ribosomes. Additional investigation of rpS21 proteins revealed N-terminal extensions that contain RNA-binding basic and aromatic residues. The anticipation of that these phage ribosomal proteins will supplant host proteins and promote competitive ribosome binding or preferential phage mRNA initiation. Lytic phages, such as T4, lyse and eradicate bacterial cells following virion multiplication. Phage offspring are capable of infecting other organisms following the cell's death. Lytic phages are more effective for therapeutic purposes. Some lytic phages prevent phage progeny from lysing out of the cell when extracellular phage concentrations are high.

This process differs from temperate phage dormancy and is usually transient. [90] The lysogenic cycle does not immediately lyse the host cell. Lysogeny-capable phages are temperate. Their viral genome integrates with host DNA and replicates harmlessly or becomes a plasmid. Endogenous phages (prophages) activate when host circumstances decrease, such as nutritional deprivation. The host cell lyses when they start reproducing. The lysogenic cycle permits the host cell to live and proliferate, replicating the virus in all offspring. *E. coli's* lambda phage follows the lysogenic and lytic cycles.[91] Prophages can help the host bacterium by adding additional functionalities to the genome during dormancy, called lysogenic conversion. Bacteriophages can turn safe strains of *Corynebacterium diphtheriae* or *Vibrio cholerae* into highly virulent ones that cause diphtheria or cholera. [92] There are ways to fight certain bacterial infections by targeting toxin-encoding prophages. [93] The viruses in this electron micrograph of bacteriophages adhering to a bacterial cell are coliphage-sized. T1

Bacterial cells' polysaccharide cell walls shield them against antibiotics and immunological host defenses. [94] Bacteriophages bind to lipopolysaccharides, teichoic acids, proteins, or flagella on bacteria to enter a host cell. Bacteriophages can only infect bacteria with receptors they can bind to, limiting their host range. Virion-associated polysaccharide-degrading enzymes breakdown their hosts' capsular outer coat during the start of a tightly regulated phage infection. Host growth parameters affect phage attachment and invasion. Phage virions cannot move autonomously, thus they must randomly contact the right receptors in solution, such as blood, lymphatic circulation, irrigation, soil water, etc. Myovirus bacteriophages inject genetic material into cells using a hypodermic syringe. The tail fibers flex to bring the base plate closer to the cell after hitting the receptor. This is reversible binding. Once fully joined, irreversible binding begins and the tail

contracts, presumably with ATP, shooting genetic material through the bacterial membrane. [95] The shaft bends to the side, contracts closer to the cell, and pushes up to inject. Unlike myoviruses, podoviruses use their short, tooth-like tail fibers to enzymatically destroy a section of the cell membrane before introducing their genetic material.

In minutes, bacterial ribosomes convert viral mRNA into protein. Early RNA replicase synthesis occurs in RNA-based phages. Proteins make bacterial RNA polymerase prefer viral mRNA. The host must produce viral products instead of proteins and nucleic acids due to disruptions. Ribosomal proteins in some dsDNA bacteriophages may influence protein translation during infection. [96] T4 phage morphogenesis requires catalytic helper proteins to build new virus particles. The foundation plates are produced first, then the tails. Separately built head capsids will spontaneously assemble with tails. Phage genes encode morphogenetic proteins that interact in a specific sequence during phage T4 virion formation. Normal phage T4 morphogenesis requires a balance in these proteins produced during viral infection. DNA is efficiently packed in heads. The entire process takes 15 minutes.

studies of bacteriophage T4 (1962–1964) revealed almost all of the genes required for laboratory development. [97] Two groups of conditional lethal mutations enabled these experiments. Amber mutants were one type. Temperature-sensitive mutants were another type of conditional lethal mutant. These two groups of mutants revealed the activities and connections of proteins involved in DNA replication, repair, and recombination and how viruses are built from protein and nucleic acid components. Sometimes phages are released via cell lysis, extrusion, or budding. Tailed phages lyse by breaking down cell wall peptidoglycan with endolysin. Filamentous phages cause host cells to release new virus particles. Free virions can infect a new bacterium unless faulty. Budding is linked to Mycoplasma phages. Unlike virion release, lysogenic phages become prophages and stay in the host. [98]

The majority of cystovirus isolates are virulent and lyse the bacterial cells of their host during reproduction. Certain dsRNA phages have the ability to produce viral particles within the host cell without causing lysis or integrating into the host chromosome. This single-cell phenomenon be referred to as a non-productive chronic infection or carrier cell in order to differentiate it from the carrier state life cycle, which should be allocated to population-level phage-host interactions. In cell cultures that are continuously evolving, cystovirus carrier cells are identified by intracellular viral genomic dsRNA molecules or viral particles. phi6 and its *P.*

syringae host were first reported to infect this way, but the phi13 variant (containing a kanamycin resistance gene) has also been found to infect Salmonella typhimurium and *E. coli*. Recently, dsRNA phage phiNY was shown to infect similarly. The continuous phiNY infection boosted Microvirgula aerodenitrificans host growth, suggesting a mutualistic, parasitic lifestyle. Interestingly, dsRNA phages persist like fungus dsRNA viruses, which do not create external infectious virions and are mostly cryptic.

Bacteriophages are essential to the human microbiome and mediate genetic exchange between pathogenic and nonpathogenic bacteria, even though they cannot infect and multiply in human cells. Transduction occurs when a bacteriophage transfers genes from one bacterial strain to another. Endocytosis pathways are key to eukaryotic cell bacteriophage uptake. Phages cannot multiply in eukaryotic cells but can be absorbed through cellular processes similar to those utilized for other particles or viruses: Phages can be taken up by clathrin-mediated, caveolin-dependent, or macropinocytosis from eukaryotic cells. Phages can enter vesicles and pass epithelial barriers via these internalization mechanisms [99]. Receptor Binding: Polysialic acid-binding E. coli phages can enter neuroblastoma cells by interacting with cell surface chemicals like polysialic acids [100]. After endocytosis, some phages can cross human epithelial cells to reach underlying tissues via intracellular trafficking processes that converge on recycling endosomes [99]. Phage Display and Cell Penetration Peptides: The introduction of TAT peptides into engineered phages is known to promote the penetration of phages into various eukaryotic cells as well as improved membrane translocation. [101] Phage uptake in eukaryotic cells has been extensively studied, detailing the various avenues by which phages could penetrate human cells, shape immunologic responses and provide therapeutic benefits.

How eukaryotic cells eat bacteriophage has been described experimentally in recent work: Mammalian cells predominantly internalize the bacteriophage T4 via macropinocytosis, which engulfs particles and extracellular fluid (2023). Intact phages accumulate into macropinosomes for endosomal processes such lysosomal degradation or exocytosis. Importantly, when phages were internalized, they sparked a cascade of protein phosphorylation that boosted cell metabolism and survival rates, without engaging the inflammatory immune pathways that sense DNA. This observation has particular implications for interactions between the microbiome and phage therapy [102]. Another study investigated the internalization of polysialic acid-binding Escherichia coli bacteriophage (PK1A2) in neuroblastoma cells. Internalization of the phage-receptor complex by the endolysosomal pathway was evidenced by fluorescence and electron imaging. Inside the cell, the phages lingered for twenty-four hours. Realizing that receptor-mediated endocytosis represents

a dominant uptake pathway, phages were diverted to vesicular compartments surrounding the nucleus. Filamentous M13 phages in mammalian cells, not only carrying infiltrating peptides as TAT also led to enhance endocytosis and intracellular trafficking. This modification of the phage surface led to better therapeutic delivery and cell penetration [103]. The study underscores the potent role of active cellular processes such as macropinocytosis or receptor-mediated endocytosis for phage internalization in human cells. The results show that phages travel through endosomes and how internalized phage may respond to cellular signaling.

Some molecular receptors eukaryotic cells use for bacteriophage binding have been explored experimentally: Polysilic acid is a well-characterized receptor on human neuroblastoma cells that bind and absorb such phages from Escherichia coli. This sialic acid polymer facilitates eukaryotic cell receptor mediated phage endocytosis [105]. Phage receptors in bacteria are more specific, but eukaryotic cells may bind phages through carbohydrate moieties on the cell surface [106]. Membrane permeabiliz Privateies: TAT and other engineered phage peptides increase absorption of mammalian cells by binding with negatively charged elements of the cell membrane, either through proteoglycans or phospholipids [107].

Many eukaryotic phage receptors' molecular identities are unknown. In contrast, bacterial phage receptors typically contain outer membrane proteins, lipopolysaccharides, teichoic acids, and polysaccharides that have been extensively studied in numerous phage-host systems [108]. Thus, polysialic acid and certain surface glycans are major bacteriophage receptors for eukaryotic cells, while cell-penetrating peptide-targeted membrane components offer tailored uptake options.

Lytic cycles with fast host cell lysis or lysogenic cycles integrating their genome into host chromosomes allow persistence and horizontal gene transfer. Some dsRNA phages cause chronic non-lytic host cell infections. Tail fibers or spikes that identify lipopolysaccharides or proteins bind to bacterial cells specifically. Phage infection involves anti-CRISPR proteins and nucleus-like compartments that protect phage DNA to avoid host resistance.

Horizontal Gene Transfer, Phages:

Phages transfer horizontal genes by universal and specialized transduction, affecting bacterial virulence, antibiotic resistance, and evolution. Lysogenic conversion via prophages encodes toxins and other virulence factors, causing bacterial disease.

Transduction:

As the host cell disintegrates from lytic replication, generalized transduction bundles random bacterial genomic DNA in phage capsids instead of phage genomic DNA. The bacterium's genome and that of its progeny cells may be altered if this phage injects this bacterial DNA into a healthy host cell and integrates into the bacterium's chromosome. When initiating a lytic replication cycle in specialized transduction, lysogenic phages that have been amplified in a population of bacteria may eliminate a portion of the bacterial DNA with their genome. Lysogens share an integration site, which is why all progeny phages transfer the same bacterial gene to their hosts.

In addition to genetic exchange, bacteriophages can affect microbial populations by preying on select bacteria species while ignoring others. This characteristic has been studied to treat pathogenic bacterial infections in humans and animals for over 100 years. Wild phages may temporarily affect wild bacterial populations, [109], but lytic bacteriophages as antimicrobial therapy (phage therapy) in humans face significant challenges. Various wild bacterial strains resist phages. Famous resistance mechanisms include the CRISPR-Cas9 system, which was created for genetic manipulation in the lab and started as a bacterial defensive mechanism against bacteriophage invasion. [110] Phages are more immunogenic than antimicrobials and removed from the blood by the reticular endothelial system quickly. If effective phage mixtures are developed, their size may limit their topical use compared to antimicrobials. Some researchers recommend using phage enzymes, which can permeate bacterial cell walls, for simplicity. [111] No randomized, controlled, double-blind trials have proved either technique works in humans.

Host Specificity and Phage-Host Interactions

Bacteriophage-human cell interactions are a new field of study that combines microbiology and immunology and reveals exciting intricacies beyond basic bacteria-phage dynamics. The gut is full of phages, viruses that infect bacteria, which regulate bacterial ecosystems and immunological activities. Phages can directly interact with human cells, affecting immunological responses, inflammation, and tissue homeostasis, according to recent studies. Phages are capable of attaching to and being internalized by eukaryotic cells, with effects on cell signaling pathways and immune activation; however, phage infections in humans occur extremely infrequently.

Beyond microbiota modulation, phages interact with human cells and have therapeutic links. Phages that enter human macrophages and epithelial cells can antibiotic-treat microbes

within cells. Due to their complicated life cycle phages practice control of bacteria which are resistant against some drugs. Besides tailoring inflammatory and antiviral defenses, phages can modulate innate immune responses through interaction with receptors on human immune cells. Both phage type and bacterial habitat, as well as host immunological status play key roles in the interaction of phages with human cells. Some phages have evolved specific tactics to bypass the human immune system or manipulate the host cell in ways that ultimately advantage the bacteria they infect. While phages do not elicit disease in human themselves, their interactions with human cells may modulate dysbiotic inflammation, immunological tolerance and gut homeostasis. Phage applications have been assessed for promising antibacterial and cell-interacting medical agents. The more in phages pathogenicity with human cells prove that they are also predators, not just a force of the bacteria. Phages guard against the spread of intracellular infections in human cells, making them a viable novel treatment through immune system regulation. Phages play a critical role in maintaining health through various cellular interactions within the microbiota. page 12–18. While phages (or bacteriophages) are known to infect bacteria, a fresh study has revealed their complex relationships with human cells. Phages play a crucial role in preventing bacterial infections and maintaining really homeostasis of microbes such as the respiratory system, skin, and gastrointestinal tract. Phages that adhere to human cell surfaces, invade cells and alter biological activity hold promise for phage therapy and immune system modulation. The internalization of phages by mammalian cells is critical for phage-human cell interaction. It was also shown in several studies that some specific phages can endocytose from human immune and epithelial tissue-derived cells. Phages can either (i) deliver genetic material, (ii) modulate signaling pathways or (iii) interact with intracellular elements. Phages therefore work only outside cells—and that is now the general view, although low numbers of intracellular phages suggest control over inflammation and host immunity might be possible.

Phages directly affect both bacterial populations and immunological cells. They induce production of cytokines and regulate inflammation through activation of pattern recognition receptors on immune cells. Phages possess immunomodulatory as well as antibacterial properties, which make them a potential candidate for novel therapeutic agents against both bacterial diseases and inflammatory diseases. Phages have been found to be largely safe for humans in clinical trials. To fight diseases caused by those bacteria resistant to antibiotics, researchers are turning to phages

which can bind to human cells. Phages are able to penetrate host cells and reach germs that have been buried beneath biofilm, which allows treatment of chronic infections more effectively.

Phage-host cell interactions are being studied to increase delivery, reduce resistance, and boost immunomodulation while maintaining safety. Besides bacterial targeting, bacteriophages and human cells interact directly, modulate the immune system, and have therapeutic potential. This new knowledge calls into question phages' role in human physiology and immunological homeostasis as well as antibacterial agents. [19-126]. Phages change the bacterial microbiome and affect human physiology and immunology without infecting cells. Endocytosis or receptors allow phages to enter tissues and cells, altering mucosal immunity and cell activity. Microbial ecology and immunological homeostasis are regulated by a complex tri-kingdom interaction network of bacteriophages, bacteria, and human cells.

Interactions with Mammalian Immunity:

Clinical aspects:

Phages are clinically significant for many reasons. First, bacteriophage genomes encode several highly pathogenic bacterial toxins, making the host bacterium only pathogenic when lysogenized. *Vibrio cholerae*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium difficile*, and *Shigella species* produce cholera, diphtheria, botulinum neurotoxin, binary, and Shiga toxins, respectively. [127]. These bacteria are either harmless or nonpathogenic without phage-encoded toxins. Phages encode these poisons for unknown reasons. Botulinum toxin paralysis seems to have the opposite effect of cholera toxin, which causes watery diarrhea and helps the phage and host find their next victim. Second, bacteriophages enable horizontal gene transfer, including antibiotic resistance. They have been developed to incorporate genes into specific strains for clinical use, but this is still in testing. [128] A third therapeutically relevant component of bacteriophages is their usage as a biomarker for their host in complicated environmental samples. This usually indicates water feces pollution. The host is likely present if the phage is. To identify bacteria in mixed environmental samples, phages have been designed to produce luciferase when they infect their host.

Bacteriophages may identify strains of the same bacterial species, making them clinically valuable even though they are generally replaced by newer technology. As humans are susceptible

to many viruses, most bacteria have multiple bacteriophage pathogens. Not all strains of a species are phage-resistant. Infecting each strain routinely with a standardized panel of phages for that species reveals its sensitivity and resistance to each phage type. To distinguish S. aureus strains, phage typing used a standardized panel of bacteriophages common internationally. Phage typing was the standard epidemiological strain monitoring approach before molecular methods like multilocus sequence typing and pulsed-field gel electrophoresis. Finally, bacteriophages were the first virus discovered and contributed to several molecular biology breakthroughs. Bacteriophages proved that DNA transferred genetic information, set up gene control, and revealed the genetic code. [129]

Immune Responses to Phages in Humans

Phages greatly influence the immune system: Innate immunity: Phages activate pattern recognition receptors, producing cytokines and modulating immunity. Adaptive immunity: Strong humoral responses generate IgM, IgG, and mucosal IgA antibodies that neutralize phages and alter clearance and therapy. Cellular adaptive responses include T cell activation. Repetition of phage therapy reduces bioavailability due to host immunological neutralization by antibodies and complement. Phages can cause pro- or anti-inflammatory immune responses depending on context and purity. Phages can influence immune cells to help eliminate microorganisms synergistically or inhibit immunological processes, demonstrating diverse immunomodulatory actions.

Phages' indirect impact on mammalian immunity:

Bacterial disease, ecology, and genetic evolution are all significantly influenced by phages. Consequently, phages indirectly influence host defense and immunological function. Bacterial virulence and human immunity are influenced by the proteins encoded by phages. Lysogenic phages encode proteins that enable their bacterial hosts to penetrate tissue barriers, which serve as the initial line of defense against infections. The temperate phage Φctx is renowned for its ability to parasitize *Vibrio cholerae* hrough the production of cholera toxin. Bacterial adhesion, colonization, tissue invasion, and biofilm formation are all facilitated by phage-derived virulence factors [130,131].

The immune-clearing phagocytes are either destroyed or discouraged by phage-encoded proteins. The phage-encoded chemotaxis inhibitory protein of Staphylococcus aureus binds to and

inhibits neutrophil receptors for complement and formylated proteins, thereby safeguarding it from neutrophil-mediated mortality. Exotoxin [132], cytotoxicity, intracellular infection [133], and superantigen are all produced or delivered by other phage-encoded proteins. Chromosome transcription factors primarily regulate phage-encoded virulence genes, which are synthesized during lysogeny, when other genes are not actively transcribed. For example, the lytic phage genes are located on the coding strand, while the λ-encoded bor of *E. coli* and the phage-encoded vir of M. arthritidis are located on the noncoding strand. Lastly, phages horizontally transfer antibiotic resistance genes within and between bacteria [134]. Prophages improve their bacterial hosts' fitness and propagation. Prophages can shed genes, including virion-producing genes, and domesticate [135]. When they still benefit, prophage-derived genomic elements can be selectively retained [136]. Thus, many phage-bacteria interactions are complex coevolutionary interactions.

Direct Interactions with Phages and Innate Immunity:

Innate immunity controls the microbial-human contact through structural and germline-encoded characteristics. Phages may be essential to our relationship with bacterial flora, as they contribute to and cross these barriers. In the mucosa, circulation, and cells, phages are identified on the cell surface and in endocytic vesicles. Phages are abundant at bacterial colonization sites and may directly contribute to mucosal barrier defenses. Barr et al. [137] discovered that mucosal surfaces can retain as many as 109 adhering phages per biopsy. Phages can be modified to facilitate these interactions. Immunoglobulin (Ig)-like domains are present in *E. coli* T4 phage capsid proteins, which interact with epithelial cell mucins and surface glycoproteins. Many phage families include Ig superfamily-like protein domains, suggesting mucosal layer enrichment of other phages [138]. Mucosal binding increased the susceptibility of some bacteria to phage-mediated lysis [139] and phage diffusion in the mucus layer [140]. Thus, mucosal phages may prevent bacterial invasion in a ubiquitous, strain-specific, and non-host-derived manner. These findings suggest a complicated interaction between phages, bacteria, and mucosal surfaces that needs additional study. Antiphage antibodies may weaken or change barrier immunity, among other questions.

In mice models of colitis, the intestinal virome affects innate immunity, which may affect outcomes. Yang et al. [141] found that antiviral medicines worsened DSS-induced colitis in mice, but gut-resident viruses detected by TLR 3 and TLR7 protected via IFN-β production. Phages make

up most of the intestinal virome, however this study did not focus on them. In contrast, Gogokhia et al. [142] found that oral phage cocktail exacerbated DSS colitis via TLR9. Phages' significance in intestinal homeostasis needs further study. Mucosal-associated phages may affect tissues beyond the epithelial barrier. Transcytosis across cells transports many phages across the gut and into systemic circulation, possibly for paracytosis at inflammatory sites. Based on in vitro studies of cell monolayers [142], this transit is expected to occur at 3.1 × 107 particles/day. Transcytosis across the Golgi apparatus allows phage T4 to absorb apically to basolaterally. Certain phages and peptide sequences may be more easily taken up [144]. T1 phage straight entering the small intestine entered gut-draining lymph and blood. Bioactivity against bacteria in circulating phages may help hosts defend against bloodstream bacteria. These investigations demonstrate the potential importance of phage interactions with mucosal immunity and how little we know about them. It is unclear how many phages transverse mucosal tissues, what processes are involved, if M cells, which sense the intestinal environment, aid transmission, or whether inflammation influences this transit.

Peripheral circulation and tissues contain several phages. The pharmacokinetics of some of these phages have been widely studied for lytic phage treatment. Regardless of method, circulating phages clear temporally and spatially. Phages last several days, with the biggest decline (>99%) in the first hour. After infusion, phages are discovered in most major organs, with the liver and spleen having the greatest and most lasting titers, indicating that these organs remove circulating phage particles. Splenic and hepatic macrophages phagocytose phage quickly and efficiently, according to research. Active phage is less persistent in the liver than in the spleen. Kupffer cells endocytose better, retain greater basal ROS levels, and produce fewer proinflammatory cytokines in response to TLR ligation than splenic macrophages [145]. These cells' phagolysosomes digested and inactivated internalized phage. Phage clearance involves two steps: quick elimination of most particles in 24 hours, followed by slower clearance of the remaining ~1% of circulating phages over many days. This slower tail may be phages trapped by antibodies or other immune response components. Inflammation and bacterial contamination may influence phage pharmacokinetics [146].

Immunologically, circulating and peripheral phages are well tolerated. Phages at immunological privilege areas including the placenta and CSF are also well tolerated. The recently obtained CSF virome shows that phage are abundant (104 pfu/mL) without inflammation [147]. This

is noteworthy since phages are related with LPS, bacterial DNA, and other powerful immune stimulants. Even with remaining bacterial contaminants, lytic phage treatment infusion is well tolerated. It is probable that mechanisms exist to facilitate the immunological reception of numerous phages in peripheral tissues and circulation. Phage capsid proteins may be engineered to mitigate immune detection. Phages that are designed to exhibit peptides for epitope identification or phage vaccines are immunogenic; however, lytic phage therapy is well-tolerated. The clearance of phages in the liver and spleen may also be influenced by tolerogenic pathways. In the liver and spleen, there is an abundance of tolerogenic macrophages and DCs. Finally, endogenous phages may possess the unique ability to stimulate tolerogenic immunological responses, such as the differentiation of M2 macrophages. Phages are absorbed by a diverse array of eukaryotic cells through nonspecific uptake, receptor-mediated endocytosis, and the uptake of bacteria-harboring prophages [148,149].

Most phages are cleared by phagocytes. This literature focuses on tumor cell lines and biotechnology-engineered phages, however wild (unmodified) phage uptake is widespread and may follow comparable routes. The capsid protein gp24 of the E. coli phage T4 is characterized by a Lys-Gly-Asp motif that interacts with β3-integrin receptors on target cells. Chondroitin sulfate proteoglycans are associated with phage absorption in other studies. The filamentous E. coli phage M13 [150] is subjected to a variety of receptor-mediated endocytosis mechanisms by various cell lines. Most phages' cellular uptake receptors and processes are unclear. Phages are degraded in endosomal vesicles, cytoplasm, nucleus, Golgi, and lysosomes after internalization. Recently, intracellular phages were demonstrated to be bioactive against intracellular bacterial infections, including *Mycobacteria abscessus* [151]. Phagocytosis can also expel Listeria from phagosomes by removing prophages. Phages can reach the nucleus and create RNA and protein, as shown by phage DNA vaccines [152]. A new review [153] details intracellular phage. Multiple cell-surface and intracellular pattern-recognition receptors (PRRs) identify phages during cellular uptake and transit [153]. Most pathways include detecting ssDNA and dsDNA and inducing IFN responses.

Several studies have linked endosomal PRR TLR9 to phage recognition. TLR9 detects unmethylated CpG patterns found in phage and bacteria DNA [154]. TLR9 activates proinflammatory and antiviral cytokines via MyD88. Recent gut phage sensing research has also linked TLR9 to phage-induced inflammation. The use of oral E. coli tailed phages enhanced IFN-

γ-producing CD4+ T cells, mediated by DC sensing of phage DNA via TLR9. The role of TLR9 in phage responses may be complicated. Hashiguchi et al. [155] found that MyD88−/− mice's weak antibody responses to M13 phage immunization indicate the importance of TLR signaling in antiphage adaptive immunity. However, TLR9−/− animals had much higher IgG levels. The authors suggested that TLR9 regulates M13 ssDNA genome sensing. The primary integrating axis for cytosolic dsDNA sensing is the STING route. STING has not been implicated in phage sensing, despite the fact that modified phages enter the cytosol. Phage sensing is also facilitated by TLR3, an endosomal RNA sensor [156]. Only TLR3 strongly induces antiviral cytokines by signaling through the adaptor Toll/interleukin-1 receptor (IL-1R) resistance (TIR) domain-containing adapter-inducing IFN-β (TRIF). Sweere et al. [156] discovered that Pf, a filamentous phage that infects P. aeruginosa, activates IFN-β in DCs through TLR3 and TRIF. In eukaryotic cells, this RNA-sensing receptor is activated by phage-derived RNA synthesis; however, the mechanism by which Pf initiates transcription in mammalian cells remains enigmatic. This was not the initial report of phage genome transcription in a eukaryotic environment, as phage DNA vaccines require RNA to generate protein. However, mammalian cell bacterial absorption is still poorly understood. It is uncertain if phages are cell type-tropic [157].

If phages attack human tissues, the immune syste responds in two systems: innate and adaptive. According to pattern recognition receptors on immune cells, the innate immune system recognizes phages and releases cytokines in tissues and induces inflammation. Uptake of phage by dendritic cells and macrophages induces adaptive immunity through delivery of phage-derived antigens on MHC intermediates to T cells. Phages induce specific antibodies in the adaptive immune system. IgM antibodies emerge first upon exposure to phage, and they do by several days. IgG antibodies are generated by the immune system to defend against subsequent phage infections upon repeated exposure. These antibodies latch onto phages and purge them from the body. Phage inactivation is magnified when antibody-mediated neutralization is combined with the complement system. While phages infect bacteria, it seems that the immune system treats them like some eukaryotic viruses (as evidenced by the antibody/complement pincer). Repeated exposure to phages reinforces antibody responses as T helper cells promote B cell expansion into plasmablasts and memory B cells upon recognition of phage antigens. Immunity to specific phages can reduce their therapeutic function as memory-mediated immune responses can facilitate expedited neutralization during subsequent exposures.

Phages may modulate immune cell activation and inflammatory equilibrium in human tissues, which could be used therapeutically. Thus, human tissues respond to phages with immediate innate recognition, cellular antigen presentation, and a powerful, adaptive antibody response that neutralizes phages and regulates the immune system. Learning these pathways is crucial to improving phage therapy and predicting immune responses to phages delivered into the body. [159-163] Human tissues respond to phages with innate and adaptive immunity. Innate immune cells produce cytokines when they recognize phages via pattern recognition receptors. Antigen-presenting cells absorb phages and present MHC molecules with phage-derived antigens to T cells, initiating adaptive immunity. Adaptive immunity develops phage-specific IgM antibodies days after exposure and stronger IgG antibodies following re-exposure. By binding and clearing phages, these antibodies work with the complement system to neutralize them. Phages, bacterial viruses, have similar immunological treatment to eukaryotic viruses, as seen by this antibody/complement action. Phage antigen-activated T helper cells differentiate B cells into antibody-producing plasma blasts and memory B cells for long-term immunity. Immunologic memory can also negate phage therapy in addition to control of immune activation and inflammation. The complex immune response to phages within human tissues includes rapid innate recognition [47], antigen presentation [48], and all the components of robust adaptive antibody responses for suppression and regulation of immunity. Understanding these interactions is critical for improving phage therapy and predicting immunological responses.

The effects of the host immune system response can play an important role in phage therapy, given that phage specific antibodies are produced by the host during treatment and may affect future treatment. On exposure, the immune system mounts an antibody response against therapeutic phages that follows an IgM to IgG and finally IgA temporal switch. These antibodies are allowed to bind to phages that they can neutralize, and then vanquish them by repeatedly binding to new target bacterial cells until the phages have some intrinsic loss of activity against subsequent doses during species/microbiome succession. Multiple injections of the phage, especially via intravenous or intra-abdominal routes, appears to elevate antibody titers in animal studies. This antibody response reduces the time that phages circulation and their ability to reach sites of infection after subsequent injections, by increasing their clearance from the bloodstream. IgG antibodies persist after treatment has abated, and upon re-exposure to the same phage IgA titers can mount quickly resulting in improved neutralization. The distribution mode determines antibody induction. Because responses to phage-specific antibodies are much more severe when

introduced intravenously rather than orally, the activity of the phages may be maintained throughout treatment. Recurrent phage therapy may be hampered by increasing immune reactions induced in response to inhalation (nebulization) and systemic injections, unless formulations or treatment regimens are modified to evade or manage the immune response. In persistent phage therapy, the effectiveness of antibodies neutralizing against use of phages can be countered through immunosuppressive techniques [38] as well as modulation of surface protein on the particles for decreased immunogenicity [39] or by modifying types (cocktails) [40]. However, in order to guide the design of effective recurrent phage therapy regimens, it is essential to understand how the pharmacokinetics of phages and kinetics of antibody induction interact. Phage-specific antibodies induce the destruction and rapid clearance of therapeutic phages, thus leading to impaired efficacy. This immune response necessitates the use of personalized treatment strategies to overcome and preserve therapeutic effectiveness. [146-168]

IgG and IgA antibodies have different impacts on phage biodistribution and efficacy due to their distinct functions and localization in the immune response. As IgG antibodies bind to specific phages in the blood and other body compartments, those phages become neutralized, cleared from circulation, and rendered less bioavailable (and thus less effective against bacteria). Elevated IgG levels, especially after parenteral administration (frantically neutralizes and clears the phage) would limit systemic efficacy of phage therapy. Most IgA antibodies exist in mucosal tissues, including those of the respiratory system and gastrointestinal tract, and in secretions. Secretory IgA prevents entry and survival of phages at mucosal barriers through localized neutralization. Since IgA restricts phage activity in the stomach, increased levels of phage-specific IgA indicate decreased passage and/or efficacy of phages if active phages are no longer detected in feces. IgA is also a poor applicant to direct protection against phages since mucosal tissues exhibit low bioavailability of IgA yet generally do not respond strongly out-of-the-gate as the primary IgA response is relatively slow and drawn-out, although with repeat exposures there may be rapid upsurge. Whereas IgA dominantly modulates phage activity in secretory mucosal tissues, IgG is the principal immunoglobulin affecting systemic phage biodistribution and elimination from circulation. Both antibody types kill phages, although systemic exposure is influenced by IgG and mucosal activity by IgA. Due to this variability, phage therapy plans should keep in mind phage route of delivery, treatment site and antibodies kinetics. [169-171]

Given that IgG and IgA antibodies exhibit differing immunological activities and localizations, they differentially influence phage biodistributions and therapeutic efficacy. IgG antibodies bind and eliminate phages from circulation and extracellular fluids, which significantly decreases the systemic availability of phages. Introducing phages directly into the systemic circulation increases their rate of clearance from the bloodstream and limits the number of microbes that they are able to kill in tissues. In contrast, IgA antibodies are designed to protect mucosal surfaces such as those in the respiratory system, digestive tract and secretions. Secretory IgA binding within the mucosa prevents phage translocation and infection. Phages are equally unlikely to survive long enough in the gut to become effective if given orally because of production of phage-specific IgA. The increased levels of IgA associate with absence of active phages in feces supporting the observation on reduced mucosal phage activity. IgA response reduces mucosal phage bioavailability the IgA response is slow upon first exposure and shortens time on subsequent exposure. So, IgA mediates phage neutralization in the mucosa, while IgG controls clearance and phage efficacy in the system. Thus, to provide a balance between phage activity and immune neutralization aspects, phage therapies can be optimized through modulating the online dosage route and targeted administration site. [172-144]

Mucosal IgA secures phage particles in the intestinal lumen and mucus to stop translocation into bacteria present within gut immune cells. IgA lyses the phages by entangling them in mucus-trapped aggregates. Because it glues itself and can't migrate or prank a bacterial host, phages get stuck to each other and are expelled by intestinal peristalsis. Phages are well covered with IgA, which prevents their penetration across the mucus barrier and maintains the homeostasis through interaction of mucosal barrier. This prevents phage from entering epithelial cells and triggering the immune system. Antigen-presenting cells and gut dendritic cells can acquire IgA-bound phages, allow them to clear pathogens and create a targeted immune response while maintaining a healthy balance between tolerance and gut elimination. As shown by several capsid-displayed Ig-like protein domains, the capacity of phages to bind mucin glycoproteins in mucus, may promote their adhesion on mucosal surfaces and provide a mutualistic antimicrobial conduit. Immunologic targeting of these particles leads to a high prevalence of specific IgA antibodies directed against phages that significantly diminish their persistence and therapeutic benefit in the gut, and thus are the major determinants of phage elimination [40]. Thus, mucosal IgA has a role in preventing

bacteria infection and promoting phages clearance by binding and aggregating gut phages into mucus while keeping immune homeostasis. [175–177].

Mucosal IgA neutralizes them by binding to gut phages in the mucus layer and blocking their access to bacteria. By binding and being immobilized in mucus, phages lose their infectivity after clustering. That is, phage particles are propelled out of the gut through intestinal peristalsis. To maintain this balance, immunological exclusion limits the ability of microbes to breach our mucosal barrier. IgA envelops phages, preventing them from accessing intestinal epithelial cells. By presenting antigens from phages with IgA, dendritic cells can modulate the immune response toward either clearance or tolerance. IgA defenses lyse and clear phages that nonspecifically bind to mucin glycoproteins through Ig-like domains, thereby enabling their growth within mucus. Consequently, mucosal IgA serves as a barrier against bacterial infections through the capturing and neutralization of gut phages within mucus. It also regulates immunological homeostasis through coordinated clearance and immune signaling. [175-177]

Viruses and Eliminating Bacteria

The influence of phages on inflammation, bacterial clearance and PRRs is contradictory regarding phage identification. The proinflammatory phage vaccines utilize different types of phages as well, such as modified T4 [178]. These phages induce a strong antimicrobial response [179], characterized by both Th1 and Th2 responses, as well as high levels of proinflammatory cytokine production. There is little research on the endotoxin levels of phage vaccines, and what exists relies on studies that utilize lysates from bacteria as immunogen [180]. These preparations could be contaminated with bacteria, leading to immunogenicity. As befits this proinflammatory effect, phages synergize with the innate immune response to eliminate pathogens. Bodner et al. Using a novel fluorescent lysis reporter system, [181] showed that E. coli lambda prophage lysed in response to macrophage phagosome ROS. It appears that phages give macrophages another tool for exterminating germs. Tiwari et al. investigated treatment of wild-type and neutropenic mice with phages for P. aeruginosa. [182]. None of the neutropenic mice survived phage injection (all wild-type mice did). Phage-neutrophil synergy is thought to be important for bacterial clearance as cocultured phage and separated neutrophils were more effective than phage alone in vitro. Using a Pseudomonas lung infection model, this study demonstrated that lymphocytes are not required for bacteria clearance at this time point [183]; treatment with the lytic phage PAK P1 yielded similar

effects in wild-type and Rag2−/−IL2rg−/− animals. The phage therapy was also ineffective in neutrophil-deficient mice and MyD88−/− mice, indicating the importance of these leukocytes. These findings suggest that the host immune system, including neutrophils and myeloid cell TLR-sensing pathways, needs to cooperate with phages to control bacterial infections.

Other investigations found limited phage-induced inflammation [ ] (sd104–106) and no phagocytosis effect [184]. Proinflammatory cytokines were not altered by T4 phages in naive and LPS-activated monocytes [185]. Similar to pure T4 and A3/R phages, monocyte and neutrophil ROS generation was low. Endogenous phages in circulation and lytic phage treatment infusions are well tolerated in people with low inflammation [185]. Phages decrease bacterial phagocytosis and reduce proinflammatory cytokine production in response to endotoxin, according to another research. In response to LPS, P. aeruginosa Pf4 phages suppressed phagocytosis and tumor necrosis factor production, contributing to chronicity of mouse wound and lung infection models and to chronic wound and lung infections in humans [187]. These effects were caused by type 1 IFN-dominated antiviral responses that inhibited bacterial clearance. T4 and F8 phages from E. coli suppressed phagocytosis and ROS generation but not phorbol myristate acetate. Jahn et al. [188] found that phage-expressed protein ANKp inhibited proinflammatory cytokine production and macrophage phagocytosis of *E. coli* producing ANKp under an inducible promoter. Van Belleghem et al. [189] observed that S. aureus and *P. aeruginosa* phage stimulation affected endotoxin-induced transcription in human peripheral blood mononuclear cells. These investigations show that some phages directly affect local immune responses, changing bacterial infection susceptibility. They also suggest that phages control local immunity to protect their bacterial hosts. How can we interpret these conflicting phage pro- and anti-inflammatory data? It is interesting that T4 phages are pro-inflammatory in some circumstances [190] and anti-inflammatory in others. The immunological response may depend on the purity of the phage preparation [191]. Phages appear to decrease the immune response to bacteria but no other inflammatory conditions, suggesting that their immunomodulatory effects may be context- and possibly phage-dependent. This could happen if numerous phages trigger IFN responses that counteract bacterial immunity but boost other inflammatory responses.

Innate phage immune responses are poorly understood. Most of our data comes from a few phages; it would be important to determine if common responses exist. It is also unknown how different phages (lytic versus filamentous) affect immune responses. Adaptive immunity targets

specific infections. Phages can induce antibody (humoral immunity) and T cell responses (cellular immunity), which affects phage display vaccines, phage therapy, and microbiome interactions. Phages induce neutralizing antibodies that aid in their absorption and elimination. Most synthetic and environmental phages generate neutralizing antibodies without adjuvant [192]. Animal experiments have shown how some phages induce antibody production. These results show that the spleen is needed for a humoral response to circulating phages and that phagocytes are crucial to APC absorption, processing, and presentation of phage antigens. After presenting the target peptides on MHC-I and MHC-II pathways, B and T cells respond to the viral or tumor antigen in vitro and in vivo [193]. Antiphage antibodies are mostly IgM, but IgG and IgA are also generated [194]. In a heterogenous phage therapy investigation, local phage delivery at the infection site induced stronger antibody responses than oral phages in people, but controlled studies have not been conducted.

The long history of study on the coliphage phiX174 as a diagnostic for adaptive immune responses gives crucial data on human antiphage antibody responses. PHX174 circulates for 3–4 days after first IV immunization in healthy controls. This time window produces phage-specific IgM antibodies that peak at 2 weeks. Secondary immunization 6 weeks later produces a higher IgG/IgM peak after 1 week. X-linked agammaglobulinemia patients, who lack functioning B cells, have extended active phage circulation and no antiphage antibody response. Because these patients cannot manufacture natural antibodies or mount an adaptive response, phage clearance is difficult. B cell depletion with anti-CD19 antibody (Rituximab) normalizes phiX174 clearance despite reduced primary and secondary antibody responses. This study emphasizes the role of humoral proteins in phage clearance as anti-CD19 therapy will not influence patients' natural IgM/IgA antibodies and complement. Antiphage antibodies may regulate phage bioactivity against our microbiome. Repeated T4 phage administration caused antiphage IgAs, which reduced bioactivity. Intravenous phage therapy may also be affected by antiphage adaptive immunity. Human antiphage antibodies are rarely studied, although one study reveals that many people have neutralizing antibodies against phages used in phage therapy. Opportunistic pathogens that are part of our commensal flora may cause several chronic infections, exposing us to their phages. Patients acquire neutralizing antibodies during phage treatment. Antiphage antibodies may affect phage therapy success, however this is uncertain. In one study of 122 individuals receiving various phages for various illnesses, robust humoral immunity did not affect treatment outcomes. The lack of

controlled trials employing standardized phage product, administration routes, and treatment time makes it difficult to monitor patient antiphage antibody formation and treatment outcome.

Peptide display techniques may make phages with changed capsid shapes more immunogenic and clearer from circulation faster [195], making the data harder to interpret. Phages given intravenously or at the infection site may be more immunogenic than gastrointestinal phages. Both complement and antiphage antibodies remove phages from peripheral circulation. Sokoloff et al. injected rats with a random-peptide T7 phage library. IgM and IgG reduction in rat serum increased phage survival by 62% and 16%, respectively. Dabrowska et al. found that complement is essential for serum antibodies to inactivate phages. These results indicate that antibody binding and complement fixation opsonize and eliminate phages. Much regarding phage antibody response is unknown. It is unclear how much humoral immunity exists against the body's larger pool of phages and how these antibodies modulate phage activity, transcytosis, or opsonization of commensal or pathogenic bacteria targeted by phages. Phage treatment and phage display vaccine development require a better grasp of these and related concepts. [196]

Phages activate cellular adaptive immunity. APCs endocytose and process phages, presenting phage-derived peptides on MHC surface molecules to induce B and T cell responses against the viral antigen in vitro and in vivo. E. coli fd phage modified to express a peptide antigen is taken up by phagocytes and displayed on cell-surface MHC-I and MHC-II molecules, resulting in CD8+ and CD4+ T cell responses. Phages can also activate T cells by secreting costimulatory molecules. Phage display vaccines induce cytotoxic CD8+ T cell responses against eukaryotic virus-infected cells and malignancies. Phages prime CD4+ Th cell immunity. Phages can induce Th1 and Th2 responses in T cell line stimulation experiments and phage vaccines. PhiX174 research shows that T cells contribute generate the initial B cell IgM phage-specific response and class-switch to IgG [197]. There are signs that endogenous phages may help train T cells and fight cancer. Fluckiger et al. [196] (sd146) found MHC-I-restricted epitopes in an Enterococcus hirae prophage that drive CD8+ T lymphocytes to attack tape measure protein, a cross-reactive tumor-associated antigen. Mice with E. hirae harboring this prophage responded to cyclophosphamide or anti–PD-1 antibodies with a TMP-specific cytotoxic T-lymphocyte response, clearing the tumor. In human renal and lung cancer patients, enterococcal prophage and tumor TMP expression linked with long-term PD-1 blocking benefit. These intriguing findings show that the phageome and immunomodulatory medications that modify antigen responses may affect anticancer immunity.

To completely understand this topic, further research is needed on patient tumor phageomes and how composition and abundance affect cancer outcomes. These findings may apply to other microbiome and cellular immunity issues. Autoimmunity coupled with PD-1 blockage may also be impacted by similar dynamics. [179]

Interactions Between phages Human Hosts

Recently, many study has shown how commensal bacteria affect host health and development. Microbiota greatly impact host immunity, metabolism, and commensal makeup and structure, according to research. Only a few studies have considered bacteria's role in this dynamic. These findings show that bacteriophages are essential to human biology. Phages directly affect bacteria immune responses and indirectly damage human cells and tissues through their hosts. Thus, phages influence immunologic-bacterial interactions. Bacteria, bacteriophages, and human cells form a microbiome network. These components' tri-kingdom interactions may determine network stability. Phages reduce colonization site microorganisms and inflammation. Phages modulate host immunity directly and indirectly to increase commensal colonization immunologic tolerance. Exogenous phages, microbial dysbiosis, and immunological dysregulation can upset this balance, impacting metabolism and immunity. Unfortunately, these encounters have limited data and options. Focus is on a few phages. Many phages have been altered for biotechnology or lytic phage therapy. Expand this research to encompass steady-state and inflammatory commensal (unmodified) phages. Current approaches are often unsuitable for examining phages' physiological impacts in vivo. These projects need prophages, free phage particles, and cell and tissue phage data. Despite computational biology advances, phage biology is still difficult to explain from sequencing data. We need well-controlled, extensive human studies to understand how phages alter immunity. Most of our evidence comes from mice. Optimizing these efforts yields huge returns. Better understanding our endogenous phages may help us understand human health and disease and these tri-kingdom links.

The evolution of phages with enormous genomes—whether they are the product of recent genome expansion within clades of normal-sized phages or a persistent strategy—is intriguing. Phylogenetic trees for big terminase subunit and main capsid proteins using public database sequences to examine. Many of our phage genome sequences form clades with significant bootstrap support. The public sequences in these clades are from phages with genomes at least 120 kb,

according to database sequence genome size analysis. The Mahaphage clade, named after Sanskrit for enormous, encompasses all of our biggest genomes plus the 540–552 kb Lak genomes from human and animal microbiomes. A Nine more clusters of enormous phages and called them "huge" in certain authors' languages. Despite modest differences in gene and dataset tree topologies, protein family and capsid studies support grouping. Large phages are reliably sorted into clades, indicating that large genomes are stable. Phages were sampled from many environments within each clade, suggesting the diversity of these massive phages and their hosts throughout ecosystems. In 20 cases, phages that are so closely related that their genomes may be matched occur in at least two cohorts or habitat types. Most main CRISPR–Cas systems in phages, including Cas9-based type II, the recently discovered type V-I [198], new variants of type V-U systems, and new subtypes of type V-F systems [199]. Phages have not been reported to have class II systems (types II and V). Most phage effector nucleases (for interference) have conserved catalytic residues, indicating function.

Unlike the well-known phage with a CRISPR system, most phage CRISPR systems lack spacer acquisition machinery (Cas1, Cas2, and Cas4) and interference genes. Two similar phages have a type I-C variant system without Cas1 and Cas2 and with a helicase protein instead of Cas3. Phages have a second system with a 750-amino-acid CasΦ (Cas12j) effector protein, located near CRISPR arrays. Phages without genes for interference and spacer integration may use host Cas proteins since they have homologous CRISPR repeats. Alternatively, systems without an effector nuclease can inhibit target sequence transcription without cleavage [200]. Spacer-repeat guide RNAs may also silence host CRISPR systems or nucleic acids they hybridize with using an RNA-interference-like mechanism. Phages encode compact CRISPR arrays (median, six repetitions). This range is much less than bacterial genomes (mean 41 repetitions for class I systems) [201]. Several phage spacers target other phages' structural and regulatory genes. To defend against competing phages, phages appear to boost their hosts' immune systems.

Some spacers in phage-encoded CRISPR loci target bacteria from the same sample or study. Other host prediction analyses corroborate our assumption that these phages host focused bacteria. Not all loci with bacterial chromosome-targeting spacers encode Cas proteins that can break the host chromosome. The phage infection cycle may benefit from targeting host genes to inhibit or change their control. Phage CRISPR spacers may block promoters or silence non-coding RNAs in bacterial intergenic regions, affecting genome control. Transcription and translation genes are

known CRISPR targets for bacterial chromosomes. For example, one phage constructs its own σ70 by targeting a transcription factor gene in its host's genome. Several large phage genomes contain anti-sigma factor-like proteins (AsiA), supporting prior reports of σ70 hijacking by phages with AsiA. In another example, a phage spacer targets the host glycyl tRNA synthetase, but the Cas14 effector lacks a catalytic residue, suggesting suppression (as a 'dCas14'). No host-encoded spacers targeting CRISPR-bearing phages were detected. However, phage CRISPR targeting of other phages that are similarly targeted by bacterial CRISPR revealed phage–host connections, which the phage taxonomic profile corroborated.

Some big Pseudomonas-infecting phages encode anti-CRISPRs (Acrs) and proteins that form a nucleus-like compartment to separate their replicating genomes from host-defense and other bacterial processes. We found proteins in large phage genomes that may be Acrs and cluster with AcrVA5, AcrVA2, AcrIIA7, and AcrIIA11. We also found tubulin homologues (PhuZ) and proteins that form a proteinaceous phage "nucleus". Recently, the phage nucleus physically blocked CRISPR–Cas degradation to protect the genome from host defense.

Clinical and Therapeutic Implications

Phage treatment may replace antibiotics for antibiotic-resistant microorganisms. However, phage mixtures and surface changes are needed to reduce immunological neutralization by phage-specific antibodies. Optimizing phage therapy, vaccine development, immunomodulation, and intracellular pathogen targeting requires understanding the immune-phage interaction. Phages' ability to cling to mucosal surfaces and modify local immunity can be used to prevent and treat disease.

Host resistance and anti-phage defense

Because bacteriophages are dangerous to bacteria, prokaryotes have developed several host resistance and anti-phage defensive mechanisms. Examples include CRISPR. Retron anti-toxin system. Thoeris' NAD+ degradation method for bacterial antiphage resistance is unique. Hailong's anti-phage defense system has two genes: HalA, a transmembrane ion channel effector, and HalB, a nucleotidyltransferase. Cell death and membrane depolarization result from phage HalA activation. This kills the sick cell but stops bacterial spread. Although phages do not infect humans, the microbiome contains many phage particles. The human phageome includes the "healthy gut

phageome" (HGP) and "diseased human phageome" (DHP). Some dozens to thousands of viruses replicate in a healthy person's active phageome. There is evidence that gut microbiome bacteriophages and bacteria interact negatively and positively. According to preliminary research, 62% of healthy people and 42% and 54% of UC and Crohn's disease patients had common bacteriophages. Older persons may have fewer phages. CrAssphages rule the gut worldwide. CrAssphages are transferred from mother to child shortly after delivery, with some local transmission. People create distinct crAssphage clusters. Non-human apes may have CrAss-like phages. Most bacteriophages infect human bacterial populations. Recent investigations have shown that bacteriophages can infiltrate eukaryotic tissues and impact the immunological, respiratory, gastrointestinal, and central neurological systems. They directly affect immune responses and indirectly affect human health and disease-causing microbes [202,203]. Phages, bacteria, and human cells form a complex tri-kingdom network, revealing their interactions with people. Phages maintain immunological homeostasis and commensal microbiota tolerance by regulating bacterial populations and minimizing overgrowth and inflammation. Phage-bacteria-human ecosystem disruptions such microbial dysbiosis or immune dysfunction can cause sickness. These interactions affect phage therapy for bacterial infections and immune control in health and sickness [204,205].

Phages control innate and adaptive immunity. These engage immunological sensors, alter cytokine production, and are eliminated by the immune system. Phage immunomodulation can treat intracellular infections and inflammation [202,204]. In conclusion, bacteriophages effect the microbiota, immunity, and health directly and indirectly. Phages should be regarded human microbiological and immunological milieu members, not just bacterial predators.

Conclusions:

The rules, however, for summarizing the results of attached bacteriophage research should be remembered:

1. Bacteriophages have been known to infect both bacteria and archaea, possessing various genomes including double-stranded DNA and double-stranded RNA. They are the most widespread viruses on the planet.

2. There are also "large phages," whose genomes rival those of the smallest bacteria, both categories revealed in recent metagenomic surveys of tremendous diversity among phages. These phages encode for complex functions such as tRNAs and CRISPR-Cas system.
3. Phylogenetic studies of significant genes (e.g. polymerases) are supplanting morphology and host specificity as the major metric used to determine phylum and realm taxonomy criteria for phages.
4. Bacteriophages are the most clinically diverse viruses that infect bacteria and archaea. They are key players in diversity and evolution through their role of regulating bacterial populations, influencing host metabolism, or facilitating horizontal gene transfer. Obviously, their complex architecture and wide spectrum of replication cycles make them an ideal candidate for chronic infections either lytic or lysogenic or non-lytic.
5. Phages indirectly affect human health through interaction with immune system and microbiota. They can influence both the innate and adaptive immune systems and be tolerated by the immune system in the bloodstream, mucosal surfaces, and tissues. 511, 641 Immunogenic viral peptides can activate specific immunological receptors (such as TLR3, TLR7, and TLR9) and induce neutralizing antibodies (IgM, IgG, and IgA), thereby limiting the efficacy of second phage therapy.
6. The identification of enormous "huge phages" containing genomes comparable to ones found in small bacteria has markedly advanced our understanding of phage biology. These phages compete with other phages and evade host defenses by encoding complex activities such as transfer RNAs (tRNAs), translation factors (TFs), CRISPR-Cas systems (Cas) and protective nucleus-like compartments.
7. All this makes phage therapy a promising weapon in the war against antibiotic bacteria, but many barriers are still left, such as up regulation of receptor sites for viral particles or their immunological neutralization; bacterial resistance to specific phages and engineering enhancers that can enhance the activity either by creating cocktails of active viral species or reprogramming them. Understanding immune responses and phage-host interactions is critical for developing therapeutic applications.
8. Advancement in metagenomics and metatranscriptomics has led to the discovery of a diverse range of phages, ranging from tailless dsDNA phages to various RNA specific viruses, contributing towards our broader understanding of phage taxonomy and evolution.

9. Specific receptors on bacterial surfaces (for example, lipopolysaccharides and outer membrane proteins) determine the possible hosts.
10. An emerging field of study is the molecular interplay between phages and eukaryotes. This novel aspect demonstrates that phages could gain access inside cells through endocytosis-like mechanisms and can exert immunomodulatory and therapeutic effects beyond their specific bacterial targets. Phages have anti-CRISPR proteins to get around the bacterial defense systems.
11. They can colonize mucosal surfaces, interact with immune cells, and traverse organs along endocytosis pathways without replicating inside human cells.
12. The impact of horizontal gene transfer mediated by phage on the evolution and pathogenicity of bacteria. Prophages contribute to bacterial pathogenicity via clinical important factors, such as toxins expressed by phages, and the carriage of anti-bacterial resistance genes.
13. Phages usually provoke a robust immune response from the body by both innate and adaptive mechanisms. Phages modulate immune responses that range from promoting inflammation, bacterial clearance and immunological homeostasis to inflammatory pathology.
14. At mucosal surfaces, mucosal IgA antibodies neutralize phages by sequestering them in mucus, preventing their access to bacteria and inhibiting local immunity.
15. Future studies should instead emphasize a more defined characterization of dsRNA and non-tl phage types, their mechanisms in the course of non-lytic infections, their interplay with bacteria and human hosts, as well as their means to escape immune clearance. This will aid in the optimization of phage therapy and biocontrol applications.
16. Chronic non-lytic infections, also referred to as carrier states, are known for several dsRNA phages.
17. Human health can be affected indirectly, as when phages alter the composition of the microbiota or trigger immunological responses. Neutralizing antibodies often render repeat phage therapy ineffective and necessitate avoidance of immune clearance.
18. Phages can be used as biomarkers, and in environmental or clinical monitoring. To inform the most effective treatment plans, it is important to understand how the immune system responds to phages in clinical settings.

19. Bacteriophages hold an enormous potential in biotechnology, immunology and medicine; they are also essential components of human microbiomes and microbial ecology. Further cross-disciplinary research will improve their use in the management of health and illness.
20. Phages hold great promise in the battle against bacteria that have evolved resistance to antibiotics. They do, however, contend with challenges such as neutralization of the human immune system and resistance from the bacteria themselves. Surely, this requires optimizing delivery and using modified phages or a phage cocktail to maximize efficacy.

Future Studies

Isolating and sequencing entire genomes of dsRNA and other phages from various environments can be used to improve taxonomy and genetic diversity. Further investigation is warranted into the ecological distribution of large-genome phages, molecular processes associated with host contact, and methods of non-lytic infection. We are just starting to understand how the immune system, the bacteria and their phages work together to shine light on health and disease that can lead to new strategies for pharmaceutical intervention. Translation will also require better methods to study endogenous phages and their physiological effects in living organisms, including humans. Today, we recognize no less than bacteriophages — a diverse class of biological agents — as occupying key positions in microbial ecology, bacterial evolution, human immunity and therapeutic innovation.

Guidelines for studying bacteriophages and their uses

Characterization of Taxa and Genomes

Continued isolation and whole-genome sequencing of dsRNA phages will further help to decipher the genetic diversity and taxonomy of this enigmatic group. Use structural approaches in addition to sequence-based phylogenetic methods to revise and enhance the classification of double-stranded RNA bacteriophages, with special consideration towards genes encoding RNA-dependent RNA polymerase. We will use metagenomic and metatranscriptomic data, integrated with cutting-edge computational and structure-based methods to discover new classes of phages (especially RNA phage that are genetically diverse but underexplored).

Research on dsRNA Phage Interactions

One example of an alternative non-lytic infection cycle of dsRNA phages is the carrier cell chronic infection mode, yet its molecular mechanisms remain to be determined. (P1) Study Well-Characterized Model Phages at Different Infection Stages Using Reverse Genetics and Related Molecular Tools: To learn about the determinants of various infection steps, well-studied model phages such as phi6 would be promising studies to investigate infection. Explore some of the internal and external factors, like host growth rate, which influence lytic versus non-lytic replication strategies.

Phage Therapy and Biocontrol Studies

Overall, this study highlights that dsRNA phages have potential as agricultural biocontrol agents and potentially as therapies for human bacterial infections, however further research needs to focus on their host range, ability to shift hosts using alternative infection mode(s), before it can be used. Phages (e.g., phi6 and phiYY) need to be tested for stability and efficacy in different clinical and environmental conditions. Characterizing emergence and effect of neutralizing anti-phage antibodies in therapeutic contexts is important for emerging phage dosage regimens and formulations. Combinations of phages or modified phages must be used to disable the host immune system and surmount bacterial resistance.

Immunomodulation and Phage-Host Interaction

There is a need to further understand the intricate relationship between phages, bacteria and host so that we can unravel the continuously evolving role of these three kingdoms in inflammation, immunological tolerance and microbiome homeostasis. Learn more about the molecular mechanisms behind how phages impact immune activation, cytokine profiles and antigen-presenting cells in context of innate and adaptive immunity. Explore phage populations and phage treatment in mucosal sites under the impact of secretory IgA and other elements of the mucosal immune system. Identify the molecular receptors and entrance routes (e.g. endocytosis and transcytosis) that enable phages to access eukaryotic cells.

Phage-Induced Anti-CRISPR Systems

Studies of CRISPR-Cas systems encoded by phages and anti-CRISPR proteins that modulate the outcomes of phage infection, as well as influence evolutionary arms races between the two bacteria and their mobile genetic elements. Explore the diversity of phage defense mechanisms used by bacteria, including novel systems such as Thoeris and Hailong, plus their genomic and functional diversity. Examine phage strategies of genomic protection from infection, including nucleus-like chambers that penetrate bacterial immune systems.

Environmental and Clinical Monitoring

Phage presence in clinical and environmental samples can serve as a biomarker for bacterial contamination. Develop revision of phage typing methods for epidemiological surveillance and differentiation of bacterial pathogens. Thus, clinical studies are needed to assess immune responses following therapeutic phages and explore the impact of cellular and humoral immunity on the efficacy of phage therapy in general.

Where to Take Future Studies

Novel, physiologically relevant and high-throughput methods are needed to investigate phage biology and interactions with their hosts in vivo, including studies on microbiomes and immune system effects. Use structural biology, microbiology, immunology, bioinformatics/genome-sequencing/genomics capable approaches to achieve an integrated understanding of phage diversity and function Help researchers overcome immune neutralization and bacterial resistance to drive the development of phage-based medicines. It is important to study the role of bacteriophages in human microbiome and immunity, optimize therapeutic use of them, and increase our molecular and ecological knowledge about this type of viruses. These developments will be integral to understanding of phage biology, biocontrol and phage therapy.

Possible Paths for Further Study:

1. It certainly needs more genome sequencing and isolation of phages, including dsRNA-based those to get better information on the diversity genetic and taxonomical aspects.
2. More investigation is needed into the molecular basis of host specificity and non-lytic infection cycles.

3. A better understanding of phage–microbe and phage-mammalian immunity interactions is vital in maximizing the efficacy of phage therapy.
4. Better krnowledge about phage-encoded CRISPR-Cas and anti-phage defense systems should aid our understanding of bacterial evolution.
5. Phage-based biomarkers and epidemiological tools will facilitate clinical as well as environmental surveillance.

References:

1. Kim A, Shin TH, Shin SM, Pham CD, Choi DK et al. 2012. Cellular internalization mechanism and intracellular trafficking of filamentous M13 phages displaying a cell-penetrating transbody and TAT peptide. *PLOS ONE* 7: 12e51813
2. Boyd EF. Bacteriophage-encoded bacterial virulence factors and phage-pathogenicity island interactions. Adv Virus Res. 2012; 82:91-118.
3. Keller R, Zatzman M. 1956. Studies on the factors concerned in the disappearance of bacteriophage particles from the animal body. *J. Immunol.* 83:167–72
4. Bilzer M, Roggel F, Gerbes AL. 2006. Role of Kupffer cells in host defense and liver disease. *Liver Int* 26:101175–86
5. Bronte V, Pittet MJ. 2013. The spleen in local and systemic regulation of immunity. *Immunity* 39:806–18
6. Mäntynen, S.; Laanto, E.; Kohvakka, A.; Poranen, M.M.; Bamford, J.K.H.; Ravantti, J.J. New enveloped dsRNA phage from freshwater habitat. *J. Gen. Virol.* 2015, *96*, 1180–1189
7. Crippen, C.S.; Zhou, B.; Andresen, S.; Patry, R.T.; Muszynski, A.; Parker, C.T.; Cooper, K.K.; Szymanski, C.M. RNA and sugars, unique properties of bacteriophages infecting multidrug resistant *Acinetobacter radioresistens* strain LH6. *Viruses* 2021, *13*, 1652
8. Chen, Y.M.; Sadiq, S.; Tian, J.H.; Chen, X.; Lin, X.D.; Shen, J.J.; Chen, H.; Hao, Z.Y.; Wille, M.; Zhou, Z.C.; et al. RNA viromes from terrestrial sites across China expand environmental viral diversity. *Nat. Microbiol.* 2022, *7*, 1312–1323
9. Li, L.; Zhong, Q.; Zhao, Y.; Bao, J.; Liu, B.; Zhong, Z.; Wang, J.; Yang, L.; Zhang, T.; Cheng, M.; et al. First-in-human application of double-stranded RNA bacteriophage in the treatment of pulmonary *Pseudomonas aeruginosa* infection. *Microb. Biotechnol.* 2023, *16*, 862–867.

10. Pinheiro, L.A.M.; Pereira, C.; Frazao, C.; Balcao, V.M.; Almeida, A. Efficiency of phage phi6 for biocontrol of *Pseudomonas syringae* pv. syringae: An in vitro preliminary study. *Microorganisms* 2019, *7*, 286.

11. Koonin, E.V.; Dolja, V.V.; Krupovic, M.; Varsani, A.; Wolf, Y.I.; Yutin, N.; Zerbini, F.M.; Kuhn, J.H. Global organization and proposed megataxonomy of the virus world. *Microbiol. Mol. Biol. Rev.* 2020, *84*, e00061-19

12. Brum, J.R.; Schenck, R.O.; Sullivan, M.B. Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. *ISME J.* 2013, *7*, 1738–1751

13. Kauffman, K.M.; Hussain, F.A.; Yang, J.; Arevalo, P.; Brown, J.M.; Chang, W.K.; VanInsberghe, D.; Elsherbini, J.; Sharma, R.S.; Cutler, M.B.; et al. A major lineage of non-tailed dsDNA viruses as unrecognized killers of marine bacteria. *Nature* 2018, *554*, 118–122.

14. Krupovic, M.; Bamford, D.H. Virus evolution: How far does the double beta-barrel viral lineage extend? *Nat. Rev. Microbiol.* 2008, *6*, 941–948.

15. Abrescia, N.G.; Bamford, D.H.; Grimes, J.M.; Stuart, D.I. Structure unifies the viral universe. *Annu. Rev. Biochem.* 2012, *81*, 795–822.

16. Krupovic, M.; Koonin, E.V. Multiple origins of viral capsid proteins from cellular ancestors. *Proc. Natl. Acad. Sci. USA* 2017, *114*, E2401–E2410.

17. Krupovic, M.; Makarova, K.S.; Koonin, E.V. Cellular homologs of the double jelly-roll major capsid proteins clarify the origins of an ancient virus kingdom. *Proc. Natl. Acad. Sci. USA* 2022, *119*, e2120620119.

18. Yutin, N.; Backstrom, D.; Ettema, T.J.G.; Krupovic, M.; Koonin, E.V. Vast diversity of prokaryotic virus genomes encoding double jelly-roll major capsid proteins uncovered by genomic and metagenomic sequence analysis. *Virol. J.* 2018, *15*, 67.

19. Mannisto, R.H.; Kivela, H.M.; Paulin, L.; Bamford, D.H.; Bamford, J.K. The complete genome sequence of PM2, the first lipid-containing bacterial virus to be isolated. *Virology* 1999, *262*, 355–363.

20. Leigh, B.A.; Breitbart, M.; Oksanen, H.M.; Bamford, D.H.; Dishaw, L.J. Genome Sequence of PM2-like phage Cr39582, induced from a *Pseudoalteromonas* sp. isolated from the gut of *Ciona robusta*. *Genome Announc.* 2018, *6*, e00368-18.

21. Kalatzis, P.G.; Carstens, A.B.; Katharios, P.; Castillo, D.; Hansen, L.H.; Middelboe, M. Complete genome sequence of *Vibrio anguillarum* nontailed bacteriophage NO16. *Microbiol. Resour. Announc.* 2019, *8*, e00020-19.

22. Oksanen, H.M.; Ictv Report Consortium. ICTV virus taxonomy profile: Corticoviridae. *J. Gen. Virol.* 2017, *98*, 888–889.

23. Krupovic, M.; Bamford, D.H. Putative prophages related to lytic tailless marine dsDNA phage PM2 are widespread in the genomes of aquatic bacteria. *BMC Genom.* 2007, *8*, 236.

24. Manrique, P.; Bolduc, B.; Walk, S.T.; van der Oost, J.; de Vos, W.M.; Young, M.J. Healthy human gut phageome. *Proc. Natl. Acad. Sci. USA* 2016, *113*, 10400–10405.

25. Townsend, E.M.; Kelly, L.; Muscatt, G.; Box, J.D.; Hargraves, N.; Lilley, D.; Jameson, E. The human gut phageome: Origins and roles in the human gut microbiome. *Front. Cell Infect. Microbiol.* 2021, *11*, 643214.

26. Shkoporov, A.N.; Clooney, A.G.; Sutton, T.D.S.; Ryan, F.J.; Daly, K.M.; Nolan, J.A.; McDonnell, S.A.; Khokhlova, E.V.; Draper, L.A.; Forde, A.; et al. The human gut virome is highly diverse, stable, and individual specific. *Cell Host Microbe* 2019, *26*, 527–541.e5.

27. Shkoporov, A.N.; Hill, C. Bacteriophages of the human gut: The “known unknown” of the microbiome. *Cell Host Microbe* 2019, *25*, 195–209.

28. Yutin, N.; Makarova, K.S.; Gussow, A.B.; Krupovic, M.; Segall, A.; Edwards, R.A.; Koonin, E.V. Discovery of an expansive bacteriophage family that includes the most abundant viruses from the human gut. *Nat. Microbiol.* 2018, *3*, 38–46.

29. Yutin, N.; Benler, S.; Shmakov, S.A.; Wolf, Y.I.; Tolstoy, I.; Rayko, M.; Antipov, D.; Pevzner, P.A.; Koonin, E.V. Analysis of metagenome-assembled viral genomes from the human gut reveals diverse putative CrAss-like phages with unique genomic features. *Nat. Commun.* 2021, in press

30. Guerin, E.; Shkoporov, A.; Stockdale, S.R.; Clooney, A.G.; Ryan, F.J.; Sutton, T.D.S.; Draper, L.A.; Gonzalez-Tortuero, E.; Ross, R.P.; Hill, C. Biology and taxonomy of crAss-like bacteriophages, the most abundant virus in the human gut. *Cell Host Microbe* 2018, *24*, 653–664.e6. nShkoporov, A.N.; Khokhlova, E.V.; Fitzgerald, C.B.; Stockdale, S.R.; Draper, L.A.; Ross, R.P.; Hill, C. PhiCrAss001 represents the most abundant bacteriophage family in the human gut and infects Bacteroides intestinalis. *Nat. Commun.* 2018, *9*, 4781.

31. Sausset, R.; Petit, M.A.; Gaboriau-Routhiau, V.; De Paepe, M. New insights into intestinal phages. *Mucosal Immunol.* 2020, *13*, 205–215.

32. Yuan Y, Gao M. Jumbo bacteriophages: an overview. Front. Microbiol. 2017; 8:403.

33. Breitbart M, Bonnain C, Malki K, Sawaya NA. Phage puppet masters of the marine microbial realm. Nat. Microbiol. 2018; 3:754–766.

34. Rascovan N, Duraisamy R, Desnues C. Metagenomics and the human virome in asymptomatic individuals. Annu. Rev. Microbiol. 2016; 70:125–141.

35. Emerson JB, et al. Host-linked soil viral ecology along a permafrost thaw gradient. Nat. Microbiol. 2018; 3:870–880.

36. Balcazar JL. Bacteriophages as vehicles for antibiotic resistance genes in the environment. PLoS Pathog. 2014;10: e1004219.

37. Penadés JR, Chen J, Quiles-Puchalt N, Carpena N, Novick RP. Bacteriophage-mediated spread of bacterial virulence genes. Curr. Opin. Microbiol. 2015; 23:171–178.

38. Brown-Jaque M, et al. Detection of bacteriophage particles containing antibiotic resistance genes in the sputum of cystic fibrosis patients. Front. Microbiol. 2018; 9:856.

39. Shkoporov AN, Hill C. Bacteriophages of the human gut: the “known unknown” of the microbiome. Cell Host Microbe. 2019; 25:195–209.

40. Devoto AE, et al. Megaphages infect Prevotella and variants are widespread in gut microbiomes. Nat. Microbiol. 2019; 4:693–700.

41. Castelle CJ, et al. Biosynthetic capacity, metabolic variety and unusual biology in the CPR and DPANN radiations. Nat. Rev. Microbiol. 2018; 16:629–645.

42. Pérez-Brocal V, et al. A small microbial genome: the end of a long symbiotic relationship? Science. 2006; 314:312–313.

43. Nakabachi A, et al. The 160-kilobase genome of the bacterial endosymbiont Carsonella. Science. 2006; 314:267.

44. Basem Al-Shayeb, Rohan Sachdeva , Lin-Xing Chen , Fred Ward , Patrick Munk , Audra Devoto , Cindy J Castelle , Matthew R Olm , Keith Bouma-Gregson , Yuki Amano , Christine He , Raphaël Méheust , Brandon Brooks , Alex Thomas , Adi Lavy , Paula Matheus-Carnevali , Christine Sun , Daniela S A Goltsman , Mikayla A Borton , Allison Sharrar , Alexander L Jaffe , Tara C Nelson , Rose Kantor , Ray Keren , Katherine R Lane , Ibrahim F Farag , Shufei Lei , Kari Finstad , Ronald

Amundson , Karthik Anantharaman , Jinglie Zhou , Alexander J Probst , Mary E Power, Susannah G Tringe , Wen-Jun Li , Kelly Wrighton , Sue Harrison , Michael Morowitz, David A Relman , Jennifer A Doudna , Anne-Catherine Lehours , Lesley Warren , Jamie H D Cate , Joanne M Santini, Jillian F Banfield Clades of huge phages from across Earth's ecosystems Nature. 2020 Feb 12;578(7795):425–431.

45. Lobry JR. Asymmetric substitution patterns in the two DNA strands of bacteria. Mol. Biol. Evol. 1996; 13:660–665.

46. . Paez-Espino D, et al. Uncovering Earth's virome. Nature. 2016; 536:425–430.

47. Paez-Espino D, et al. IMG/VR: a database of cultured and uncultured DNA viruses and retroviruses. Nucleic Acids Res. 2017;45: D457–D465

48. Ivanova NN, et al. Stop codon reassignments in the wild. Science. 2014; 344:909–913.

49. Ignatiou, A. · Brasilès, S. · El Sadek Fadel, M. Structural transitions during the scaffolding-driven assembly of a viral capsid *Nat. Commun.* 2019; 10:4840

50. Dröge, A. · Santos, M.A. · Stiege, A.C. Shape and DNA packaging activity of bacteriophage SPP1 procapsid: Protein components and interactions during assembly *J. Mol. Biol.* 2000; 296:117-132

51. Orlova, E.V. · Gowen, B. Dröge, A. Structure of a viral DNA gatekeeper at 10 A resolution by cryo-electron microscopy *EMBO J.* 2003; 22:1255-1262

52. Veesler, D. · Robin, G. · Lichière, J. Crystal structure of bacteriophage SPP1 distal tail protein (gp19.1): A baseplate hub paradigm in gram-positive infecting phages *J. Biol. Chem.* 2010; 285:36666-36673

53. Mahony, J. · Stockdale, S.R. · Collins, B. *Lactococcus lactis* phage TP901–1 as a model for *Siphoviridae* virion assembly *Bacteriophage.* 2016; 6, e1123795

54. Kizziah, J.L. · Manning, K.A. · Dearborn, A.D. Structure of the host cell recognition and penetration machinery of a Staphylococcus aureus bacteriophage *PLoS Pathog.* 2020; 16, e1008314

55. Goulet, A. · Spinelli, S. · Mahony, J. ... Conserved and diverse traits of adhesion devices from siphoviridae recognizing proteinaceous or saccharidic receptors *Viruses.* 2020; 12:1-21

56. sx30.Xu, J. · Hendrix, R.W. · Duda, R.L. Chaperone-protein interactions that mediate assembly of the bacteriophage lambda tail to the correct length *J. Mol. Biol.* 2014; 426:1004-1018

57. Langlois, C. · Ramboarina, S. · Cukkemane, A. Bacteriophage SPP1 tail tube protein self-assembles into β-structure-rich tubes *J. Biol. Chem.* 2014; 290:3836-3849

58. Chaban, Y. · Lurz, R. · Brasilès, S. Structural rearrangements in the phage head-to-tail interface during assembly and infection *Proc. Natl. Acad. Sci. U. S. A.* 2015; 112:7009-7014

59. Boulanger, P. · Jacquot, P. · Plançon, L. Phage T5 straight tail fiber is a multifunctional protein acting as a tape measure and carrying fusogenic and muralytic activities *J. Biol. Chem.* 2008; 283:13556-13564

60. Fang, Q. · Tang, W.C. · Tao, P. Structural morphing in a symmetry-mismatched viral vertex *Nat. Commun.* 2020; 11:1713

61. sx39.Leiman, P.G. · Kanamaru, S. · Mesyanzhinov, V.V. Structure and morphogenesis of bacteriophage T4 *Cell. Mol. Life Sci.* 2003; 60:2356-2370

62. Qin, L. · Fokine, A. · O'Donnell, E. Structure of the small outer capsid protein, Soc: A clamp for stabilizing capsids of T4-like phages *J. Mol. Biol.* 2010; 395:728-741

63. Rossmann, M.G. · Mesyanzhinov, V.V. · Arisaka, F. The bacteriophage T4 DNA injection machine *Curr. Opin. Struct. Biol.* 2004; 14:171-180

64. Taylor, N.M. · Prokhorov, N.S. · Guerrero-Ferreira, R.C. ... Structure of the T4 baseplate and its function in triggering sheath contraction *Nature.* 2016; 533:346-352

65. Zheng, W. · Wang, F. · Taylor, N.M.I. ... Refined cryo-EM structure of the T4 tail tube: Exploring the lowest dose limit *Structure.* 2017; 25:1436-1441.e2

66. Fokine, A. · Zhang, Z. · Kanamaru, S. The molecular architecture of the bacteriophage T4 neck *J. Mol. Biol.* 2013; 425:1731-1744

67. Yap, M.L. · Klose, T. · Arisaka, F. ... Role of bacteriophage T4 baseplate in regulating assembly and infection *Proc. Natl. Acad. Sci. U. S. A.* 2016; 113:2654-2659

68. Washizaki, A. · Yonesaki, T. · Otsuka, Y. Characterization of the interactions between Escherichia coli receptors, LPS and OmpC, and bacteriophage T4 long tail fibers *Microbiologyopen.* 2016; 5:1003-1015

69. Hu, B. · Margolin, W. · Molineux, I.J. Structural remodeling of bacteriophage T4 and host membranes during infection initiation *Proc. Natl. Acad. Sci. U. S. A.* 2015; 112:E4919-E4928

70. 1.sCai, X.; Tian, F.; Teng, L.; Liu, H.; Tong, Y.; Le, S.; Zhang, T. Cultivation of a lytic double-stranded RNA bacteriophage infecting *Microvirgula aerodenitrificans* reveals a mutualistic parasitic lifestyle. *J. Virol.* 2021, *95*, 0039921.

71. Li, D.; Li, Y.; Li, P.; Han, Q.; Zhang, T.; Yang, B.; Wu, W.; Yang, H. Phage phiZ98: A novel tri-segmented dsRNA cystovirus for controlling *Pseudomonas* strains with defective lipopolysaccharides in foods. *Food Res. Int.* 2022, *162*, 112197.

72. Ilca, S.L.; Sun, X.; El Omari, K.; Kotecha, A.; de Haas, F.; DiMaio, F.; Grimes, J.M.; Stuart, D.I.; Poranen, M.M.; Huiskonen, J.T. Multiple liquid crystalline geometries of highly compacted nucleic acid in a dsRNA virus. *Nature* 2019, *570*, 252–256.

73. Jäälinoja, H.T.; Huiskonen, J.T.; Butcher, S.J. Electron cryomicroscopy comparison of the architectures of the enveloped bacteriophages phi6 and phi8. *Structure* 2007, *15*, 157–167.

74. Hu, G.B.; Wei, H.; Rice, W.J.; Stokes, D.L.; Gottlieb, P. Electron cryo-tomographic structure of cystovirus phi12. *Virology* 2008, *372*, 1–9.

75. Huiskonen, J.T.; de Haas, F.; Bubeck, D.; Bamford, D.H.; Fuller, S.D.; Butcher, S.J. Structure of the bacteriophage phi6 nucleocapsid suggests a mechanism for sequential RNA packaging. *Structure* 2006, *14*, 1039–1048.

76. Sun, Z.; El Omari, K.; Sun, X.; Ilca, S.L.; Kotecha, A.; Stuart, D.I.; Poranen, M.M.; Huiskonen, J.T. Double-stranded RNA virus outer shell assembly by bona fide domain-swapping. *Nat. Commun.* 2017, *8*, 14814.

77. Ilca, S.L.; Kotecha, A.; Sun, X.; Poranen, M.M.; Stuart, D.I.; Huiskonen, J.T. Localized reconstruction of subunits from electron cryomicroscopy images of macromolecular complexes. *Nat. Commun.* 2015, *6*, 8843.

78. El Omari, K.; Sutton, G.; Ravantti, J.J.; Zhang, H.; Walter, T.S.; Grimes, J.M.; Bamford, D.H.; Stuart, D.I.; Mancini, E.J. Plate tectonics of virus shell assembly and reorganization in phage phi8, a distant relative of mammalian reoviruses. *Structure* 2013, *21*, 1384–1395.

79. Miyazaki, N.; Salaipeth, L.; Kanematsu, S.; Iwasaki, K.; Suzuki, N. Megabirnavirus structure reveals a putative 120-subunit capsid formed by asymmetrical dimers with distinctive large protrusions. *J. Gen. Virol.* 2015, *96*, 2435–

80. Mönttinen, H.A.M.; Ravantti, J.J.; Poranen, M.M. Structure unveils relationships between RNA virus polymerases. *Viruses* 2021, *13*, 313.

81. Gottlieb, P.; Alimova, A. RNA packaging in the cystovirus bacteriophages: Dynamic interactions during capsid maturation. *Int. J. Mol. Sci.* 2022, *23*, 2677.

82. Zhang, C.; Li, Y.; Samad, A.; Zheng, P.; Ji, Z.; Chen, F.; Zhang, H.; Jin, T. Structure and mutation analysis of the hexameric P4 from *Pseudomonas aeruginosa* phage phiYY. *Int. J. Biol. Macromol.* 2022, *194*, 42–49

83. Mäntynen, S.; Sundberg, L.R.; Poranen, M.M. Recognition of six additional cystoviruses: Pseudomonas virus phi6 is no longer the sole species of the family *Cystoviridae*. *Arch. Virol.* 2018, *163*, 1117–1124.

84. Poranen, M.M.; Tuma, R.; Bamford, D.H. Assembly of double-stranded RNA bacteriophages. *Adv. Virus Res.* 2005, *64*, 15–43.

85. Poranen, M.M.; Mäntynen, S. Cystoviridae. In *The Online Report of the International Committee on Taxonomy of Viruses*; International Committee on Taxonomy of Viruses (ICTV): Online, 2019; Available online: http://taxonomy.cvr.gla.ac.uk/PDF/Cystoviridae.pdf (accessed on 1 September 2023).

86. International Committee on Taxonomy of Viruses Executive. The new scope of virus taxonomy: Partitioning the virosphere into 15 hierarchical ranks. *Nat. Microbiol.* 2020, *5*, 668–674.

87. Simmonds, P.; Adriaenssens, E.M.; Zerbini, F.M.; Abrescia, N.G.A.; Aiewsakun, P.; Alfenas-Zerbini, P.; Bao, Y.; Barylski, J.; Drosten, C.; Duffy, S.; et al. Four principles to establish a universal virus taxonomy. *PLoS Biol.* 2023, *21*, e3001922.

88. Yang, Y.; Lu, S.; Shen, W.; Zhao, X.; Shen, M.; Tan, Y.; Li, G.; Li, M.; Wang, J.; Hu, F.; et al. Characterization of the first double-stranded RNA bacteriophage infecting *Pseudomonas aeruginosa*. *Sci. Rep.* 2016, *6*, 38795.

89. Neri, U.; Wolf, Y.I.; Roux, S.; Camargo, A.P.; Lee, B.; Kazlauskas, D.; Chen, I.M.; Ivanova, N.; Zeigler Allen, L.; Paez-Espino, D.; et al. Expansion of the global RNA virome reveals diverse clades of bacteriophages. *Cell* 2022, *185*, 4023–4037.e18.

90. *Abedon ST (October 2019).* "Look Who's Talking: T-Even Phage Lysis Inhibition, the Granddaddy of Virus-Virus Intercellular Communication Research". Viruses. 11 (10): 951.

91. Mason KA, Losos JB, Singer SR, Raven PH, Johnson GB (2011). Biology. New York: McGraw-Hill. p. 533. ISBN 978-0-07-893649-4.

92. 71.g Charles RC, Ryan ET (October 2011). "Cholera in the 21st century". Current Opinion in Infectious Diseases. 24 (5): 472–477.

93. Keen EC (December 2012). "Paradigms of pathogenesis: targeting the mobile genetic elements of disease". Frontiers in Cellular and Infection Microbiology. 2: 161

94. Drulis-Kawa Z, Majkowska-Skrobek G, Maciejewska B (2015). "Bacteriophages and phage-derived proteins--application approaches". Current Medicinal Chemistry. 22 (14): 1757–1773

95. Maghsoodi A, Chatterjee A, Andricioaei I, Perkins NC (December 2019). "How the phage T4 injection machinery works including energetics, forces, and dynamic pathway". Proceedings of the National Academy of Sciences of the United States of America. 116 (50): 25097–25105. Bibcode: 2019 PNAS.11625097M.

96. Mizuno CM, Guyomar C, Roux S, Lavigne R, Rodriguez-Valera F, Sullivan MB, et al. (February 2019). "Numerous cultivated and uncultivated viruses encode ribosomal proteins". Nature Communications. 10 (1) 752. Bibcode:2019 NatCo..10..752M. .

97. Edgar RS Conditional lethals: in Phage and the Origins of Molecular Biology (2007) Edited by John Cairns, Gunther S. Stent, and James D. Watson, Cold Spring Harbor Laboratory of Quantitative Biology, Cold Spring Harbor, Long Island, New York ISBN 978-0-87969-800-3

98. Henrot C, Petit MA (November 2022). "Signals triggering prophage induction in the gut microbiota". Molecular Microbiology. 118 (5): 494–502.

99. Nguyen S, Baker K, Padman BS, Mazel D, Cambillau C, Desnues C, et al. Endocytosis of Bacteriophages. J Virol. 2022 Feb;96(3):e0112421.

100. Frunzke J, Mußmann K, Liess B, et al. Internalization of a polysialic acid-binding Escherichia coli bacteriophage into eukaryotic neuroblastoma cells. Nat Commun. 2017 Dec 3;8(1):1508.

101. Piotukh K, Persson MA, Liu B, Paschen A, Hammarström S, Fredericson M, et al. Cellular Internalization Mechanism and Intracellular Trafficking of Filamentous M13 Phages Displaying a Cell-Penetrating Transbody and TAT Peptide. PLoS One. 2012 Dec 13;7(12):e51813.

102. Nguyen S, Baker K, Padman BS, Mazel D, Cambillau C, Desnues C, et al. Mammalian cells internalize bacteriophages and use them as a resource to enhance cellular growth and survival. PLoS Biol. 2023 Oct 26;21(10): e3002341.

103. Frunzke J, Mußmann K, Liess B, et al. Internalization of a polysialic acid-binding Escherichia coli bacteriophage into eukaryotic neuroblastoma cells. Nat Commun. 2017 Dec 3;8(1):1508.

104. Piotukh K, Persson MA, Liu B, Paschen A, Hammarström S, Fredericson M, et al. Cellular Internalization Mechanism and Intracellular Trafficking of Filamentous M13 Phages Displaying a Cell-Penetrating Transbody and TAT Peptide. PLoS One. 2012 Dec 13;7(12): e51813.

105. Frunzke J, Mußmann K, Liess B, et al. Internalization of a polysialic acid-binding Escherichia coli bacteriophage into eukaryotic neuroblastoma cells. Nat Commun. 2017 Dec 3;8(1):1508.

106. Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. FEMS Microbiol Lett. 2016 May;363(4): fnv121.

107. Piotukh K, Persson MA, Liu B, Paschen A, Hammarström S, Fredericson M, et al. Cellular Internalization Mechanism and Intracellular Trafficking of Filamentous M13 Phages Displaying a Cell-Penetrating Transbody and TAT Peptide. PLoS One. 2012 Dec 13;7(12): e51813.

108. Majewska J, Kaźmierczak Z, Legut M, et al. Interactions of Bacteriophages with Animal and Human Organisms. Int J Mol Sci. 2021 Aug 19;22(16):8937.

109. De Sordi L, Lourenço M, Debarbieux L. The Battle Within: Interactions of Bacteriophages and Bacteria in the Gastrointestinal Tract. Cell Host Microbe. 2019 Feb 13;25(2):210-218.

110. Christin JR, Beckert MV. Origins and Applications of CRISPR-Mediated Genome Editing. Einstein J Biol Med. 2016;31(1-2):2-5.

111. Maciejewska B, Olszak T, Drulis-Kawa Z. Applications of bacteriophages versus phage enzymes to combat and cure bacterial infections: an ambitious and also a realistic application? Appl Microbiol Biotechnol. 2018 Mar;102(6):2563-2581.

112. Górski A, et al. Use of Bacteriophages to Target Intracellular Pathogens. Clin Infect Dis. 2023;77(Suppl_5): S423-S431.

113. Barr JJ. Phages in the Human Body. Front Microbiol. 2017; 8:566.

114. Lehti TA, et al. Interactions between Bacteriophages and Eukaryotic Cells. J Immunol. 2020; 2020:3589316.

115. Roach DR, Debarbieux L. Interactions between Bacteriophage, Bacteria, and the Mammalian Immune System. Viruses. 2018;10(1):10.

116. Shkoporov AN, Hill C. Bacteriophages as New Human Viral Pathogens. Viruses. 2018;10(6):317.

117. Hargreaves KR, et al. Special Issue: Phage–Bacteria Interplay in Health and Disease. Viruses. 2022;14(5):970.

118. McCallin S, Oechslin F. Bacteriophage strategies for overcoming host antiviral immunity. Front Microbiol. 2023; 14:1211793.

119. Phages in the Human Body," Frontiers in Microbiology, 2017.

120. "New Insights Regarding Bacteriophage-Mammalian Cell Interactions," PubMed, 2021.

121. "Phages and Human Health: More Than Idle Hitchhikers," PMC, 2019.

122. "Use of Bacteriophages to Target Intracellular Pathogens," Clinical Infectious Diseases, 2023.

123. "Interactions between Bacteriophages and Eukaryotic Cells," Wiley, 2020.

124. "Interactions of Bacteriophages with Animal and Human Organisms-Safety Issues," PubMed, 2021.

125. "Therapeutic Phages as Modulators of the Immune Response," Clinical Infectious Diseases, 2023.

126. "Special Issue: Phage–Bacteria Interplay in Health and Disease," PMC, 2022.

127. Pham TD, Nguyen TH, Iwashita H, Takemura T, Morita K, Yamashiro T. Comparative analyses of CTX prophage region of Vibrio cholerae seventh pandemic wave 1 strains isolated in Asia. Microbiol Immunol. 2018 Oct;62(10):635-650

128. Motlagh AM, Bhattacharjee AS, Goel R. Biofilm control with natural and genetically-modified phages. World J Microbiol Biotechnol. 2016 Apr;32(4):67.

129. Laura M. Kasman; La Donna Porter. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) Bookshelf ID: NBK493185 PMID: 29630237 (http://creativecommons.org/licenses/by-nc-nd/4.0/),

130. Vaca Pacheco S, Garcıća González O, Paniagua Contreras GL 2006. The *lom* gene of bacteriophage λ is involved in *Escherichia coli* K12 adhesion to human buccal epithelial cells. *FEMS Microbiol. Lett.* 156:1129–32

131. Secor PR, Sweere JM, Michaels LA, Malkovskiy AV, Lazzareschi D et al. 2015. Filamentous bacteriophage promotes biofilm assembly and function. *Cell Host Microbe* 18:549–59

132. do Vale A, Cabanes D, Sousa S. 2016. Bacterial toxins as pathogen weapons against phagocytes. *Front. Microbiol.* 1:42

133. asechnek A, Rabinovich L, Stadnyuk O, Azulay G, Mioduser J et al. 2020. Active lysogeny in *Listeria monocytogenes* is a bacteria-phage adaptive response in the mammalian environment. *Cell Rep* 32:4107956

134. Goh S, Hussain H, Chang BJ, Emmett W, Riley TV, Mullany P 2013. Phage ϕC2 mediates transduction of Tn*6215*, encoding erythromycin resistance, between *Clostridium difficile* strains. *mBio* 4:6e00840-13

135. Wang X, Kim Y, Ma Q, Hong SH, Pokusaeva K et al. 2010. Cryptic prophages help bacteria cope with adverse environments. *Nat. Commun.* 1:9147

136. Gebhart D, Williams SR, Bishop-Lilly KA, Govoni GR, Willner KM et al. 2012. Novel high-molecular-weight, R-type bacteriocins of *Clostridium difficile*. *J. Bacteriol.* 194:226240–47

137. Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML et al. 2013. Bacteriophage adhering to mucus provide a non-host-derived immunity. *PNAS* 110:2610771–76T4 phages are shown to interact with mammalian mucosa through capsid immunoglobulin-like domains.

138. Fraser JS, Yu Z, Maxwell KL, Davidson AR. 2006. Ig-like domains on bacteriophages: a tale of promiscuity and deceit. *J. Mol. Biol.* 359:2496–507

139. Almeida GMF, Laanto E, Ashrafi R, Sundberg LR. 2019. Bacteriophage adherence to mucus mediates preventive protection against pathogenic bacteria. *mBio* 10:601984-19

140. Barr JJ, Auro R, Sam-Soon N, Kassegne S, Peters G et al. 2015. Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters. *PNAS* 112:4413675–80

141. Yang JY, Kim MS, Kim E, Cheon JH, Lee YS et al. 2016. Enteric viruses ameliorate gut inflammation via Toll-like receptor 3 and Toll-like receptor 7-mediated interferon-β production. *Immunity* 44:4889–900

142. Gogokhia L, Buhrke K, Bell R, Casjens SR, Longman RS, Round JL. 2019. Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. *Cell Host Microbe* 25:285–99Germ-free mice treated with a phage cocktail exhibit TLR9-dependent immune cell activation

143. Barr JJ. 2017. A bacteriophages journey through the human body. *Immunol. Rev.* 279:1106–22

144. Pacífico C, Hilbert M, Sofka D, Dinhopl N, Pap IJ et al. 2019. Natural occurrence of *Escherichia coli*-infecting bacteriophages in clinical samples. *Front. Microbiol.* 10:2484

145. Guillot A, Tacke F. 2019. Liver macrophages: old dogmas and new insights. *Hepatol. Commun.* 3:6730–43

146. Hodyra-Stefaniak K, Miernikiewicz P, Drapała J, Drab M, Jonczyk-Matysiak E et al. 2015. Mammalian host-versus-phage immune response determines phage fate in vivo. *Sci. Rep.* 5:114802

147. Ghose C, Ly M, Schwanemann LK, Shin JH, Atab K et al. 2019. The virome of cerebrospinal fluid: viruses where we once thought there were none. *Front. Microbiol.* 10:2061

148. ecor PR, Michaels LA, Smigiel KS, Rohani MG, Jennings LK et al. 2017. Filamentous bacteriophage produced by *Pseudomonas aeruginosa* alters the inflammatory response and promotes noninvasive infection in vivo. *Infect. Immun.* 85:1e00648-16

149. Zaczek M, Górski A, Skaradzińska A, Łusiak-Szelachowska M, Weber-D'browska B. 2019. Phage penetration of eukaryotic cells: practical implications. *Future Virol* 14:745–60

150. Tian Y, Wu M, Liu X, Liu Z, Zhou Q et al. 2015. Probing the endocytic pathways of the filamentous bacteriophage in live cells using ratiometric pH fluorescent indicator. *Adv. Healthc. Mater.* 4:3413–19

151. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K et al. 2019. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. *Nat. Med.* 25:5730–33

152. Manickan E, Karem KL, Rouse BT. 2017. DNA vaccines—a modern gimmick or a boon to vaccinology? *Crit. Rev. Immunol.* 37:2–6483–98

153. Bodner K, Melkonian AL, Covert MW. 2021. The enemy of my enemy: new insights regarding bacteriophage–mammalian cell interactions. *Trends Microbiol* 29:6528–41

154. Hess KL, Jewell CM. 2020. Phage display as a tool for vaccine and immunotherapy development. *Bioeng. Transl. Med.* 5:1e10142

155. Kawasaki T, Kawai T. 2014. Toll-like receptor signaling pathways. *Front. Immunol.* 5:461

156. Hashiguchi S, Yamaguchi Y, Takeuchi O, Akira S, Sugimura K. 2010. Immunological basis of M13 phage vaccine: regulation under MyD88 and TLR9 signaling. *Biochem. Biophys. Res. Commun.* 402:119–22

157. Sweere J M, Van Belleghem J D, Ishak H, Bach M S, Popescu M et al. 2019. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* 363:6434 eaat 9691 Filamentous Pf phage downregulates myeloid cell inflammation in a TLR3-dependent manner

158. Basu R, Zhai L, Contreras A, Tumban E. 2018. Immunization with phage virus-like particles displaying Zika virus potential B-cell epitopes neutralizes Zika virus infection of monkey kidney cells. *Vaccine* 36:101256–64

159. Wang L, Gao J, Lan X, Zhao H, Shang X et al. 2019. Identification of combined T-cell and B-cell reactive *Echinococcus granulosus* 95 antigens for the potential development of a multi-epitope vaccine. *Ann. Transl. Med.* 7:22652

160. Popescu et al., "Bacteriophages and the Immune System," Annual Review of Virology, 2021.

161. Majewska et al., "Mammalian Host-Versus-Phage Immune Response," Scientific Reports, 2015.

162. Popescu et al., "Phage Interaction with Adaptive Immune Cells," Frontiers in Microbiology, 2023.

163. Gorski et al., "Therapeutic Phages as Modulators of the Immune Response," Clinical Infectious Diseases, 2023.

164. Roach and Debarbieux, "Bacteriophages and the Immune System," PubMed, 2021.

165. Huo et al., "Phage-specific antibodies: are they a hurdle for the success of phage therapy?" Essays in Biochemistry, 2024.

166. Majewska et al., "Induction of Phage-Specific Antibodies by Two Therapeutic Staphylococcal Bacteriophages," Frontiers in Immunology, 2019.

167. Merril et al., "Anti-phage serum antibody responses and the outcome of phage therapy," Frontiers in Immunology, 2020.

168. Klimuk et al., "Phage K exposure generates phage-neutralizing activity," Antimicrobial Agents and Chemotherapy, 2024.

169. Gorski et al., "Contribution of the Immune Response to Phage Therapy," Journal of Immunology, 2018

170. Danis-Wlodarczyk et al., "Induction of Phage-Specific Antibodies by Therapeutic Phages," Frontiers in Immunology, 2019.

171. Górski et al., "Pharmacokinetics and Biodistribution of Phages and their Applications," PMC, 2023.

172. Brandtzaeg, "Translocalized IgA mediates neutralization and stimulates innate immunity," PNAS, 2014.

173. Danis-Wlodarczyk et al., Frontiers in Immunology, 2019.

174. Górski et al., PMC, 2023.

175. Brandtzaeg, PNAS, 2014.

176. Bunker JJ et al., "Microbiota-antibody interactions that regulate gut homeostasis," Cell Host Microbe, 2021.

177. Barr JJ et al., "Bacteriophage adhering to mucus provide a non–host-derived immunity," PNAS, 2013.

178. Gutzeit C et al., "Roles of Secretory Immunoglobulin A in Host-Microbiota Interactions in the Gut Ecosystem," Frontiers in Microbiology, 2022.

179. Carvalho GB, Costa LE, Lage DP, Ramos FF, Santos TTO et al. 2019. High-through identification of T cell-specific phage-exposed mimotopes using PBMCs from tegumentary leishmaniasis patients and their use as vaccine candidates against *Leishmania amazonensis* infection. *Parasitology* 146:3322–32

180. Iwagami Y, Casulli S, Nagaoka K, Kim M, Carlson RI et al. 2017. Lambda phage-based vaccine induces antitumor immunity in hepatocellular carcinoma. *Heliyon* 3:900407

181. Kumar AB, Mishrad AAK, Prakash C, Priyadarshini A, Rawat M 2018. Immunization with *Salmonella* Abortusequi phage lysate protects guinea pig against the virulent challenge of SAE-742. *Biologicals* 56:24–28

182. Bodner K, Melkonian AL, Barth AIM, Kudo T, Tanouchi Y, Covert MW. 2020. Engine ered fluorescent *E. coli* lysogens allow live-cell imaging of functional prophage induction triggered inside macrophages. *Cell Syst* 10:3254–64

183. Tiwari B R, Kim S, Rahman M, Kim J. 2011. Antibacterial efficacy of lytic *Pseudomonas* bacteriophage in normal and neutropenic mice models. *J. Microbiol.* 49:6994–99

184. Roach DR, Leung CY, Henry M, Morello E, Singh D et al. 2017. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host Microbe* 22:138–47. e4Phages work synergistically with neutrophils to protect in a murine model of *Pseudomonas aeruginosa* pneumonia.

185. Jończyk-Matysiak E, Łusiak Szelachowska M, Kłak M, Bubak B, Międzybrodzki R et al. 2015. The effect of bacteriophage preparations on intracellular killing of bacteria by phagocytes. *J. Immunol. Res.* 2015:482863

186. Bocian K, Borysowski J, Zarzycki M, Wierzbicki P, Klosowska D et al. 2016. LPS-activated monocytes are unresponsive to T4 phage and T4-generated *Escherichia coli* lysate. *Front. Microbiol.* 7:1356

187. Zhvania P, Hoyle NS, Nadareishvili L, Nizharadze D, Kutateladze M. 2017. Phage therapy in a 16-year-old boy with Netherton syndrome. *Front. Med.* 4:94

188. Burgener EB, Sweere JM, Bach MS, Secor PR, Haddock N et al. 2019. Filamentous bacteriophages are associated with chronic *Pseudomonas* lung infections and antibiotic resistance in cystic fibrosis. *Sci. Transl. Med.* 11:488eaau9748

189. Jahn MT, Arkhipova K, Markert SM, Stigloher C, Lachnit T et al. 2019. A phage protein aids bacterial symbionts in eukaryote immune evasion. *Cell Host Microbe* 26:4542–50.e5

190. Van elleghem J D, Clement F, Merabishvili M, Lavigne R, Vaneechoutte M et al. 2017. Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by *Staphylococcus aureus* and *Pseudomonas aeruginosa* phages. *Sci. Rep* 7:18004

191. Tao P, Zhu J, Mahalingam M, Batra H, Rao VB. 2019. Bacteriophage T4 nanoparticles for vaccine delivery against infectious diseases. *Adv. Drug Deliv. Rev.* 145:57–72

192. Dufour N, Henry M, Ricard J-D, Debarbieux L, Khan Mirzaei M et al. 2016. Commentary: morphologically distinct *Escherichia coli* bacteriophages differ in their efficacy and ability to stimulate cytokine release *in vitro*. *Front. Microbiol.* 7:1029

193. Dąbrowska K, Miernikiewicz P, Piotrowicz A, Hodyra K, Owczarek B et al. 2014. Immunogenicity studies of proteins forming the T4 phage head surface. *J. Virol.* 88:2112551–57

194. Lotfi Z, Golchin M, Khalili-Yazdi A, Khalili M. 2019. Immunological properties of the SLLTEVET epitope of Influenza A virus in multiple display on filamentous M13 phage. *Comp. Immunol. Microbiol. Infect. Dis.* 65:76–80

195. Majewska J, Kaźmierczak Z, Lahutta K, Lecion D, Szymczak A et al. 2019. Induction of phage-specific antibodies by two therapeutic staphylococcal bacteriophages administered *per os*. *Front. Immunol.* 14:102607

196. Hodyra Stefaniak K , Lahutta K , Majewska J , Kaźmierczak Z , Lecion D et al. 2019. Bacteriophages engineered to display foreign peptides may become short-circulating phages. *Microb. Biotechnol.* 12:4730–41

197. Sartorius R, Pisu P, D'Apice L, Pizzella L, Romano C et al. 2008. The use of filamentous bacteriophage *fd* to deliver MAGE-A10 or MAGE-A3 HLA-A2-restricted peptides and to induce strong antitumor CTL responses. *J. Immunol.* 180:63719–28

198. Medeea Popescu, Jonas D. Van Belleghem[1], Arya Khosravi[1] and Paul L. Bollyky[1] ANNUAL REVIEW OF VIROLOGY Volume 8, 2021 Vol. 8:415-435

199. Yan, W. X. et al. Functionally diverse type V CRISPR–Cas systems. *Science* 363, 88–91 (2019).

200. Shmakov, S. et al. Diversity and evolution of class 2 CRISPR–Cas systems. *Nat. Rev. Microbiol*. 15, 169–182 (2017).

201. Stachler, A.-E. & Marchfelder, A. Gene repression in Haloarchaea using the CRISPR (clustered regularly interspaced short palindromic repeats)–Cas I-B system. *J. Biol. Chem*. 291, 15226–15242 (2016).

202. Toms, A. & Barrangou, R. On the global CRISPR array behavior in class I systems. *Biol. Direct* 12, 20 (2017).

203. Popescu M, Van Belleghem JD, Khosravi A, Bollyky PL. Bacteriophages and the Immune System. Annu Rev Virol. 2021;8(1):415–35.

204. Majewska J, Kaźmierczak Z, Legut M, et al. Interactions of Bacteriophages with Animal and Human Organisms. Int J Mol Sci. 2021 Aug 19;22(16):8937. Górski A, Międzybrodzki R, Borysowski J, et al. Phage therapy: Concepts and applications. Folia Microbiol (Praha). 2019;64(2):123–31.

205. Roach DR, Debarbieux L. Phage therapy: awakening a sleeping giant. Emerg Top Life Sci. 2017;1(1):93–103.