Title: Phageome transfer from gut to circulation and its regulation by human immunity

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Abstract

Bacteriophages are a critical part of the human microbiome, especially in the gastrointestinal tract. While phage translocation beyond the gut is known, its regulation within phageomes and interaction with immune responses remain unclear. This study explores the taxonomic structure of the phageome, lymphatic filtration, and phage-specific IgG induction as factors regulating phage translocation from the gut to the bloodstream.

Mucosal intestinal biopsies and serum samples from 38 patients were analyzed to determine gut and blood phageome compositions. Phage epitopes targeted by IgGs were identified via immunoprecipitation of a phageome-wide epitope library and high-throughput sequencing. In vivo, phage arrest in lymph nodes was studied using a mouse model.

Tequatroviruses and Kayviruses were abundant in the gut mucosa and frequently translocated to blood circulation. However, gut-blood phageome correlation was moderate. Phage-specific IgGs reduced targeted phages in both blood and gut mucus, limiting translocation. Lymphatic tissues also sequestered phages in vivo.

We propose that translocation involves multiple steps, beginning with epithelial transcytosis, followed however by filtration in lymphatic tissues, which limits translocation dynamics in vivo. While the gut phageome significantly contributes to circulating phages, other sources also play a role. These findings improve our understanding of phageome functions and their medical applications.

Introduction

Phages constitute the largest known component of microbiomes, with an estimated 10^{31} bacteriophages existing worldwide (Bakhshinejad & Ghiasvand, 2017), making them the most abundant biological entities on Earth. This hypothesis was originally based on data from ocean microbiotas. However, a revised calculation in 2016 provided a virus-to-microbe ratio (VMR) ranging from 2.6 up to 160, significantly exceeding earlier estimates, which suggested viruses were ten times more abundant than their bacterial hosts. The widespread bacteriophage host-virus relationships, along with their transition to prophage forms, play a pivotal role in regulating bacterial biomass, maintaining biodiversity, and driving biogeochemical cycles within Earth's biosphere (Wigington et al., 2016).

Likewise, phages are a critical component in the human microbiome, particularly in the intestinal tract. The density of phages in the large intestine ranges between 10^8 and 10^{10} phage virions per gram of feces (Guerin & Hill, 2020; Sutton & Hill, 2019; Zuppi et al., 2021). The term 'gut phageome' refers to the community of phages present in this region. Extensive research has highlighted the abundance of phages in the gut, underscoring their significance. These phages engage in complex interactions with the gut bacterial community, influencing microbial diversity, population dynamics, and functional capabilities. By selectively infecting and controlling specific bacterial species, phages contribute to the maintenance of microbial balance and stability. Phages also facilitate horizontal gene transfer among gut bacteria, potentially shaping the evolution and adaptation of these microbial populations. Understanding the intricate role of phages in the gut is essential for comprehending the dynamics of the gut microbiome and its implications for human health (Breitbart et al., 2008). Bacteriophages persist in the environment, exhibiting two main lifestyles: lytic and lysogenic. The predominant lifestyle observed in gut phages is the lysogenic

one (Benler et al., 2021; Manrique et al., 2017). Most gut phages exist as prophages within bacterial cells, becoming active under specific environmental conditions. Despite their primarily lysogenic nature, abundance of phage virions in the gut remains remarkable.

Studies aim at identifying the most abundant phage groups within the gut microbiome, have largely relied on traditional phage taxonomy based on morphology. Most bacteriophages identified in the gut belonged to the order *Caudovirales* and represented families *Siphoviridae*, *Podoviride*, and *Myoviridae* (Lin & Lin, 2019; Townsend et al., 2021). The most isolated virus-like particles (VLPs) targeted bacterial hosts like *Escherichia/Shigella*, *Enterococcus* sp, and *Clostridioides*. High-throughput analyses of phageome using next-generation sequencing have revealed that 80%-95% of phage sequences remained neither annotated in existing databases nor assigned to known taxonomic groups or bacterial hosts (Barr et al., 2013a, 2013b, 2015; Benler et al., 2021; Wu et al., 2012).

In addition to well-known body niches inhabited by phages, such as the gut, which harbors a rich bacterial microbiome, phages have also been observed in the body where a normal bacterial microbiome is absent, such as the bloodstream. The concept of translocation proposes that phages originating from the gut or mucosal surfaces can breach host barriers, enter the bloodstream, and disseminate to various organs. Indeed, phages have been observed in the blood and other tissues many times (Dąbrowska, 2019; Majewska et al., 2019a). Barr and colleagues investigated mechanisms underlying this phenomenon and demonstrated that bacteriophages could adhere to and be transported across epithelial cells via transcytosis. This suggests that phages exploit cellular machinery to traverse host barriers(Barr et al., 2013a; Bichet et al., 2021). Further, phages can be enclosed within intracellular vesicles, thus being protected during transit (Van Belleghem et al., 2019). Phages in the blood stream appear, to some extent, to depend on systemically dispersed bacteria. In the blood of septic patients, phages showed specific patterns associated with different pathogens (Bichet et al., 2021; Haddock et al., 2023).

The translocation of phages is linked to immune responses, as might be expected during their systemic dissemination and resulting challenge of the immune system. Immune responses play a critical role in regulating phage activity within the human body (Dąbrowska & Abedon, 2019). Conversely, the gut exhibits a prominent phenomenon known as immunological tolerance, which

profoundly impacts the microbiota. By maintaining tolerance, the immune system allows the microbiota to thrive, supporting microbial diversity and ecological balance. Disruption of immunological tolerance can result in dysbiosis and altered microbial composition, exacerbating pathological symptoms (Barr et al., 2013a; Belkaid & Hand, 2014; Round & Mazmanian, 2009). The gut employs several mechanisms to establish and sustain immunological tolerance. These include the induction of regulatory T cells (Tregs) (Belkaid & Hand, 2014), the presence of a mucosal barrier that physically separates the microbiota from underlying cells, and the production of secretory IgA antibodies that prevent microbiota from adhering to the gut epithelium and anchor microbiota within the mucus layer (Chen et al., 2020; MacPherson et al., 2018; Rogier et al., 2014). However, it remains unclear whether translocating bacteriophages induce specific immune responses or if they benefit from the phenomenon of tolerance. Studies have shown that phages can translocate from the gut to systemic circulation, but this process is influenced by factors such as phage dose, morphology, and host physiological status (Dąbrowska, 2019). Notably, the efficiency of translocation and immunoreactivity of phages within the context of the entire phageome have yet to be fully characterized.

Here, we report a high-throughput study on phageome translocation from mucosal surfaces in the human gut to circulation, along with the identification of specific immune responses to translocating phages. The study involves human donors of large intestine mucosal bioptates and blood serum, allowing for comparisons between the phageome composition identified in the mucosa and blood serum of the same individual. We identified major phage antigens recognized by specific IgGs in these patients. We sought to explore the phenomenon of translocated phages, like the age-old question of which came first, the chicken or the egg: does the presence of phage induce antibodies that effectively neutralize the phage, thereby preventing the detection of targeted phages in blood samples containing specific IgGs? Or conversely, must phages be present in the blood to sustain detectable levels of phage specific IgGs?

Materials and Methods

Biological samples

Biopsies from the colon mucosa were collected in the Endoscopy Laboratory of the Regional Specialist Hospital in Wrocław (Poland) by an expert physician from adult patients. These patients

were also blood donors (Figure 1), with blood collected into clotting tubes, and blood serum separated and stored. The biopsies were performed following hospital procedures and guidelines to ensure accurate diagnosis and treatment of gastrointestinal conditions. Patients were recruited as qualified for colonoscopy examination due to adverse symptoms from the GI tract; Also, patients who in the interview reported antibiotic treatment in last six months or diabetes treated with metformin were excluded from the study. The group of patients included 22 women and 16 men (average age as 61, N=38) (Figure S1).

Bioethics statements

The study was conducted in accordance with the principles of the Declaration of Helsinki. The research was approved by the local Bioethical Commission of the Regional Specialist Hospital in Wroclaw (approval number: KB/nr8/rok2017). During the individual interview, all information about the study was provided and written consent was obtained from each participant. The written consent form was accepted by the local Bioethical Commission of the Regional Specialist Hospital in Wroclaw (approval number: KB/nr8/rok2017).

Next Generation Sequencing of blood and mucosal VLPs

Bioptates obtained during colonoscopy examination were floated in PBS for 2h and then forwarded to Cesium Chloride (CsCl) ultracentrifugation, preceded by filtration through 0.22 µm pore membrane filters. CsCl density gradient ranged from the highest density 1.7g/ml, followed by 1.5 g/ml, 1.35 g/ml and 1.15 g/ml. Centrifugation was performed overnight at 62,000 x g at 4°C in a swinging bucket rotor. Half a milliliter of content between 1.35 and 1.5 of CsCL solution was collected with a syringe for further phage DNA isolation. DNA isolation was performed with Sherlock AX (A&A Biotechnology) kit. Samples were proceeded to Genomiphi V2 DNA Amplification (Cytiva).

Whole blood collected from patients was centrifuged at 2000 RCF for 10 minutes at 4°C. The top layer, which contained the serum, was collected for total DNA isolation using the Sherlock AX kit (A&A Biotechnology). The samples were then subjected to GenomiPhi V2 DNA Amplification (Cytiva) before preparing the sequencing library.

Thirty-eight samples were analyzed by shotgun sequencing. Sequencing was carried out in the sequencing facility of the Biobank laboratory of the University of Lodz or in the sequencing facility

unit of the Institute of Immunology and Experimental Therapy, Wrocław Poland. Both facilities make use of Illumina NextSeq 500/550 v2.5 Kit (Medium) for sequencing and DNA library preparation kit by Vazyme. A computer cluster available at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences was used to analyze FASTQ sequences. The designed data analysis process at the very beginning rejected low-quality sequences using Trimmomatic 0.32 software. Then, obtained reads were mapped to the human GRCh38.1 genome from the UCSC (University of California Santa Cruz) repository. The program Bowtie2 2.4.5 was used for this purpose. Only sequences that did not map with the *"–very-sensitive"* parameter were used for further analysis. Alignment of bacteriophage sequences to the obtained reads was carried out using the BLAST (Basic Local Alignment Search Tool) algorithm. The BLAST algorithm was used for alignment along with an indication for e-value of 1e-50 and sequence identity of 99. The database of bacteriophage sequences for the alignment step was created from resources available in the NCBI (National Library of Medicine) repository. Viruses containing genetic material in the form of RNA were not included into the analysis. Analysis of the data from the reports generated by the BLAST algorithm was carried out using python language libraries: "pandas 1.4.3", "numpy 1.23" and "seaborn 0.11.2". DNA viruses that are specific to human eukaryotic cells were excluded from the final transcytosis analysis. Only viruses specific to bacteria - bacteriophages - were subjected to statistical analysis. Normalization against the number of reads obtained from the sequencer was used to correctly visualize the amount of penetration of each type of virus.

Potential correlation between the number of reads generated in the sequencing and the number of identified translocated phage genera was assessed, to validate the methodology for the identification of phage translocation. The analysis revealed the r-Pearson correlation coefficient of 0.236, with a significance level of 0.154. Similarly, the rho-Spearman correlation coefficient was calculated as 0.283, with a significance level of 0.085. These values demonstrate that correlation is unlikely, indicating that the number of reads per sample cannot be considered as a determining factor in the detection of translocating phages.

Library of phage-derived immune reactive epitopes

Identification of bacteriophage oligopeptides interacting with specific IgGs is presented in Figure 2. It was performed in accordance with the modified protocol published by Xu et al., 2015and adapted for our research using coding sequences of the investigated bacteriophages as the source for library design. The GenBank database (National Center for Biotechnology Information) was searched for all available phage genomes (accessed on: June 1, 2018). For further research, annotated open reading frames (ORFs) were selected, which were described with the keywords: 'structural', 'tail', 'decoration', 'capsid', 'scaffold', 'baseplate', 'coat', 'virion', 'structure', 'lysin', 'muramidase' or 'holin' but did not contain the keywords: 'initiator', 'chaperone', 'maturation protease', 'morphogenesis'. Phage enzyme-coding sequences were used as a reference for possible comparisons between structural proteins and propagation cycle-related proteins of phages. The amino acid sequences of selected ORF were virtually cut into 56 aa long fragments tailing through protein sequences, starting every 28 aa from the first amino acid (50% overlap was applied to avoid splitting potential epitopes). Sequences were deduplicated and then reverse-translated into DNA sequences using codons optimized for expression in *E. coli*; as a result, 277 051 oligopeptide sequences were included in the library. The oligopeptide library was synthesized using the SurePrint technology for nucleotide printing (Agilent). These oligonucleotides were used to create a phage display library using the T7Select 415-1 Cloning Kit (Merck Millipore) using Liga5™ (A&A Biotechnology) instead of T4 ligase and default restriction sites (EcoRI and HindIII).

Immunoprecipitation

Specific reactions between phage-derived oligopeptides and antibodies present in the investigated sera were identified by immunoprecipitation. Immunoprecipitation of the library was performed in accordance with a previously published protocol (Xu et al., 2015) . Briefly, the phage library was amplified in a standard culture as described in the manufacturer's manual, and then purified using a hollow fiber filtration membrane. In the final step, the purified and concentrated library was suspended in a Phage Extraction Buffer (20 mM Tris-HCl, 100 mM NaCl, 60 mM MgSO4, pH 8.0). All plastic containers (96-well plates) used for immunoprecipitation were prepared by blocking with 3% Bovine Serum Albumin (BSA) in TBST buffer overnight on a rotator (40 rpm, 4°C). A sample representing an average of $2x10^5$ copies of each clone in 250 µL was mixed with 1 µL of human serum (two technical replicates were applied) and incubated overnight at 4°C

with rotation (50 rpm). A 20µL aliquot of a 1:1 mixture of Protein A and Protein G Dynabeads (Invitrogen) was added and incubated for 4h at 4°C with rotation (50 rpm). The liquid in all wells was separated from Dynabeads on a magnetic stand and removed. Beads were washed 5 times with 280µL of a wash buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20) and beads were resuspended in 60µL of water to elute the immunoprecipitated bacteriophages from the beads.

The immunoprecipitated part of the library was then used for amplification of the insert region according to the manufacturer's instructions with a Phusion Blood Direct PCR Kit (Thermo Fisher Scientific). The primers T7_Endo_Lib_LONG_FOR GCCCTCTGTGTGAATTCT and T7_Endo_Lib_LONG_REV GTCACCGACACAAGCTTA were used, and a second round of PCR was carried out with the IDT for Illumina UD indexes (Illumina Corp.) to add adapter tags. Sequencing of the amplicons in accordance with Illumina next generation sequencing (NGS) technology was outsourced (Genomed, Warszawa). A FASTA file containing the selected oligonucleotide sequences was utilized to construct a BLAST database. This was achieved by executing the "makeblastdb" command with the "nucl" dbtype option. Reads from sequencing were annotated using BLAST 2.12 algorithm.

Animal model of phage translocation

C57BL/6J normal male mice (N=8) were obtained from the Medical University of Bialystok and bred in the Animal Breeding Centre of the Hirszfeld Institute of Immunology and Experimental Therapy (HIIET) in an isolated area in SPF conditions. All animal experiments were performed according to EU directive 2010/63/EU for animal experiments and were approved by the 1st Local Committee for Experiments with the Use of Laboratory Animals, Wrocław, Poland (project no. 76/2011). The authors followed the ARRIVE (Animal Research: Reporting of in vivo Experiments) guidelines.

The T4 phage was purchased from American Type Culture Collection (ATCC) (Rockville, Maryland, USA), cultured on an *Escherichia coli* B host obtained from the Collection of Microorganisms at the HIIET, culture medium was LB-Broth high salt (Sigma, Europe). The culture was conducted 8-10 hours in 37ºC. Phage lysates were filtered through polysulfone membrane filters 0.22 µm (Millipore, Europe) and purified phage preparations were obtained by gel filtration on Sepharose 4B

(Sigma-Aldrich, Poland) followed by dialysis against PBS on 1000 kDa-pore membranes and filtered with 0.22 µm PVDF filters (Millipore, Europe). Phage concentration was determined by the double-layer plate method. Mice were treated with the phage added to drinking water ($5x10^9$ /ml). Murine blood, mesenteric lymph nodes, gut content, and mucosal layer (middle and distal parts of the intestine) were collected at 26 hours post-starting phage administration. Whole blood: phage concentration was measured using two-layer plates (100 µl blood/plate). Lymph nodes: tissue sample (weighed in PBS 10^{-1} v/o) was homogenized, serial dilutions were prepared, and phage concertation in the tissue was tested using the double-layer plate method. Gut content: intestinal sample (weighed in PBS 10^{-1} v/o) was manually homogenized, shaken at 22°C for 15 minutes using an Eppendorf Thermomixer at 500 rpm, and serial dilutions were prepared and spotted on bacterial layer plate to identify the phage concentration (routine test dilution). Gut mucosa: intestinal mucus sample (weighed in PBS 10^{-1} v/o) was manually homogenized, shaken at 22°C for 15 minutes using an Eppendorf thermomixer at 500 rpm, and serial dilutions were prepared and spotted on bacterial layer plate to identify the phage concentration (routine test dilution). In control mice (N=3, limited number of animals due to ethical reasons), PBS was added to drinking water and the same procedures and samples were processed to confirmed that no T4-like phages were naturally present in these mice.

Results

Phage genera detected in human gut mucosa and in blood serum

Phages present in gut mucosa and in blood serum were identified in the group of 38 human patients. These patients served as both colon mucosa bioptate donors and blood serum donors (Figure 1). Mucosal biopsies were specifically chosen instead of the gut content to exclude transient phages from food or water, as these phages may have limited access to the transcytosing endothelium. To ensure the analysis focused on intact phage particles, only DNA encapsulated within VLPs was investigated, rather than DNA originating from disrupted capsids or lysed cells. This approach allowed the exclusion of partially digested or degraded phage particle remains that could not represent phages capable of translocation. Each mucosal sample underwent CsCl separation to isolate VLPs, followed by NGS shotgun sequencing. The circulating (blood) phageome

was identified as total cell-free DNA (cfDNA), to include phages that might have been neutralized by phage-specific antibodies. The obtained NGS results were subsequently analyzed to identify bacteriophage sequences by referring to the NCBI database.

Phage genera that were most abundant in the investigated group of patients (as a whole) were identified in patients' blood and mucosa. Full list of phage genera detected in the investigated patients is presented in Table S1. The ten most abundant genera are presented in Table 1, with the first 3 in gut including *Tequatrovirus*, *Kayvirus*, and *Skunavirus*, representing coliphages, staphphages, and lactococcal phages, respectively (Table 1 column 3.). In turn, phage genera found to be the most abundant in human blood were *Tequatrovirus*, *Pahexavirus* (bacterial host: *Cutibacterium*, previously *Propionibacterium*), and *Kayvirus*, as outlined in Table 1 (column 4.). While *Tequatroviruses* and *Kayviruses* have the most probably been translocated from gut where they represent dominating fraction of bacterial viruses, the origin of *Pahexaviruses* in blood does not correlate to their abundance in gut. Since these phages predate on bacteria often present on the skin, phageome transfer between skin and blood circulation can play a role in shaping blood phageome, though further studies would be necessary to elucidate this phenomenon.

Phage transcytosis from human gut mucosa to blood serum

Further analysis of phage translocation was conducted in individual patients. Bacteriophage translocation was considered positive if the same bacteriophages were present in both mucosal and serum samples of the same individual. This was detected by comparing NGS reads in mucosal and serum samples from the same patient. Overall, Translocation of any phage genus was confirmed in 33 out of 38 patients (86%) (Table 2). Like the whole group analysis, the most translocated group of bacteriophages turned out to be *Kayviruses* (myoviruses predating on *Staphylococcus*). They were detected in both gut mucosa and blood serum in 23 different individuals. Very similar frequency of translocation was observed for *Tequatrovirus* (myoviruses, specific to *Enterobacteriaceae*, in the majority *Escherichia coli*) detected in 22 individuals. These were followed by the group *Gamaleyavirus* (in the majority podoviruses predating on *Escherichia coli*), detected in 12 samples.

The frequency of translocation in the investigated individuals (Table 2) overall correlated to phage abundance in the investigated individuals (Table 1), for instance two most often translocated genera of bacteriophages (*Kayviruses* and *Tequatoviruses*) were the groups most abundant in human gut. However, Pearson test (0.58, $p<0.05$) shows, that correlation between phage abundance in the gut and phage translocation frequency was moderate. If so, other factors like the physicochemical properties of phage particles or their interactions with the human immune system may have a significant effect on phage's ability to translocate from gut to circulation. Also, some phage groups were detected only in serum while not present in the gut mucosa, including *Foxborovirus* (bacterial host: *Gordonia*), *Corndogvirus* (bacterial host: *Mycobacterium*), *Paopuvirus* (bacterial host: *Microbacterium*), *Omarvirus* (bacterial host: *Streptomyces*), and *Salasvirus* (bacterial host: *Bacillus*) (Table S1), again suggesting that gut phageome shapes the phageome found in circulation only in part. Since the majority of these phages are linked to environmental bacteria, they have probably been able to enter circulation from environmental sources, such as food, water, dust, etc.

Antibodies specific to bacteriophage proteins

Due to significant homologies between some phage proteins, antibodies targeting phages (that is: targeting phage proteins) often demonstrate cross-reactions between different phage strains (Gembara & Dąbrowska, 2021).To avoid ambiguous results, we investigated specific immune responses to the translocating phages at phage protein level. We used adapted VirScan method (Xu et al., 2015), with phage-derived epitope library containing 277 051 oligopeptides covering the structural proteome of phages available in GenBank (for details see Materials and Methods section and Figure 2). Briefly, we applied the library immunoprecipitation with each patient serum sample, followed by massive sequencing of the immunoprecipitated part of the library to identify phage proteins recognized by the patients' sera.

First, we investigated potential trends for selected phage proteins or specific phage taxa to induce immune responses more efficiently. However no significant trend was observed. The immunoreactive phage proteins represented diverse protein functions and taxonomic groups of bacteriophages (Figure 3). Next, translocation of phage antigens from gut to blood was assessed.

Similarly to the identification of phage genera translocation presented in previous section, phage proteins were also identified as translocated based on sequencing results from gut and serum phageomes. In total, 201 specific proteins were identified as crossing gut-blood barrier. The comparison of the abundance of identified protein groups based on their function is presented in Figure 4A.

We further investigated how phage-specific immune responses regulate phageome that has been translocated to blood. We aimed to determine which mechanism predominated: (i) the efficient neutralization and removal of targeted phages mediated by phage-specific antibodies, leading to the absence of these phages in blood samples with significant levels of specific IgGs; or (ii) a correlation between the presence of phages in the blood and the detection of phage-specific IgGs, suggesting that the continued presence of these phages was necessary to sustain significant levels of phage-specific IgGs. We used GenBank metadata on phage protein functions or annotated structural homologies. We compared the functional profile of the proteins targeted by specific IgGs, as identified by immunoprecipitation with the phage epitope library (Figure 2), to the profile of phage proteins identified as present in the blood of investigated patients (Figure 4B). Most proteins targeted by specific antibodies were not detectable in blood phageome; only some major capsid proteins maintained noticeable representation within the blood phageome despite pronounced pressure from specific IgGs (Figure 4B). These results were consistent with those observed when protein annotated structural homologies were analyzed instead of functions (Figure S2). Further, no significant Pearson correlation was observed between phage proteins found in blood and those recognized by IgGs (p-value > 0.05). These observations strongly suggest that the first proposed mechanism of interaction between translocating phageome and human immunity plays a major role: phages that induce specific antibodies are effectively neutralized and removed, thus undetectable in circulation.

Phage arrest in mesenteric lymph nodes

T4 phage representing *Tequatroviruses*, that were found frequently translocating phages in this study, was used as the model phage in the animal model of phage translocation. The

translocation experiment *in vivo* included four major phage populations potentially involved in the multi-step translocation process: phages in gut content, phages on the mucosal surface (those potentially contacting the translocation mediating epithelium), phages in mesenteric lymph nodes (capturing material transcytosed by gut epithelial cells), and phages circulating in the blood, representing efficiently translocated ones. Mice were administered $5x10^9$ pfu/ml of purified T4 phage in drinking water, continuously. After 26 hours phage concentration was assessed in gut content, in scrabbed intestinal mucus, in homogenized lymph node tissue, and in blood. As demonstrated in Figure 5, phage concentration in gut content exceeded $2x10^8$ pfu/g which was approx. 1 order of magnitude higher that phage concentration in intestinal mucus. Significant concentration of phage was observed in lymphatic tissue (approx. $1x10^3$ pfu/g); taking massive degradation of transcytosed material in the lymph nodes (Roozendaal et al., 2008), we hypothesize that phage inactivation in this tissue is fast and intensive. If so, active phages that were detected probably represent only a small fraction of phages captured by the lymph nodes, with the vast majority being degraded and used for phage antigen presentation to immunological cells. Only very few phages escape that, thus resulting amount of phages successfully reaching the blood was very low comparing to the initial phage concentration in gut content (Figure 5).

Discussion

The study of bacteriophage translocation and transcytosis in animals and humans represents a relatively new area of phage biology. Nguyen et al. (2017) demonstrated transcytosis of a few different types of tailed phages *in vitro* in epithelial and endothelial cell lines including mucosal-related ones. Those phages efficiently travelled between two chambers of a culture device via a cellular monolayer. Moreover, cellular uptake of phages was later evidenced by visualization within cells (Bichet et al., 2021). This indicates that phages detected in a mucosal layer in the gut should also be detected in the blood sera of animals and humans. Many animal models and human trials confirmed that for individual phages applied orally (Majewska et al., 2015a; Monsur et al., 1970; Oliveira et al., 2009; Wolochow et al., 1966), but so far it remains unclear how transcytosis is regulated in the context of phageomes as the whole, as well as how it interplays with immune responses to phages. Here we have compared phageome composition in the

human gut mucosa and in blood of the same patients. In the gut, we observed a significant contribution of bacteriophages targeting typical gut bacteria, like *Lambdavirus*, *Teseptimavirus*, *Tequatrovirus,* and *Mosigvirus*, that parasitize mainly *Escherichia coli*, *Salmonella,* and *Shigella,* or *Skunavirus* that parasitize *Lactobacilli* (Table 1). Another highly represented group of gut phages were *Kayviruses* which are staphylococcal phages; this is in line with the common identification of *Staphylococcus* as an asymptomatically carried member of human microbiomes including gut (Raineri et al., 2022) .

We further compared gut phageomes composition to blood phageomes. In the majority, the expectation that phages found abundant in gut microbial communities can also be found circulating in blood was confirmed. *Tequatrovirus* and *Kayvirus,* identified within the three most abundant phage groups in gut phageomes, were also found within the three most abundant phage groups in the patients' sera. Further, individual analyses of each patient's data confirmed the translocation of these groups in 22 and 23 patients (out of 38), respectively. To some extent, other phage groups were also consistently represented both in the gut mucosa and in blood sera (Table 1), however, Pearson test (0.58, $p<0.05$) demonstrated, that the overall correlation was moderate. Also, some phage groups found abundant in gut, like lactococcal phages from *Skunaviruses*, were less abundant in blood, or conversely, those less represented in the gut were however numerous in blood, like *Pahexaviruses.* If so, other factors, such as physicochemical properties of phage particles or their interactions with the human immune system, may significantly affect the ability of phages to translocate from the gut to the circulation. Also, contribution of other (than gut) sources of phages to the composition of blood phageomes may play a role. That includes phages that parasitize environmental bacteria, meaning they are more likely to be found abundantly in the environment rather than in the human body. Examples observed in this study include *Foxborovirus* (bacterial host: *Gordonia*), *Corndogvirus* (bacterial host: *Mycobacterium*), *Paopuvirus* (bacterial host: *Microbacterium*), *Omarvirus* (bacterial host: *Streptomyces*), and *Salasvirus* (bacterial host: *Bacillus*) (Table S1).

Phage transcytosis has been postulated as a dynamic and intense phenomenon (Nguyen et al., 2017). In our study, the number of phages detected in blood serum was rather low, and efficient

detection of phageome composition required viral DNA amplification. Also, only 21 phage groups (out of 51 detected in the mucus layer) were detected simultaneously in the serum (Table S1). What is more, a high abundance of bacteriophages in the human gut was found only moderately positively correlated with abundance of phage in blood. The overall frequency of translocation observed in patients individually (Table 2) was moderate. This may suggest that translocation is not as intensive as it could be suggested by *in vitro* models. Importantly, transcytosis indeed demonstrated as relatively dynamic (Nguyen et al., 2017), is the first step in translocation of phage from gut lumen to the systemic blood circulation. Before reaching circulation, infiltrates from the gut are first collected by lymphatic vessels and directed to lymph nodes or harvested by immunological cells like or dendritic cells or possibly macrophages (Kiernan et al., 2019; Zhuang et al., 2024). Lymph nodes, in turn, act as filters with a 70-kilodalton cutoff for molecules whereas phages are typically measured in megadaltons (Hons & Sixt, 2015). Concordantly, previously reported i*n vivo* experiments indicated phage arrest in lymph nodes (Dąbrowska, 2019). Here we assessed the ability of lymph nodes to filtrate phages translocating from gut to blood circulation in a mouse model using a model phage T4 (representing *Tequatrovirus* genus found within the most frequently translocated phage groups in this human study). We observed that despite high T4 concentration in murine intestinal content, its translocation to blood demonstrated limited efficacy and phage harvest in lymphatic tissue was marked (Figure 5). This suggests that even an intensive transcytosis at gut epithelium monolayer may be insufficient for the abundant presence of phages in blood serum due to immunological barriers preventing phage from entering circulation. Of note, lymph nodes are the body sites where immune responses to foreign antigens can be initiated, due to antigen presentation by professional cells (like dendritic cells) and resulting activation of antigen-specific lymphocytes. This is the key step both in promoting tolerance (in that case regulatory T-cells are activated) and in developing antigen-specific antibody production by B cells.

Phage-specific IgGs have been demonstrated as easily inducible for virtually all phages and potentially efficient in systemic phage neutralization. (Gembara & Dąbrowska, 2021; Ledeboer et al., 2002). In this study we sought to understand if phages constantly present in the blood sustain detectable levels of phage specific IgGs or conversely, once antibodies have been induced, they

prevent targeted phages from efficient translocation. To find the predominant mechanism, we identified specificity of patients' antibodies, using IgGs interaction with a whole phage proteome-wide library of phage epitopes (technology modified from (Xu et al., 2015), for details see Materials and methods section). Next, we compared the sequences of identified IgG-targeted phage epitopes with those detected in phageomes circulating in the patients' blood. This comparison (Figure 4B) revealed that phage antigens targeted by specific IgGs in the vast majority were very different to those detected in blood phageome. Also, no significant Pearson correlation was observed, which strongly suggests that once induced, phage-specific antibodies predominate phage translocation and make phage delivery to circulation inefficient. Of note, in this study we used total metagenomic sequencing of serum samples to detect the potential presence of phages, even those inactivated by specific antibodies. Genetic methods based on phage DNA analysis (in contrary to microbiological cultures) are capable of detecting phages even when captured by specific antibodies (Kłopot et al., 2017). Here, in the patients' blood, we found almost no phage DNA encoding epitopes targeted by IgGs. We conclude that phages targeted by specific IgGs were indeed prevented from entering circulation not only "hidden" by phage-specific antibodies.

Another observation is that the majority of phage antigens currently recognized by circulating IgG antibodies are not detectable among phage antigens present in the gut phageomes of these patients (Figure 4A and B). This observation suggests a potential mechanism by which IgG regulates the phageome, resembling the immunological control of certain bacterial microbiota. The role of systemic IgG responses to symbiotic bacteria under homeostatic conditions remains poorly understood (Zeng et al., 2016). In general, the predominant antibody response to gut microbiota is mediated by IgA. This includes both T-independent induction of IgA targeting non-invasive commensals and T-dependent induction of IgA directed at more invasive commensals or pathogens. Additionally, selective symbiotic gut bacteria have been shown to induce IgG responses, often linked to translocating microbiota (Chen et al., 2020; Vujkovic-Cvijin et al., 2022; Zeng et al., 2016). By analogy, it is plausible that translocating phages may also induce phage-specific IgG responses via a similar mechanism.

However, IgG responses, even under homeostatic (non-inflammatory) conditions, possess a greater potential for neutralizing and eradicating microbiota compared to IgA, which primarily acts to regulate and promote tolerance. Consequently, mucosal microbiota targeted by IgG are often depleted (Chen et al., 2020; Vujkovic-Cvijin et al., 2022; Zeng et al., 2016). Importantly, IgG's antimicrobial action in gut is likely restricted to mucosal surfaces, including its involvement in transcytosis, rather than affecting all microbes within the gut. This is supported by two observations: first, IgG concentrations in gut contents are generally low; second, previous studies indicate that even high systemic levels of IgG targeting gut phages do not significantly reduce phage concentrations in the gut (Majewska et al., 2015b, 2019b).

Based on these considerations, we propose a mechanism for the regulation of phage translocation, as illustrated in Figure 6. This mechanism includes:

(i) phage translocation across the epithelial barrier, as demonstrated by Nguyen et al. (2017);

(ii) subsequent redirection of translocated phages to gut-associated lymph nodes, where they appear to induce strong mucosal tolerance, as supported by their abundance in mucus (Figure 4A);

(iii) in rare instances, some phages elicit systemic IgG responses, potentially due to intrinsic properties of these phages or co-occurrence of these phages translocation with bacterial infections or other inflammatory events;

(iv) phages targeted by IgGs are neutralized and removed, thereby becoming only sporadically represented in mucus and systemic circulation.

Bacteriophages are a crucial part of the body's microbiome, and they are considered potential antibacterial agents that could enhance the medical arsenal against bacterial infections (Kutter et al., 2015; Schooley & Strathdee, 2020; “The Promise of Phages,” 2023). However, it becomes evident that each phage should be studied within the context of the entire body phageome. The key features of phage as a drug, such as pharmacokinetics and bioavailability, may not solely depend on the body's response to a single therapeutic phage, but rather on complex interactions within the patient's phageome and immune system. Here we demonstrate phage groups that either follow or avoid simple extrapolation from gut phageome to systemically disseminated

phages. We also propose the limiting effects of lymph node filtration and the exclusion effects of specific antibodies targeting certain antigens within phage populations. We however believe that our observations call for further investigation of phage interaction with the human body, particularly in the context of body phageome and human immunity responses.

Conclusions

- *Tequatroviruses* and *Kayviruses* have been found among most represented phage groups in gut mucosa of patients investigated herein, and at the same time they have been found most frequently translocated to blood circulation.
- Overall correlation between phageome composition in gut and translocated phage groups is moderate, thus other factors (than phage concentration in gut) may affect efficacy of phage translocation.
- The gut phageome contributes to the composition of the phageome circulating in the blood, but other sources of phages also appear to contribute, including environmental ones.
- We propose to consider epithelial transcytosis of phage as the first step of multi-step phenomenon of phage translocation from gut to blood circulation; since transcytosis is followed by filtration in lymphatic tissue, resulting phage translocation *in vivo* is limited.
- Significant levels of phage-specific IgGs were negatively correlated to the presence of targeted phage antigens in blood circulation and in the gut mucus layer, thus phage-specific antibodies are the key limiting factor for translocating phages.

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Conflicts of interest

Authors declare no conflicts of interest.

Data Access

https://www.ncbi.nlm.nih.gov/sra/PRJNA874475

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Figures and Tables

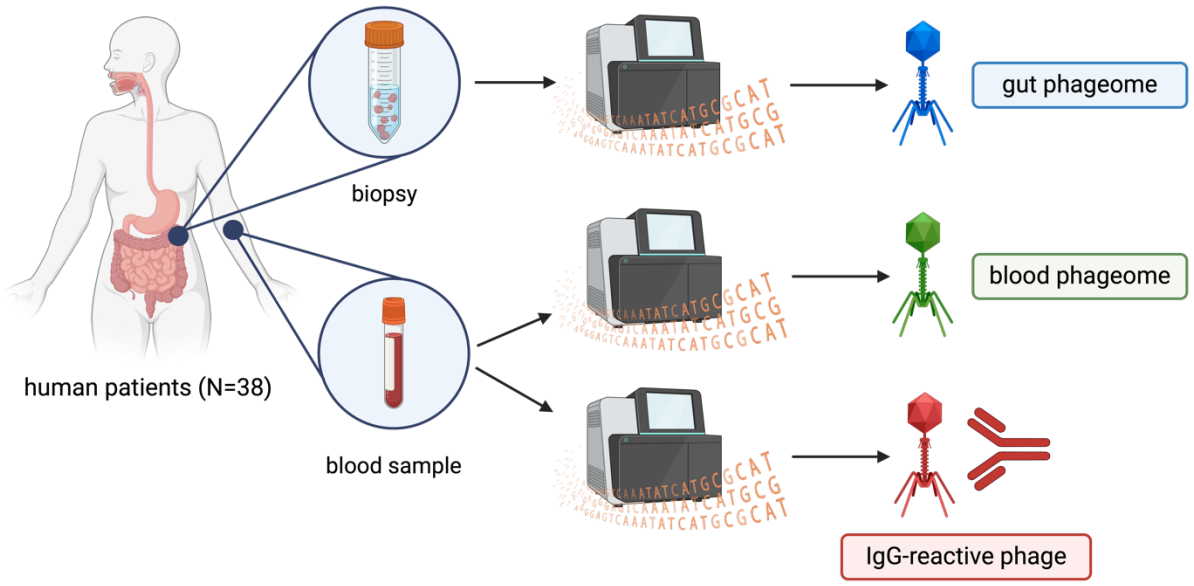


Figure 1. Human samples used in this study to investigate composition of gut and blood phageomes, and IgG antibody responses to the phageomes (BioRender).

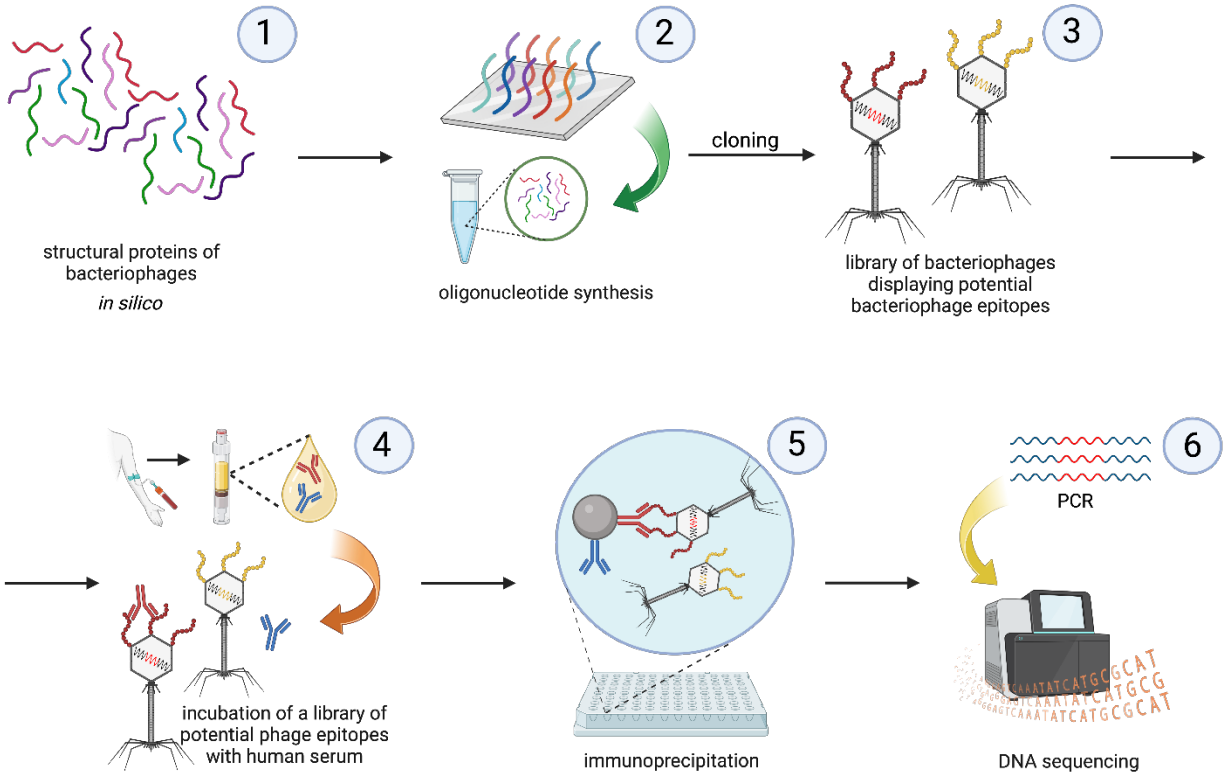


Figure 2. PhageScan technology (adapted from VirScan, Xu et al. (2015) (BioRender).

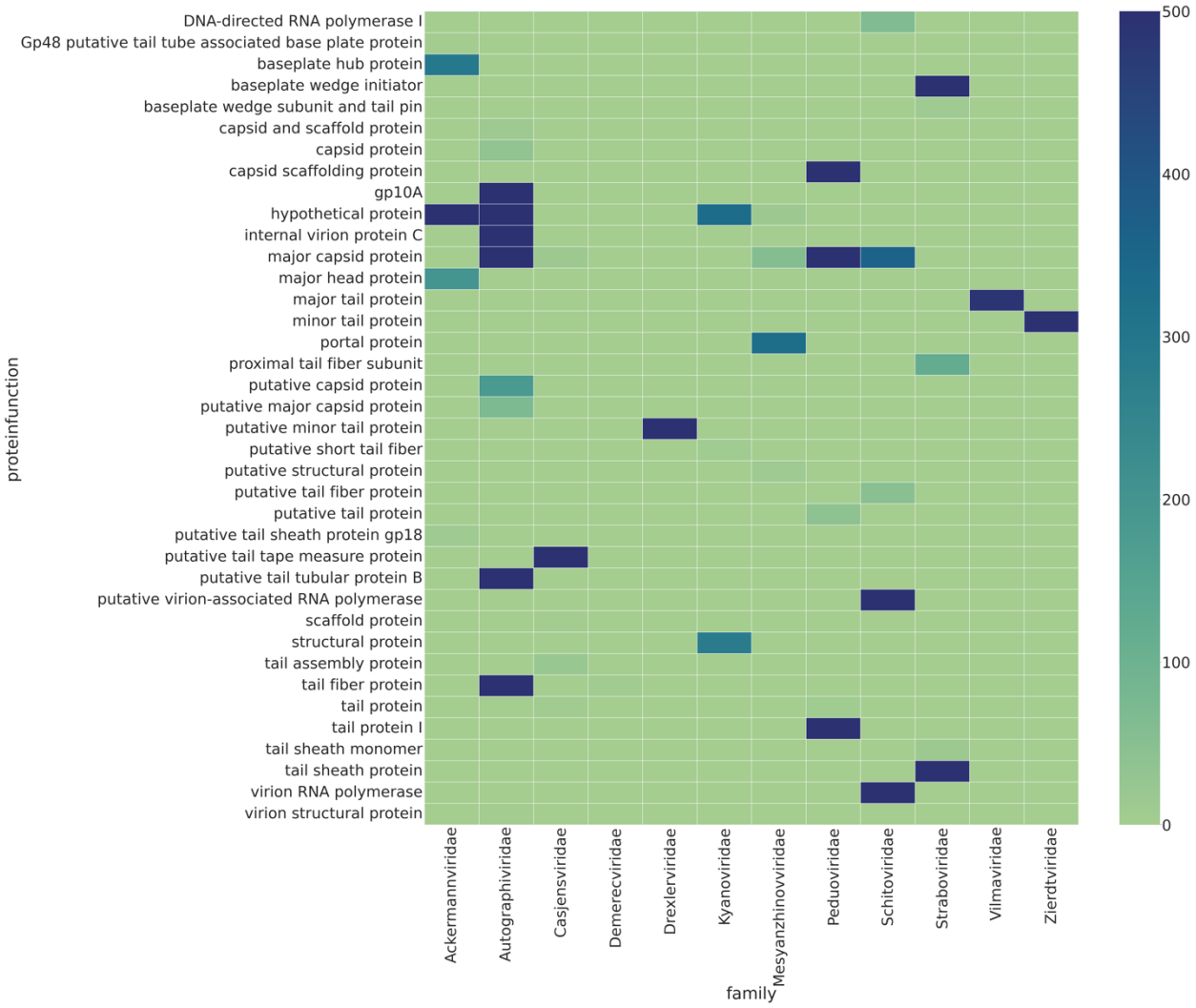


Figure 3. Antibodies targeting specific protein epitopes across various bacteriophage families. The x-axis represents the bacteriophage families, the color gradient indicates the quantity of antibodies, with lighter green representing lower quantities and darker blue representing higher quantities. This variability suggests distinct immune recognition patterns associated with the epitopes of these bacteriophage proteins.

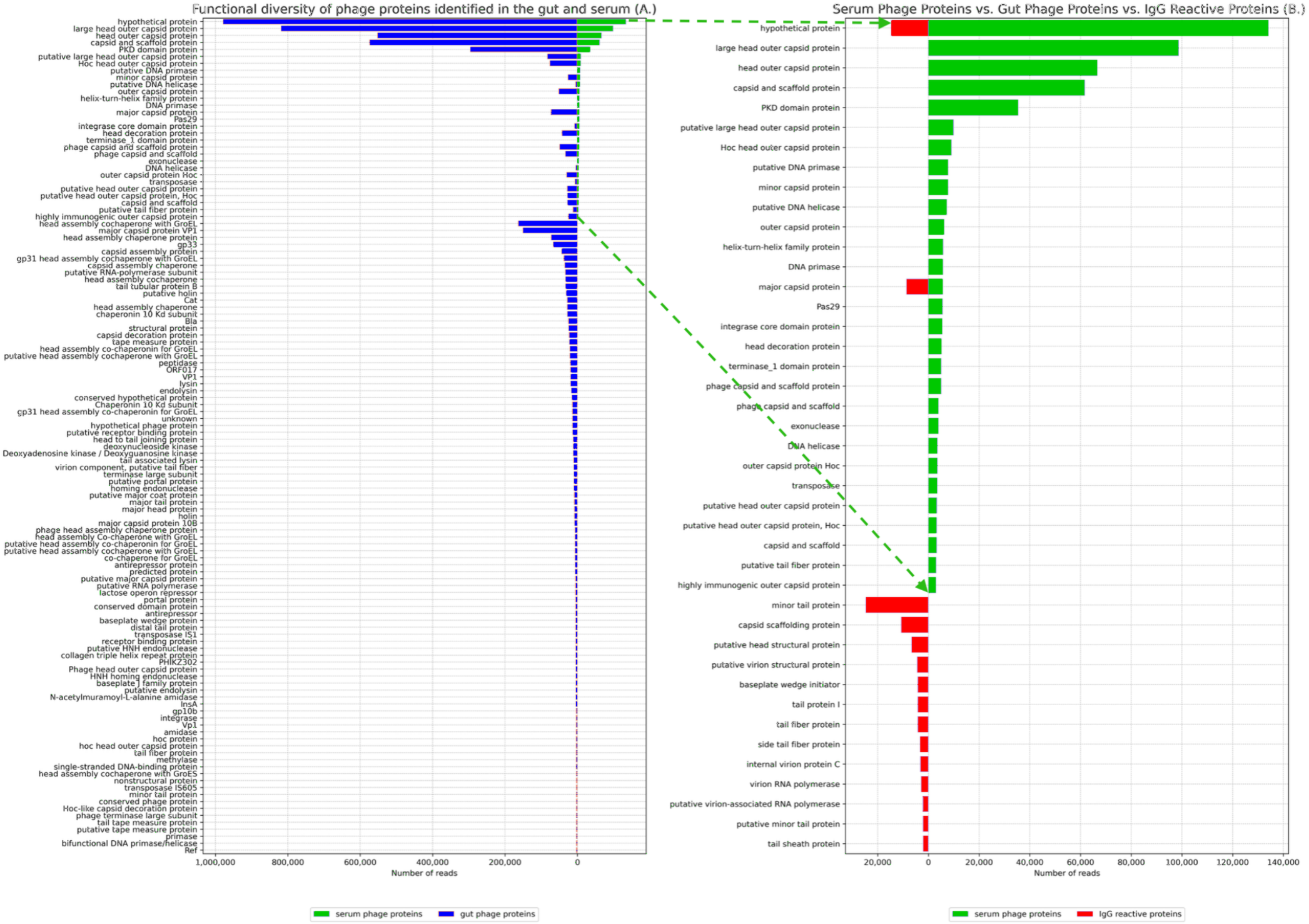

Functional diversity of phage proteins identified in the gut and serum (A.)
hypothetical protein
large head outer capsid protein
head outer capsid protein
capsid and scaffold protein
PKD domain protein
putative large head outer capsid protein
Hoc head outer capsid protein
putative DNA primase
minor capsid protein
putative DNA helicase
outer capsid protein
helix-turn-helix family protein
DNA primase
major capsid protein
Pas29
integrase core domain protein
head decoration protein
terminase_1 domain protein
phage capsid and scaffold protein
phage capsid and scaffold
exonuclease
DNA helicase
outer capsid protein Hoc
transposase
putative head outer capsid protein
putative head outer capsid protein, Hoc
capsid and scaffold
putative tail fiber protein
highly immunogenic outer capsid protein
head assembly cochaperone with GroEL
major capsid protein VP1
head assembly chaperone protein
gp33
capsid assembly protein
gp31 head assembly cochaperone with GroEL
capsid assembly chaperone
putative RNA-polymerase subunit
head assembly cochaperone
tail tubular protein B
putative holin
Cat
head assembly chaperone
chaperonin 10 Kd subunit
Bla
structural protein
capsid decoration protein
tape measure protein
head assembly co-chaperonin for GroEL
putative head assembly cochaperone with GroEL
peptidase
ORF017
VP1
lysin
endolysin
conserved hypothetical protein
Chaperonin 10 Kd subunit
gp31 head assembly co-chaperonin for GroEL
unknown
hypothetical phage protein
putative receptor binding protein
head to tail joining protein
deoxynucleoside kinase
Deoxyadenosine kinase / Deoxyguanosine kinase
tail associated lysin
virion component, putative tail fiber
terminase large subunit
putative portal protein
homing endonuclease
putative major coat protein
major tail protein
major head protein
holin
major capsid protein 10B
phage head assembly chaperone protein
head assembly Co-chaperone with GroEL
putative head assembly co-chaperonin for GroEL
putative head assembly cochaperone with GroEL
co-chaperone for GroEL
antirepressor protein
predicted protein
putative major capsid protein
putative RNA polymerase
lactose operon repressor
portal protein
conserved domain protein
antirepressor
baseplate wedge protein
distal tail protein
transposase IS1
receptor binding protein
putative HNH endonuclease
collagen triple helix repeat protein
PHIKZ302
Phage head outer capsid protein
HNH homing endonuclease
baseplate J family protein
putative endolysin
N-acetylmuramoyl-L-alanine amidase
InsA
gp10b
integrase
Vp1
amidase
hoc protein
hoc head outer capsid protein
tail fiber protein
methylase
single-stranded DNA-binding protein
head assembly cochaperone with GroES
nonstructural protein
transposase IS605
minor tail protein
conserved phage protein
Hoc-like capsid decoration protein
phage terminase large subunit
tail tape measure protein
putative tape measure protein
primase
bifunctional DNA primase/helicase
Ref
1,000,000
800,000
600,000
400,000
200,000
0
Number of reads
serum phage proteins
gut phage proteins
Serum Phage Proteins vs. Gut Phage Proteins vs. IgG Reactive Proteins (B.)
hypothetical protein
large head outer capsid protein
head outer capsid protein
capsid and scaffold protein
PKD domain protein
putative large head outer capsid protein
Hoc head outer capsid protein
putative DNA primase
minor capsid protein
putative DNA helicase
outer capsid protein
helix-turn-helix family protein
DNA primase
major capsid protein
Pas29
integrase core domain protein
head decoration protein
terminase_1 domain protein
phage capsid and scaffold protein
phage capsid and scaffold
exonuclease
DNA helicase
outer capsid protein Hoc
transposase
putative head outer capsid protein
putative head outer capsid protein, Hoc
capsid and scaffold
putative tail fiber protein
highly immunogenic outer capsid protein
minor tail protein
capsid scaffolding protein
putative head structural protein
putative virion structural protein
baseplate wedge initiator
tail protein I
tail fiber protein
side tail fiber protein
internal virion protein C
virion RNA polymerase
putative virion-associated RNA polymerase
putative minor tail protein
tail sheath protein
20,000
0
20,000
40,000
60,000
80,000
100,000
120,000
140,000
Number of reads
serum phage proteins
IgG reactive proteins

Figure 4**:** Functional diversity of phage proteins identified in the human intestinal mucosa compared to those identified in sera from the same patients (A), and phage proteins targeted by phage specific IgGs compared to phage protein presence in the same human sera (B). Phage protein sequences were analyzed based on annotations found in the NCBI database. The y-axis lists various phage protein functions based on NCBI annotations, while the x-axis represents the number of reads for each protein function.
A) The left panel displays the functional diversity of phage proteins identified in both the gut and serum of patients. (B) The right panel compares the presence of specific phage proteins in the serum (green bars) to those targeted by IgG reactive proteins.

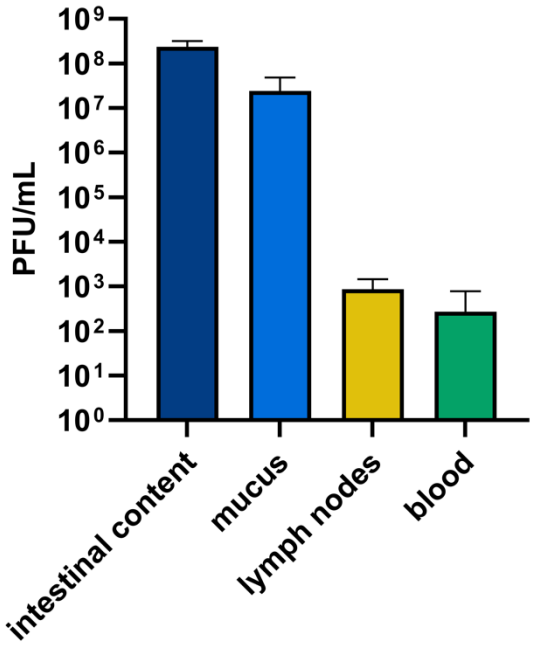


Figure 5. Mouse model of phage translocation and dissemination from gut. C57BL/6J normal male mice (N=8) were treated with Sepharose-purified T4 phage (ATCC) added to drinking water ($5x10^9$ pfu/ml) continuously for 26 hours. Murine blood, mesenteric lymph nodes, gut content, and scraped intestinal mucus were collected at 26 hours post-starting phage administration. Phage concentration was assessed by double-layer plate method or routine test dilution series. One exemplary experiment out of two conducted ones is presented.

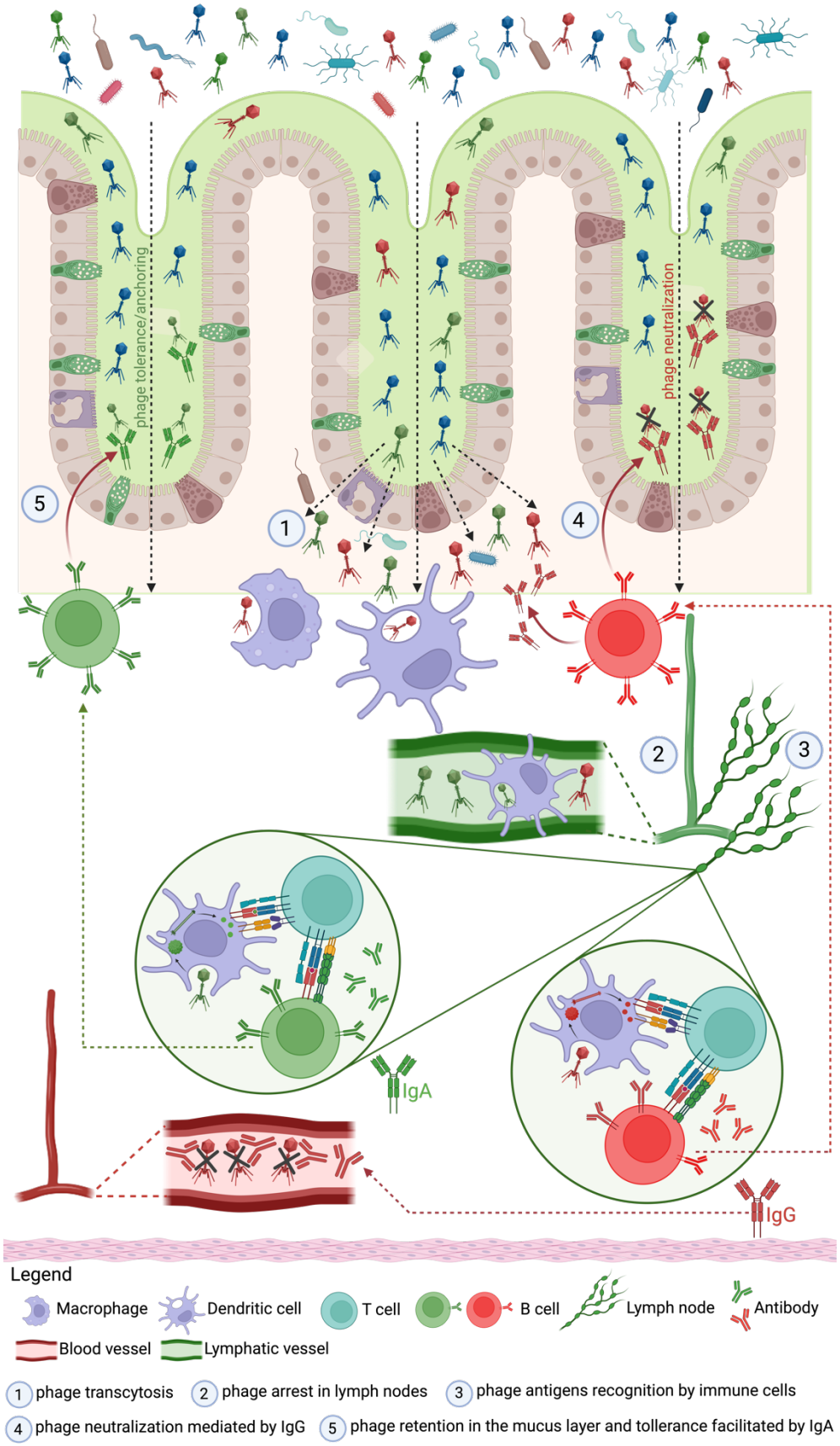

phage tolerance/anchoring
phage neutralization
5
1
4
2
3
IgA
IgG
Legend
Macrophage
Dendritic cell
T cell
B cell
Lymph node
Antibody
Blood vessel
Lymphatic vessel
1 phage transcytosis
2 phage arrest in lymph nodes
3 phage antigens recognition by immune cells
4 phage neutralization mediated by IgG
5 phage retention in the mucus layer and tollerance facilitated by IgA

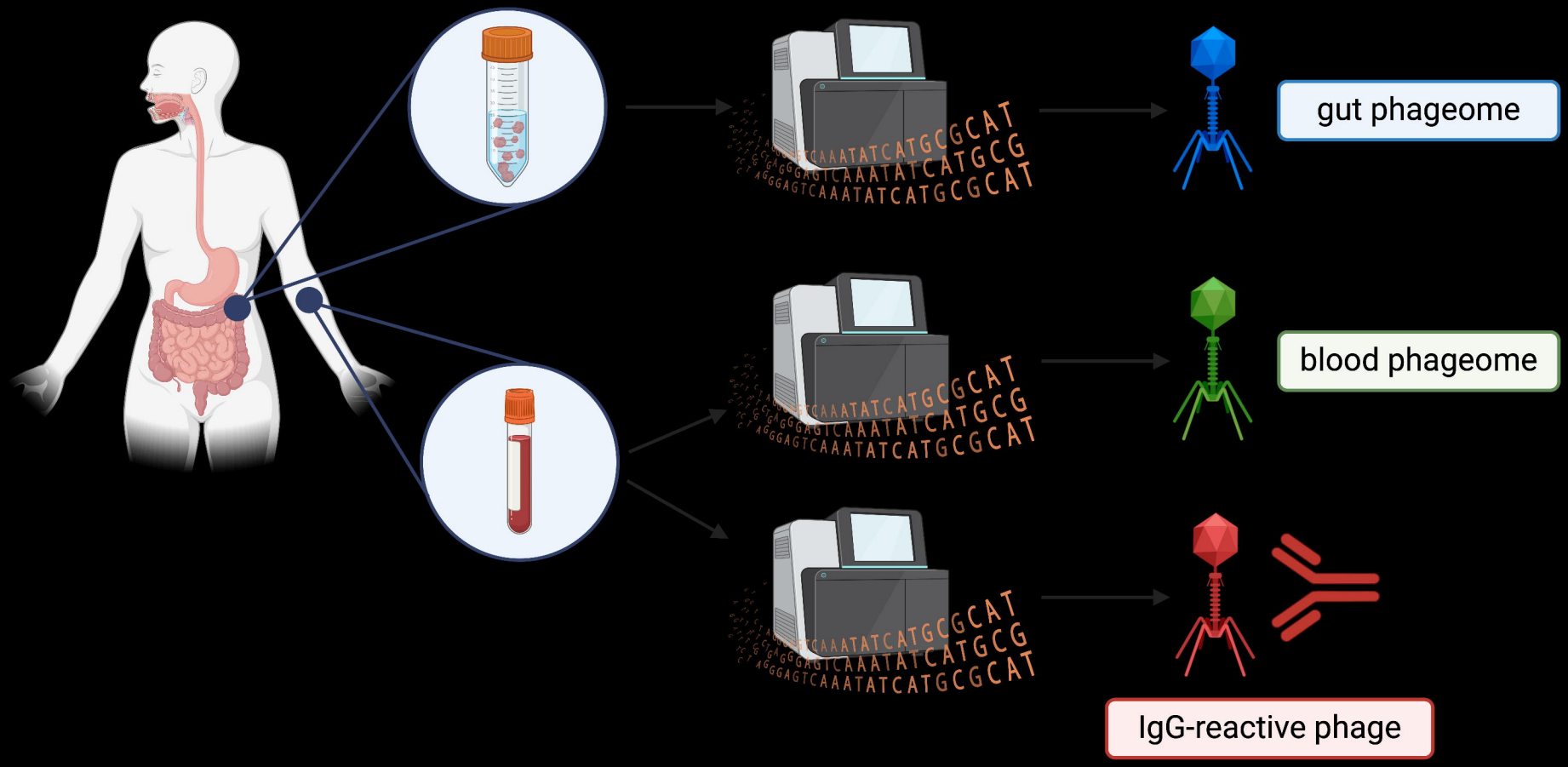
Figure 6. Proposed immunological mechanism regulating phage translocation. 1. phage translocation across the epithelial barrier; 2. redirection of translocated phages to lymph nodes with the primarily effect of mucosal tollernace; 3. some phages elicit systemic IgG responses; 4. phages targeted by IgGs are neutralized.

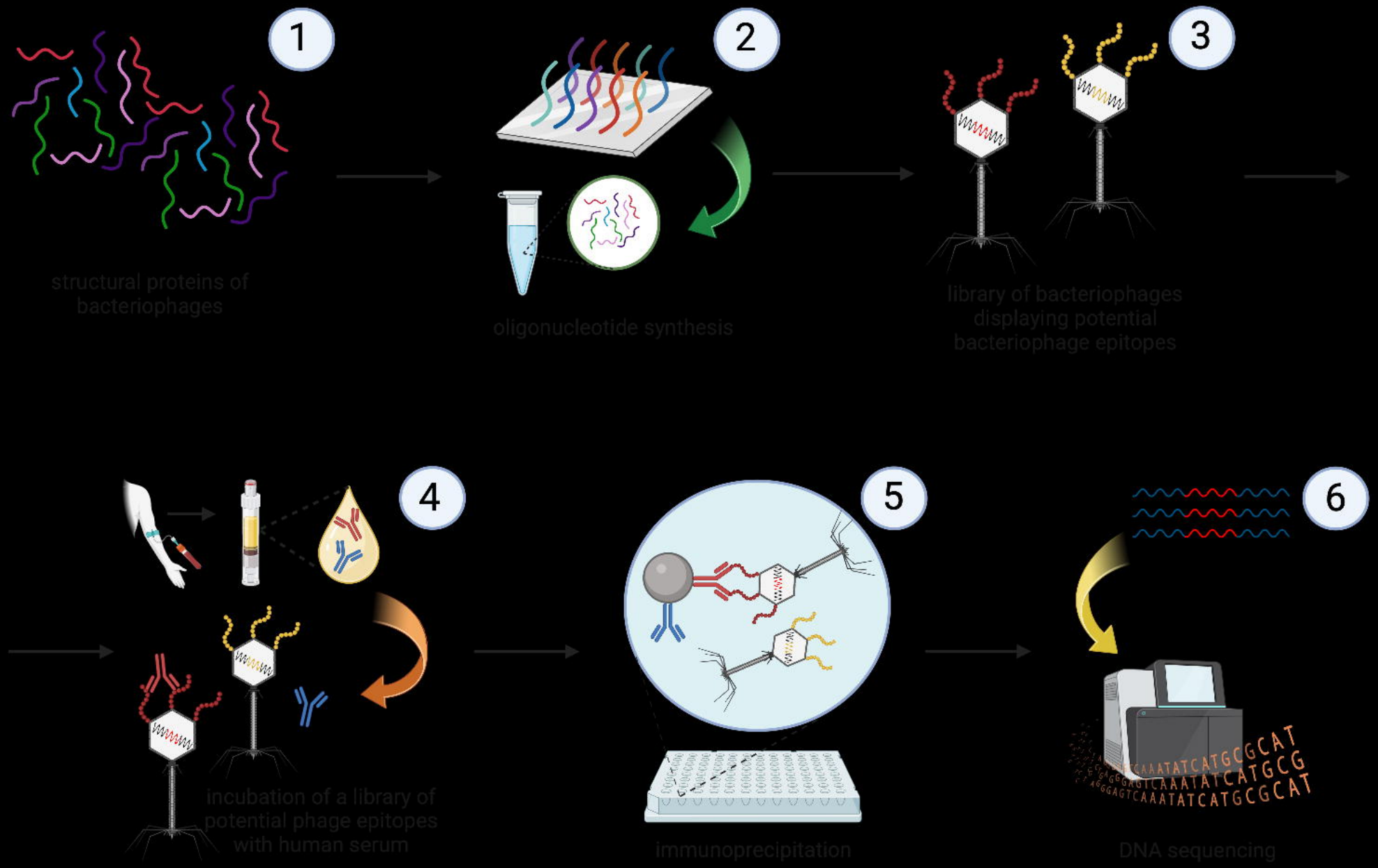
Table 1 Most abundant genera of bacteriophages in patients' large intestines' mucosa. Ratio was calculated based on readings assigned to a genus of virus relative to all reads in the samples.

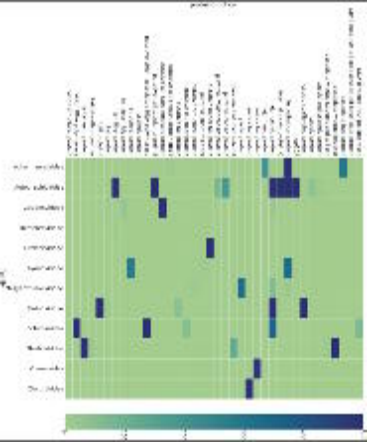
No.	**Genus**	**Major hosts**	**Presence ratio – large intestines**	**Presence ratio - serum**	**Morphology**
1	*Tequatrovirus*	*Escherichia coli sp.*	0.436254	0.362355	myovirus
2	*Kayvirus*	*Staphylococcus sp.*	0.140325	0.085876	siphovirus
3	*Skunavirus*	*Lactobacillus sp.*	0.039254	0.001108	siphovirus
4	*Mosigvirus*	*Escherichia coli sp.*	0.034812	0.026797	podovirus
5	*Shenzhenvirus*	*Aeromonas sp.*	0.023566	0.000040	myovirus
6	*Lambdavirus*	*Escherichia coli sp*	0.012607	0.001990	siphovirus
7	*Teseptimavirus*	*Escherichia coli sp.*	0.011867	0.030024	podovirus
8	*Rockefellervirus*	*Staphylococcus sp.*	0.009914	0.000671	siphovirus
9	*Dhakavirus*	*Escherichia coli sp.*	0.009354	0.004235	myovirus
10	*Kehishuvirus*	*Uncultured crAss-phage*	0.008801	0.000009	non-enveloped, head-tail structure.
11	*Chlamydiamicrovirus*	*Chlamydia*	0.008516	0.000001	non-enveloped, round, T=1 icosahedral symmetry
12	*Traversvirus*	*Escherichia spp.*	0.005853	0.011539	head-tail
13	*Pahexavirus*	*Cutibacterium sp.*	0.005143	0.094851	siphovirus
14	*Berlinvirus*	*Escherichia spp.*	0.004132	0.006762	head-tail
15	*Sepunavirus*	*Staphylococcus sp.*	0.000364	0.006788	myovirus
16	*Diegovirus*	*Shigella sp.*	0.000138	0.007664	podovirus
17	*Hemphillvirus*	*Bacillus sp*	0.000042	0.004976	non-enveloped, head-tail structure
18	*Affertcholeramvirus*	*Vibrio sp*	0.000005	0.007562	inovirus

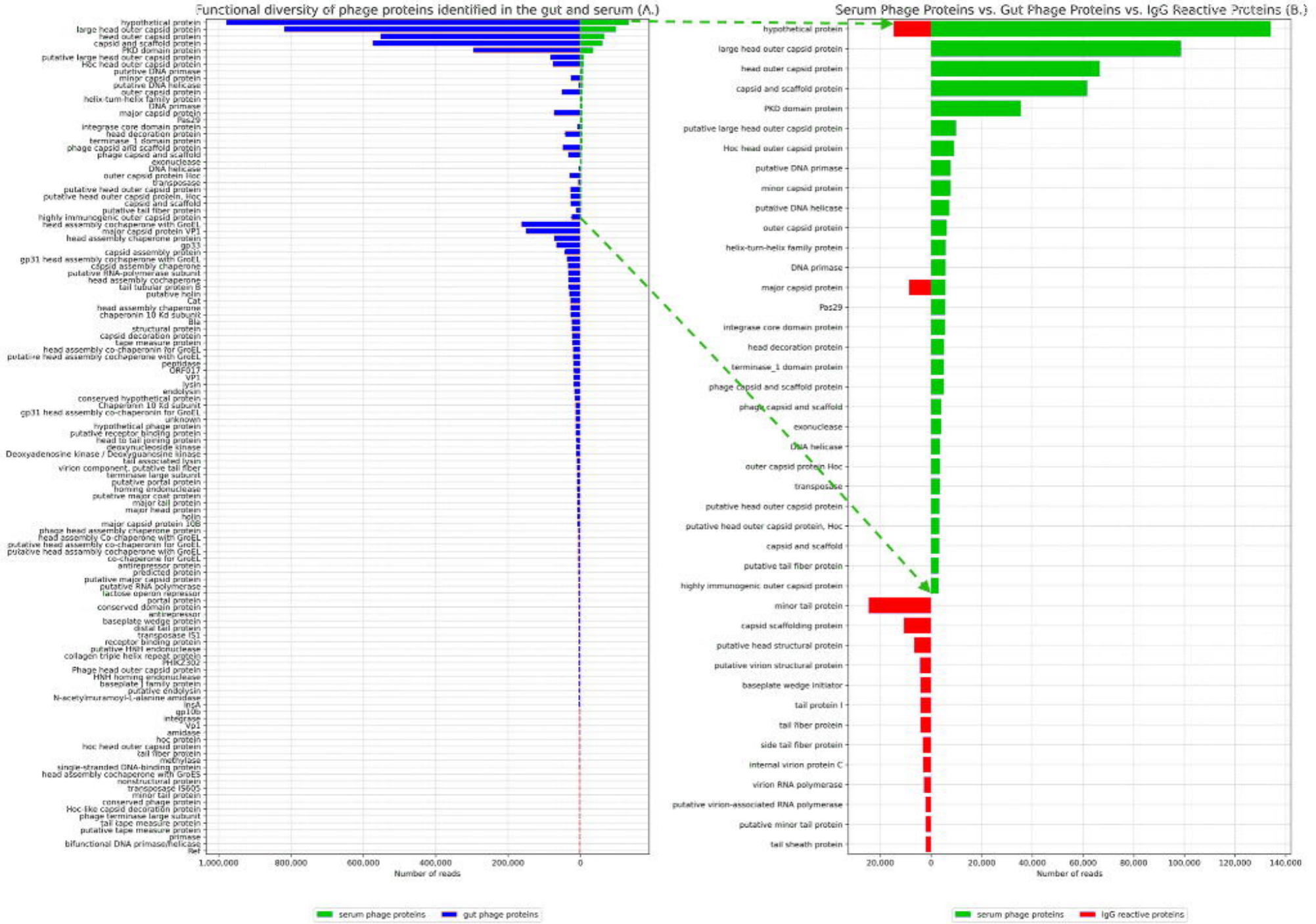
Table 2 The genus of bacteriophages most frequently transcytosed in the investigated patients. In each patient gut phageome sequencing reads were aligned to blood phageome sequencing reads and positive alignment was considered as detected transcytosis.

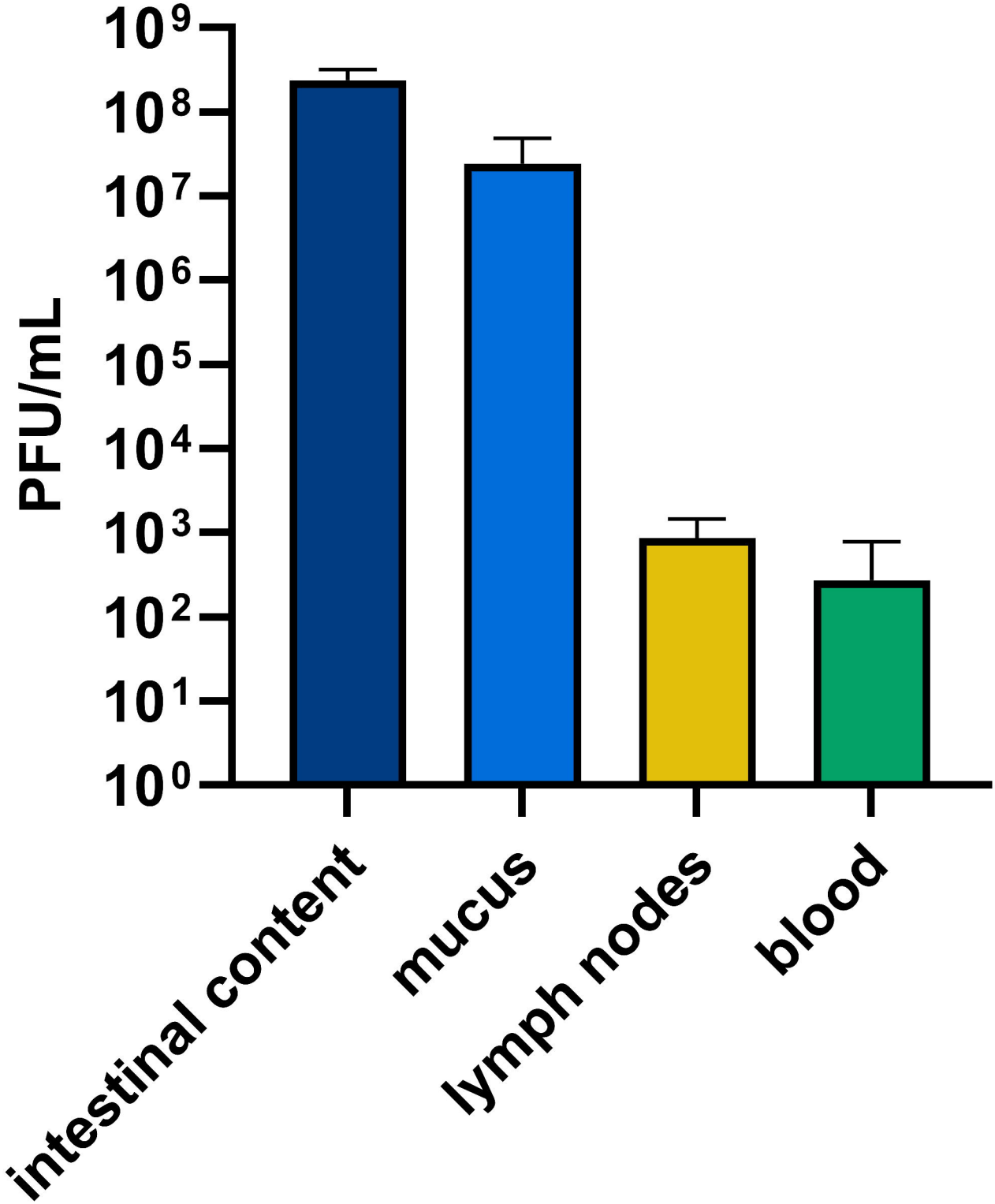
No.	Genus	No of samples where transcytosis was observed	Major host	Morphology
1	*Kayvirus*	23	*Staphylococcus sp.*	myovirus
2	*Tequatrovirus*	22	*Escherichia sp.*	myovirus
3	*Gamaleyavirus*	12	*Escherichia sp.*	myovirus
4	*Atlauavirus*	11	*Synechococcus sp.*	myovirus
5	*Punavirus*	9	*Escherichia sp.*	myovirus
6	*Lambdavirus*	8	*Escherichia sp.*	siphovirus
7	*Pahexavirus*	7	*Propionibacterium sp.*	myovirus
8	*Lowelvirus*	7	*Synechococcus sp.*	myovirus
9	*Teseptimavirus*	7	*Escherichia sp.*	podovirus
10	*Phietavirus*	7	*Staphylococcus sp.*	myovirus
11	*Traversvirus*	7	*Escherichia sp.*	myovirus
12	*Nereusvirus*	7	*Synechococcus sp.*	myovirus
13	*Namakavirus*	7	*Synechococcus sp.*	myovirus
14	*Karamvirus*	6	*Enterobacter sp.*	myovirus
15	*Dubowvirus*	6	*Staphylococcus sp.*	myovirus
16	*Skunavirus*	6	*Lactococcus sp.*	myovirus
17	*Mosigvirus*	6	*Escherichia sp.*	myovirus
18	*Bcepfunavirus*	6	*Burkholderia sp.*	myovirus
19	*Jiaodavirus*	5	*Klebsiella sp.*	myovirus
20	*Dhakavirus*	5	*Escherichia sp.*	myovirus
21	*Harbinvirus*	5	*Lactobacillus sp.*	myovirus
22	*Gaprivervirus*	5	*Escherichia sp.*	myovirus

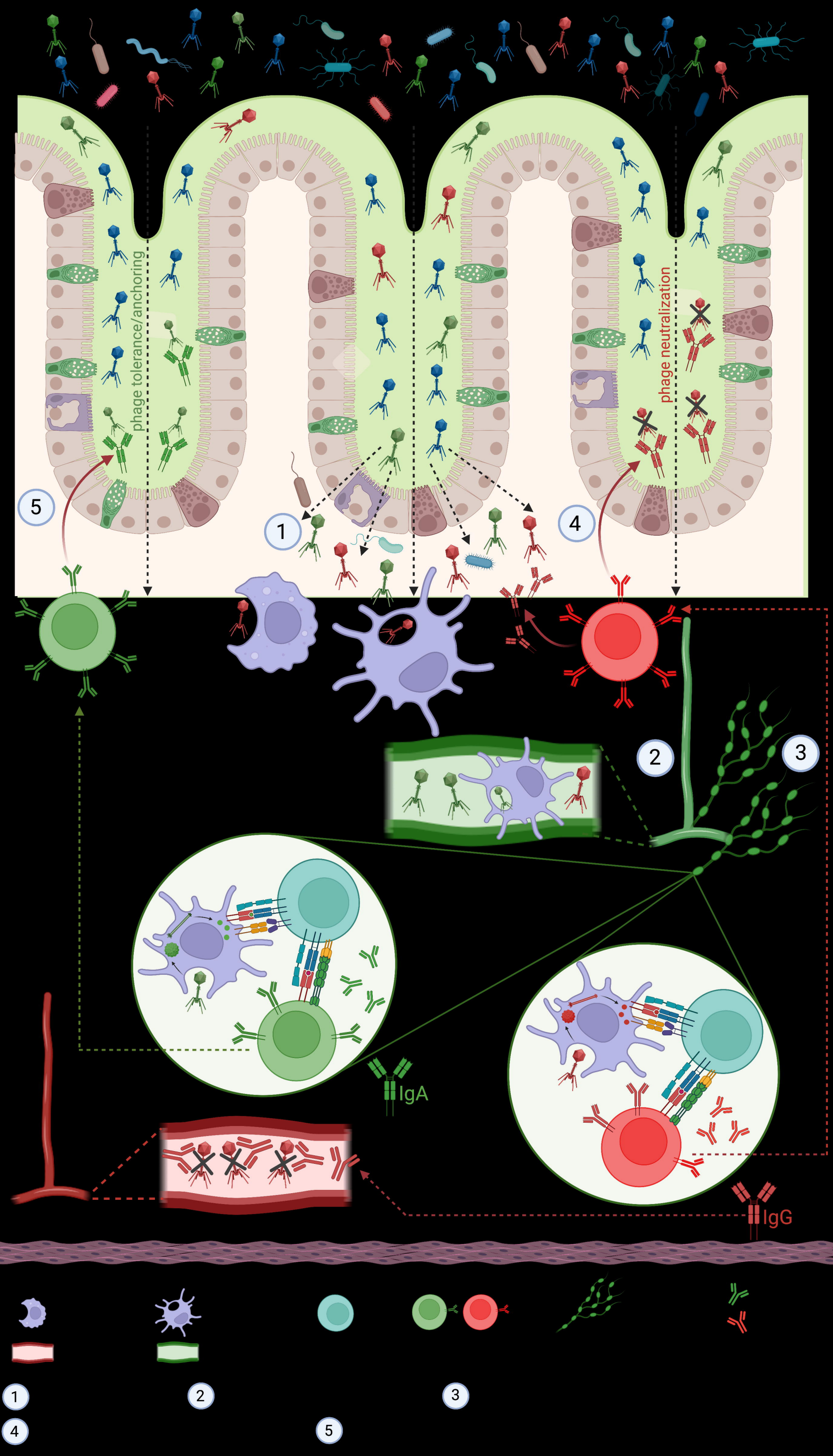

gut phageome
blood phageome
IgG-reactive phage


1
structural proteins of bacteriophages
2
oligonucleotide synthesis
3
library of bacteriophages displaying potential bacteriophage epitopes
4
incubation of a library of potential phage epitopes with human serum
5
immunoprecipitation
6
DNA sequencing




Functional diversity of phage proteins identified in the gut and serum (A.)
hypothetical protein
large head outer capsid protein
head outer capsid protein
capsid and scaffold protein
PKD domain protein
putative large head outer capsid protein
Hoc head outer capsid protein
putative DNA primase
minor capsid protein
putative DNA helicase
outer capsid protein
helix-turn-helix family protein
DNA primase
major capsid protein
Pas29
integrase core domain protein
head decoration protein
terminase_1 domain protein
phage capsid and scaffold protein
phage capsid and scaffold
exonuclease
DNA helicase
outer capsid protein Hoc
transposase
putative head outer capsid protein
putative head outer capsid protein, Hoc
capsid and scaffold
putative tail fiber protein
highly immunogenic outer capsid protein
head assembly cochaperone with GroEL
major capsid protein VP1
head assembly chaperone protein
gp33
capsid assembly protein
gp31 head assembly cochaperone with GroEL
capsid assembly chaperone
putative RNA-polymerase subunit
head assembly cochaperone
tail tubular protein B
putative holin
Cat
head assembly chaperone
chaperonin 10 Kd subunit
Bla
structural protein
capsid decoration protein
tape measure protein
head assembly co-chaperonin for GroEL
putative head assembly cochaperone with GroEL
peptidase
ORF017
VP1
lysin
endolysin
conserved hypothetical protein
Chaperonin 10 Kd subunit
gp31 head assembly co-chaperonin for GroEL
unknown
hypothetical phage protein
putative receptor binding protein
head to tail joining protein
deoxynucleoside kinase
Deoxyadenosine kinase / Deoxyguanosine kinase
tail associated lysin
virion component, putative tail fiber
terminase large subunit
putative portal protein
homing endonuclease
putative major coat protein
major tail protein
major head protein
holin
major capsid protein 10B
phage head assembly chaperone protein
head assembly Co-chaperone with GroEL
putative head assembly co-chaperonin for GroEL
putative head assembly cochaperone with GroEL
co-chaperone for GroEL
antirepressor protein
predicted protein
putative major capsid protein
putative RNA polymerase
lactose operon repressor
portal protein
conserved domain protein
antirepressor
baseplate wedge protein
distal tail protein
transposase IS1
receptor binding protein
putative HNH endonuclease
collagen triple helix repeat protein
PHK2302
Phage head outer capsid protein
HNH homing endonuclease
baseplate J family protein
putative endolysin
N-acetylmuramoyl-L-alanine amidase
insA
gp10b
integrase
Vp1
amidase
hoc protein
hoc head outer capsid protein
tail fiber protein
methylase
single-stranded DNA-binding protein
head assembly cochaperone with GroES
nonstructural protein
transposase IS605
minor tail protein
conserved phage protein
Hoc-like capsid decoration protein
phage terminase large subunit
tail tape measure protein
putative tape measure protein
primase
bifunctional DNA primase/helicase
RecF
1,000,000
800,000
600,000
400,000
200,000
0
Number of reads
serum phage proteins
gut phage proteins
Serum Phage Proteins vs. Gut Phage Proteins vs. IgG Reactive Proteins (B.)
hypothetical protein
large head outer capsid protein
head outer capsid protein
capsid and scaffold protein
PKD domain protein
putative large head outer capsid protein
Hoc head outer capsid protein
putative DNA primase
minor capsid protein
putative DNA helicase
outer capsid protein
helix-turn-helix family protein
DNA primase
major capsid protein
Pas29
integrase core domain protein
head decoration protein
terminase_1 domain protein
phage capsid and scaffold protein
phage capsid and scaffold
exonuclease
DNA helicase
outer capsid protein Hoc
transposase
putative head outer capsid protein
putative head outer capsid protein, Hoc
capsid and scaffold
putative tail fiber protein
highly immunogenic outer capsid protein
minor tail protein
capsid scaffolding protein
putative head structural protein
putative virion structural protein
baseplate wedge initiator
tail protein I
tail fiber protein
side tail fiber protein
internal virion protein C
virion RNA polymerase
putative virion-associated RNA polymerase
putative minor tail protein
tail sheath protein
20,000
0
20,000
40,000
60,000
80,000
100,000
120,000
140,000
Number of reads
serum phage proteins
IgG reactive proteins


PFU/mL
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
intestinal content
mucus
lymph nodes
blood


phage tolerance/anchoring
phage neutralization
5
1
4
2
3
IgA
IgG
1
2
3
4
5