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Master thesis

Gut Virome and Impact on Health

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Methods

While trusted sources such as UpToDate and Frontiersin has been consulted at relevant areas, the publications utilized have been sourced from NCBI and PubMed. Initial key search words included: virus, clinic, treatment, utility, and has hence evolved with the authors developing insight to the field of virology to encompass specialized key words. Selected resources were limited to peer reviewed publications with strong emphasis towards modern and recent publications, although older texts has to a limited degree been utilized when relevant and suitable. Research accessible in its complete form has been sought. Both quantitative and qualitative data has been reviewed and summarized, highlighting trends and commonalities within the literature. A broad scope of papers has been read and reviewed, included or excluded depending on factors highlighted above and degree of relevancy towards this paper's aim and scope. Excessively low sample size publications were selected against, and only included briefly when pertinent to the structure and purpose of this review, with due written notation.
Ethical considerations has not presented issues during the writing of this literature review.
Abbreviations has been streamlined within the text itself when relevant.

Key words: Virome, bacteriophage, clinical, diagnostics, treatment, mutualistic

Introduction

Viruses are submicroscopic infectious entities primarily consisting of nucleic acids protected in a proteinaceous envelope sequencing a set of viral components. No virus possesses innate metabolic or reproductive ability, hence a long-standing debate whether viruses are living or non-living matter. To reproduce, viruses highjack intracellular machinery of invaded host cells, establishing viruses as obligate intracellular parasites, although symbiosis can be formed if beneficial traits are conferred to the host. Potential hosts range from protists to animals as well as bacteria, in which case viruses are termed bacteriophages.
Dependent on the nature of the viral genome, different components of the host cell is used, e.g an RNA virus requires reverse transcriptase prior to DNA polymerase activity, while DNA viruses does not require this preliminary step. Following reproduction, the new viruses, virions, are released e.g. via exosomes or cell membrane lysis, releasing newly assembled viruses into the environment to locate a new host cell, repeating this process ad infinitum.

Gastrointestinal bacterial fauna is now well-established and a recognized factor of high importance to human health, and the innate human bacteria coexist in symbiotic and competitive relationships with the virome. Viral colonization and its impacts on human health holds significant blind spots in the quest to fully comprehend the human biomedical field, reasons including the novelty of the field and requirements of advanced technology. Furthermore, due to the inseparability between the virome and host cells, studying the organism without its environment yields information with high uncertainty regarding vivo reproducibility.

This literature review will briefly present viromal qualities and their classification systems, the procedures utilized for diagnostics and novel discoveries, and evidence from the existing body of literature of viral health contributions. The objective is to demonstrate beneficial qualities and utility of viruses to human health, both by natural coexistence and scientific modifications and application. This aims to present the importance of viral contributions to maintain homeostasis and highlight avenues of utilization in clinical medicine, current and future, establishing viruses as more than undesirable pathogenic entities, but a mutualistic entity to the human species.

Classification

Viruses can be described according to morphology, biochemical composition and replication strategy. Single- or double-stranded DNA and RNA genomes in either linear or circular orientation construct the main framework for classification by biochemical and/or replicative qualities. Genome enclosement strategy by capsid or envelope is another recognized factor. Classification by composition and resulting morphology, existence of a lipid membrane enclosure, and structural morphologies of icosahedral, enveloped, helical or complex creates further classifications (1).

- **Helical**: Tube-like structure composed of proteins in helical orientation, creating a central cavity protecting the nucleic acids. Tube size depends on the content genome, and average dimensions are 15-19nm width and 300-500nm length. The tobacco mosaic virus is a helical virus (1).
- **Icosahedral:** The icosahedron encloses the genome with 20 equilateral triangles with 12 vertices coalesced to a near-spherical appearance with fivefold rotational symmetry, meaning every structural feature will within a 360^{o} rotation around either of the five axes repeat five times. The majority of viruses in this class have structural polypeptide chains

in oligomeric clusters called capsomeres, the number and pattern of which create further grounds for icosahedral virion classification. Icosahedral viruses cause eventual host cell lysis. Some of our most frequent viral pathogens exist in this category, e.g adenovirus and rhinovirus. (1)

- **Enveloped:** Can be either icosahedral or helical, but are enveloped by one or more lipid bilayer membranes. Instead of host cell lysis, cell membrane budding releases new lipid-encapsulated virions. The new envelope becomes an important component of viral infectivity. HIV, hepatitis C and influenza exist within this category (1).
- **Complex**: The icosahedral and helical structures complex together, forming capsid and sheath respectively, along with a collar, baseplate and tail fibers, creating the category bacteriophages reside within, example phages being T4 and lambda. The helical structure becomes both a ligation and penetration device, as well as a tube through which nucleic acids from the icosahedral head pass through (1).

Alongside morphology, genome characteristics and modes of replication are employed in the nomenclature and classification of viruses. Additional classification is based on whether the genome is monopartite or multipartite. RNA viruses may be further distinguished by differing replicative strategies. Antisense RNA is utilized as a template from which an RNA reverse transcriptase produces a complementary RNA strand to serve as mRNA, while sense RNA is readily utilizable by host ribosomes.

Bacteriophages are classifiable according to their reproductive cycle. Lytic bacteriophages reproduce in the sequence of penetration, phage protein replication, and cell lysis. A lysogenic cycle involves integration of phage nucleotides to host genome. The new genome is called prophage, and the host itself a lysogeny. If integrated to a plasmid, the prophage is called a phagemid (2). Upon the hosts natural replication, the prophage is transcribed and represented in bacterial daughter cells. Some phages utilize a gemmation strategy, where phage particles bud off in vesicles constructed from host lipoprotein membrane, termed "budding" (3). This presents a third phage replication strategy, highlighting diversity of approaches to classification. Phages may also be classified according to their composing structures, e.g whether the tail is contractile or not, if a lipid envelope is present, and on basis of general shape, examples including circular, filamentous or rod shaped. Genome characteristics is another stratum for characterization, and

while the majority possess linear double stranded DNA, circular, segmented and single strand DNA is also represented within the phageome.

David Baltimore developed a simplified classification system in 1971, accordingly named the Baltimore classification. This system groups viruses according to the nature of the genomic material pre-mRNA synthesis. The resulting seven groups are listed below (4).

Table 1 – Baltimore classification classes, nucleic acid nature and example species.

Class	Nucleic acid	Example
I	**dsDNA**	**Herpes viruses, poxvirus, Adenovirus, papillomavirus**
II	**ssDNA**	**Adeno-associated virus**
III	**dsRNA**	**Reovirus**
IV	**(+)ssRNA**	**Togavirus, Poliovirus, Hepatitis C and A**
V	**(-) ssRNA**	**Influenza virus**
VI	**Reverse RNA**	**HIV**
VII	**Reverse DNA**	**Hepatitis B**

The International Committee on Taxonomy of Viruses, ICTV, is a modernized and current body providing viral classifications. As of July 2021, ICTV recognized 6 realms, 17 phyla, 39 classes, 65 orders, 233 families, 2606 genera and 10434 species, with further subgroups. Compared with estimates of 10^{31} species globally (6, 7, 8), this modern system of taxonomy highlights the remaining body of analysis to develop a comprehensive picture of existing viral diversity and further yet, human health implications. The ICTV classification is a more traditional taxonomic approach of segregation depending on genomic evolution over time. A brief example of the ICTV classification system is outlined below.

Table 2 – ICTV classification examples

* Due to data input limitations, the classification appendixes has been subtracted from entry. In same order of entry as top row, the appendixes are as follows: -Viria, -virae, -viricota, -viricetes, -virales, -viridae, -virus.

Realm	Kingdom	Phylum	Class	Order	Family	Genus	Examples
Adna	**Zillig**	**Sale**	**Toki**	**Maximon** **Ligamen** **Prima**	**Ahmun** **Tristroma** **Rudi**	**Alphalipothri** **Yumkaox** **Alphatristroma**	**SFV3** **Y. pescaderonse** **PFV1/2**
Duplodna	**Heunggong**	**Peplo** **Uro**	**Hervi** **Caudo**	**Herpes** **Magro**	**Alloherpes** **Anaerodi**	**Auri** **Metfor**	**A.haliotidmalaco1** **Metforvirus Drs3**
Monodna	**Loeb** **Sanger** **Shotoku** **Trapa**	**Phixi**	**Malgranda**	**Peti**	**Micro**	**Alphatre** **Gequatro** **Bdellomicro** **Chlamydiamicro**	**Alphatrevirus ID21** **Gequatrovirus G4** **Bdellomicrov. MAC1** **Chlamydiav. CHP1**
Ribo	**Orthorna** **Pararna**	**Duplorna** **Artver**	**Amabili** **Revtra**	**Cryppa** **Blueber** **Orter**	**Mito** **Hepadna** **Belpao**	**Duamito** **Avihepadna** **Semoti**	**Duamitovirus alal1** **Duck hepatitis B** **Anopheles gambiae Moose virus**
Ribozy	-	-	-	-	**Kalmio**	**Daaz** **Dagaz** **Dee** **Delta**	**Daazvirus cynopis** **Dagazvirus schedorhinotermitis** **Deevirus Actinopterygii** **Deltavirus Japanese**
Varidna	**Bamford** **Helvetia**	**Nucleocyto** **Preplasmi** **Divido**	**Mega** **Ainoa** **Laser**	**Alga** **Lautamo** **Halopani**	**Phycodna** **Finnlake** **Simulo**	**Coccolitho** **Finnlake** **Yingchgeng**	**Emiliana huxleyi virus 86** **Finnlakevirus FLiP** **Yingchengvirus SNJ1**

Mokili et al estimated in 2012 (5) that 1% of total viral genome had been sequenced, emphasizing the remaining endeavors of viromal characterization and potential discoveries of significance to human health and disease comprehension. As of March 2023, 11555 completed viral genomes are available in the National Center for Biotechnology Information Genomes database, a small fraction of estimated global diversity (6, 7, 8).

Estimating global viral species depends on the habitats considered. Anthony et al performed in 2013 viral biodiversity estimation statistics for vertebrate and plants, and analyzed various samples from *Pteropus* giganteus, a documented zoonotic virus host. Statistics and PCR analysis indicated 58 and 55 viral species respectively, within nine sampled families. Extrapolated to all mammalian species, estimated global mammalian virome is estimated to ~ 320.000, rising to ~1.75 million for all multicellular species (9). Considering this estimate is inconsiderate of viral

species in unicellular organisms nor inclusive of the other 13 viral families, alongside the global total viral particle estimate of 10^{31} (6, 7, 8, 10) there are several arguments pointing towards potential orders of magnitude of underestimation. With only 219 known viral species capable of infecting humans (11), advancing viromal characterization is likely to provide novel clinical contributions.

When performing virological studies one must distinguish between viruses and virus-like particles (VLPs). The main difference lie in the replication incompetence of the latter. Viral particles are sequestered from cellular material through consolidations of enzymatic applications alongside filtration and centrifugation in order to exclude free nucleic acids, yielding non-cellular nucleic acids separated by order of size and density (12), providing some preliminary categorization for sequence analysis results. The majority of metagenomic results possess high taxonomic uncertainty, and sequences of undetermined replication competency are therefore attributed as VLPs. Replication incompetence does not exclude other qualities such as e.g immune signaling disturbance, participation in host cell destruction or affecting the processes of co-infecting replication competent viruses (13). Any genomic sample may contain an unknown quantity of viral sequences with potential to cause physical or biochemical effects without being attributable to a recognized species. How to overcome this hurdle of separating virus and VLP when sequencing studies yields giant quantities of unstructured genomic data is an impressive technological challenge. VLP and virus quantification studies on human fecal matter estimate a virus to bacteria ratio of 0.1-10, suggesting human viral and bacterial specimen counts are of similar magnitude (5, 6). Understanding practical qualities of different viral genomic sequences is a prerequisite to infer the significance to human health. Therefore, technologies distinguishing viruses from VLP, and studies targeted at the relationship between VLP, virus and host are paramount to the field of virology.

Viromal sequence databases are recent in conception and inadequate in representation of global genomic content. The research required to develop comprehensive and accurate databases is crucial to comprehend virus-host interactions, which further propagates benefits to clinical medicine and additional fields. This absent viral information has been coined “viral dark matter” (14), to which both uncharacterized viruses and VLPs contribute. Herein lies a major issue with developing this body of research, as current viral classification and distinguishment relies on

protein sequence similarities (15), the difficulty of which is reflected in database inadequacies and e.g nucleic acid interchangeability, reading frames, polymorphisms or intraspecies variations. Thus, even the purest sample with optimal sequencing precision is subject to much uncertainty. Advanced software with capacity to indicate sequences statistically improbable to present without contributions from viral replication strategies is an important forefront to characterize viruses within a sequencing study and subsequent protein function investigations.

Diagnostic methods of viral identification

Advances in high-throughput sequencing technology and analytical software has enabled genome sequencing of previously unavailable time and cost efficiency. A good representation of this is the human genome project, utilizing Sanger sequencing, costing ~ 2.7 billion US dollars over 13 years to sequence our 3.2 billion base pair genome (16). With technological leaps to the current era, as an example the company Illumina is a current market leader in high-throughput sequencing technologies and offers a sequencer which in “rapid mode” is capable of sequencing 30 human genomes in 27 hours(17). It’s also capable of both genomic and RNA sequencing (18).

Shell-vial culture (SVC) utilizes low speed centrifugal forces to expediate the process of viral cellular entry and propagation before incubating for ~36 hours (19). The theoretical basis for the effect of centrifugation is the induction of minor trauma to cellular membranes by mechanical forces, permitting expediated viral entry and hence infection and detectability (19). Flat-bottomed shell vials permits the establishment of a cellular monolayer for clinical specimen inoculation. Viral antigens can now be detected via immunofluorescent antibody application to the antigen-precenting cell monolayer before any eventual cytopathic effects appear on light microscopy. SVC combines cellular cultivation and molecular antigen documentation with application of theoretical enhancement strategies. It has yielded expedience to rapid clinical detection of several medically important viruses without compromising sensitivity. Influenza A and B, HSV, RSV, adenoviruses and parainfluenza viruses 1-3 are examples of pathogens clinically identifiable by SVC (19, 20). Sensitivity and specificity varies with sample quality, procedure execution and which sampled virus, but studies document ranges between 66-92% and 98-100% respectively (21, 22).

Direct diagnostic tools employs strategies of amino acid or immunological nature. No viral cultivation is required since material directly from source of suspected infection is utilized. The

polymerase chain reaction (PCR) (23) permits viral DNA or RNA detection by treating viral samples with primers complimentary to 3' and 5' ends of a target DNA sequence followed by DNA synthesis cycles by polymerase, typically the DNA polymerase I, or Taq. PCR produces millions of copies of target viral genetic material if present in the sample, allowing identification by comparing gel electrophoresis results with established viral band databases.
Real time quantitative PCR allows measurement of target sequence throughout the process. The target sequence is termed an amplicon. High-specificity primers are used to exclude non-target sequences, as these would be included when results are quantified. Further enhancement of specificity is achieved by utilizing probes requiring specific region ligations to initiate fluorescence, examples of which include hydrolysis (24) and hybridization probes (25). The specificity achieved by using high specificity primers and probes allows for high diagnostic certainty, providing clinical information that can be interpreted towards e.g prognostic indicators and treatment options. Sensitivity and specificity of up to 92.3% and 97.5% respectively establishes PCR as a robust diagnostic tool when applicable (24).
The PCR technique was further developed with loop-mediation isothermal amplification, or LAMP. LAMP employs at least four primers, which create loop-stem structures, the sequences of which are amplified through strand-displacement polymerases. The process is repeated and results in large amounts of copies (26). By altering certain parameters, LAMP presents a reaction time of 10 minutes compared to the 30-60 minutes of standard PCR. Further detailing of the mentioned or other nucleic acid-based detection methods are beyond the scope of this paper, but additional techniques such as recombinase polymerase dependent- , rolling circle dependent-, and helicase-dependent amplification techniques and a CRISPR-based nucleic detection method have been developed (27).

Viral cultures is a common diagnostic tool, with embryonated chicken eggs a good example environment. This technique is utilized for diagnostic investigations and vaccine production by providing a high quality viral growth environment. The egg is an inherently aseptic environment of low temperature fluctuation, allowing rapid viral replication (28). Due to tissue tropism of viruses, the anatomical site of inoculation in the egg is important, e.g herpes simplex viruses 1 and 2 are inoculated on the chorioallantoic membrane (29). Viral detection and characterization from this controlled environment yields high diagnostic certainty.

Utilizing shorter peptide sequences for antibody production rather than complete proteins displaying multiple epitopes, has allowed increased specificity in antigen determination and preventing cross-reactive interactions (30), both highly beneficial to high-specificity immunoassay development.

Enzyme-linked immunosorbent assays (ELISA) is one such assay. By direct or indirect contact between antigen and an antibody labelled with e.g an enzyme or a fluorophore, permits detection and diagnosis with high sensitivity and specificity. Peterhoff et al (31) utilized ELISA during the covid-19 pandemic, demonstrating its ability to document protective immunity. With high sensitivity and specificity, ELISA could determine the presence of IgA, IgM and IgG antibodies to SARS-CoV-2, and exclude possibility of cross reactions with seasonal corona virus antigens, demonstrating capabilities in “seroepidemiological surveys“ (31). Other ELISA variants exists with specific purposes, e.g competitive ELISA for small antigens incapable of binding multiple antibodies, or sandwich ELISA, where two antibodies bind differing epitopes, one for antigen capture and one for detection. Sensitivity is thereby further increased, and this modality is suitable to exclude cross-reactivity between similar viral strains and hypersensitivity diagnostics (30, 32). If the viral sample was collected at an early infectious stage, antigen levels could be insufficient to produce a positive result, yielding a false negative. Alongside being a time consuming procedure, this highlights the main drawbacks to a virological research modality of otherwise high value. ELISA provides good reliability with sensitivity and specificity of 87% and 73% respectively. Lower reliability compared with nucleic acid assays might be mitigated through recombinant antibody technology and production of high-affinity antibodies with low cross reactivity (33).

Metagenomic whole-genome shotgun sequencing is a next-generation sequencing (NGS) method, serving as a good high-throughput example technology as it entails the fragmentation and sequencing of all genomic material in a sample before recombination is performed by sophisticated software. Hence, host cell and viral genome fragments likely become mixed, a limitation resolvable via software-based elimination of known host sequences (34), or by molecular removal of host DNA (35). Metagenomic sequences contains information on viral species and potentially functional capabilities (36). One major limitation lies in the need for a high data volume to extrapolate significant results and conclusions. Significant viral dark matter proportions along insufficient quality and quantity of reference viromes poses a significant

challenge (35), yet highly valuable as viromal characterization studies promises benefits to several fields, perhaps to clinical medicine first and foremost.

Finally, rapid antigen tests detect specific antigens and indicate active infection. Providing results within minutes has established these tests as a vital screening procedure in hospitals and other high-risk areas for efficiency of practice and protection of patient and staff environments. Efficiency compromises sensitivity, ranging from 65-97%, while specificity remains high, usually above 95% (37, 38).

Viral contributions to human health

Conferred benefits from viral coinfection.

Viruses contribute to human health in a symbiotic and mutualistic manner. Human reservoirs provides niches with parameters inducive to viral propagation. A beneficial virus contributes by definition to overall host fitness. A 2016 literature review by Pradeau categorized viral benefits to either "development, protection, and invasion" (39). Regarding the former, Mallet et al demonstrated in 2004 the conservation of a retroviral locus encoding the envelope protein Env across several primate species (40). The expressed Env gene on the ERVWE1 locus, member of human endogenous retrovirus (HERV) family W, encodes the glycoprotein syncytin. The multinucleated syncytiotrophoblast layer of blastocysts is generated by fusion of cytotrophoblasts, a process executed in part by syncytin (40, 41). Env is concomitantly expressed in chorionic villi where nutrient and ion exchange between mother and fetus occurs, as well as secretion of e.g hCG and placental lactogen, essential to pregnancy continuation (40). Mallet demonstrated that 155 individuals presented this locus with identical positioning on chromosome 7, with a notably well-preserved open reading frame (ORF) and long terminal repeats (LTR). Only five polymorphic Env variants were discovered, all maintaining compatibility with life. The degree of conservation demonstrated by low to absent genetic variation between samples, preserved chromosomal location and expression in cells crucial to fetal development, highlights a viral contribution to the placentation required for human life. This is supported by in vitro demonstrations of antisense oligonucleotide treatment of trophoblasts resulting in decreased cell membrane fusion and hence cellular differentiation, including an 80% reduction in hCG secretion (42). Given the involvement of syncytin in chorionic villi it reasons that related pathologies could see abnormal expression, and indeed placentas from preeclamptic mothers

present a histologic cytotrophoblast dominance and errors in aggregation to syncytiotrophoblasts (40, 42, 43). Preeclampsia increases risk of preterm delivery which is a risk factor for e.g breathing and feeding difficulties, cerebral palsy and developmental delays. Hence, the proper expression of this retroviral protein is crucial to human health and development.
HERVs are now abundantly expressed in the human germline due to endogenization (41), currently accounting for an estimated 8% of the total human genome (44). HERV-W Env serves as an excellent example of retroviral expression to human benefit. Due to ubiquitous expression of HERVs, proving causative relationships to health and pathology is challenging. HERV LTR has been shown to regulate host cell genome expression, and might therefore contribute to human homeostasis, supported by associations of HERV expression to debilitating pathologies such as cancer (45, 46), and presents an avenue of research to further comprehend the role of appropriate retroviral expression for homeostasis. One example benefit could be gene therapies to treat cancers with HERV LTR-mediated expression of oncogenes as pathophysiology (41).

Human cells may serve as long-term viral hosts by other means than endogenization, well known from pathologic events of latent virus reactivations and in immunocompromised patient cases. Cytomegalovirus (CMV) is a beta-herpes virus with an estimated prevalence of 45-100% in the adult population (47), and is the most common opportunistic infection seen in AIDS (48). However, Furman et al demonstrated in 2015 an enhanced immune response to influenza across multiple parameters in subjects seropositive for CMV compared to seronegative individuals (49).

Baseline and post-influenza vaccination peripheral blood samples were collected from a cohort of 91 individuals. The study encompassed the evaluation of 236 immune parameters, including antibody response, multiple chemokine and cytokine levels, immune cell phenotyping, and

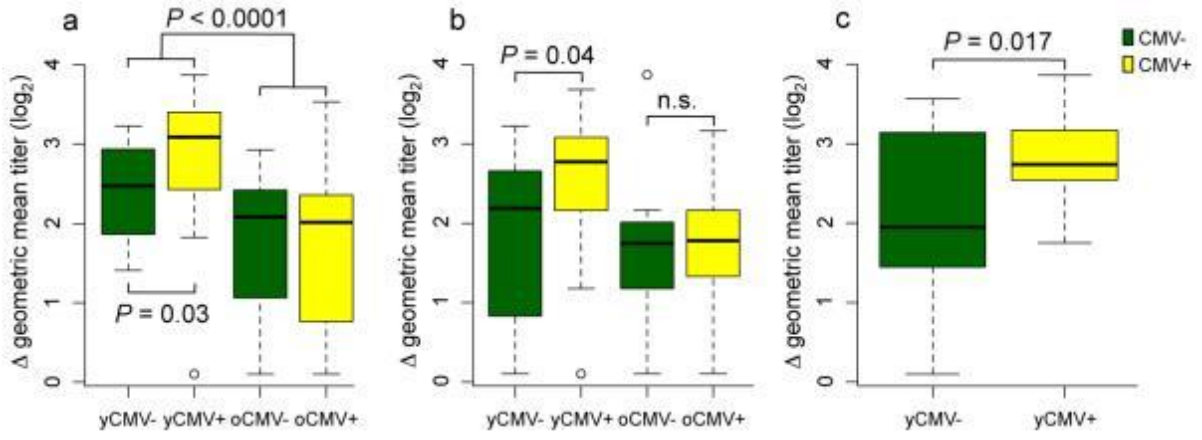


Figure 1: Antibodies produced in response to seasonal inactivated influence vaccine between young (y) and old (o) patients measured via hemagglutinin inhibition assay, quantified and represented by geometric mean titers.. Furham et al (2015) “Young but not old CMV+ individuals have a better response to influenza vaccination” https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4505610/ (64)

assessment of cellular responses to specific cytokines. Antibody responses were assessed via hemagglutinin inhibition assays. Results indicated a statistically significant increase in antibody production among CMV seropositive individuals compared to seronegative counterparts, particularly among participants categorized as "young," meaning between 19-44 years and old > 60 years. This fact was sustain across first and second year of study as well as in an independent validations study, respectively represented in Figure 1 as images A-C. Noteworthy findings included elevated serum levels of INFγ, IL-13, and IL-6, alongside a heightened CD8+ response to IL-6, evidenced by elevated transcription factors pSTAT1 and 3 levels (49). IL-6 plays a pivotal role in the acute immune response to infection, influencing responses such as acute reactant synthesis, hypoferremia induction, and differentiation of Th17 and CD8+ T cells from naïve CD4+ cells(50).

However, these enhancements were not statistically significant in the older cohort, indicating that the benefits of CMV latency are contingent upon preserved immune competency, which is known to decline with age (51). This underscores a symbiotic relationship between CMV and the immune system under specific conditions. Furman et al. bolstered their findings by replicating the results in a murine model, and conducting analogous analyses for latent Epstein-Barr virus, wherein a comparable enhancement of immune response was not observed. This demonstrates a unique symbiotic relationship with CMV in young, immunocompetent individuals, conferring both quantitative and qualitative enhancements to immune responses with conferred benefits beyond influenza vaccination response. This study provides a tangible example of protection-type mutualism, as stated in Pradeu's 2016 paper, elucidating the intricate interplay between pathogens and the host immune system in shaping immune responses.

Expanding on symbiotic protective interactions, favorable clinical outcomes have been associated with coinfection of GB virus C (GBV-C) in individuals with HIV. GBV-C, formerly known as hepatitis G, is a non-pathogenic single-stranded RNA flavivirus sharing approximately 30% sequence homology with hepatitis C virus (52). Coinfection is frequent due to shared routes of dissemination for GBV-C and HIV, such as horizontal transmission through sexual contact or

contaminated blood exposure, and vertical transmission (52), with coinfection rates estimated between 15-50% (53).

Investigations commenced after studies by Toyoda et al. and Heringlake et al. in 1998 demonstrated coinfection conferring lower mean HIV RNA levels, improved survival rates, and decelerated disease progression (52). Subsequently, a multicenter study conducted by Williams et al. further substantiated this relationship by examining the GBV-C status of 271 HIV-positive male patients and tracking disease outcomes. Their findings revealed that patients without coinfection exhibited a 2.78 times higher mortality rate 5-6 years after seroconversion (95% CI 1.34-5.76, $p = 0.006$), a risk not observed at 12-18 months. Additionally, GBV-C RNA clearance by the 5-6 year mark presented the poorest prognosis, with a relative risk of 5.87 ($p = 0.003$) (54). These findings were corroborated by e.g. Xiang et al., who over a mean assessment period of 4.1 years demonstrated a significantly higher mortality rate in GBC-V RNA negative patients ($p< 0.001$), and an increased relative all-cause mortality risk of 3.4 (95% CI 2.5-5.4) in a cohort of 362 patients (55).

However, Björkman et al. presented contradictory findings in 2004, demonstrating statistically significant increases in all-cause and HIV-associated mortality, and AIDS development (p values 0.019, 0.007, and <0.001, respectively) among patients with acquired, persistent presence or absence of GBV-C compared to patients seronegative for GBV-C antibodies, specifically anti-E2 (56). The current literature does however present an inclination towards a beneficial relationship for GBV-C coinfection in HIV patients (57), particularly when assessing parameters beyond survival and AIDS progression.

Souza et al. sought to determine the relationship between GBV-C viremia and antiretroviral therapy (ART), and utilized retrospective plasma assays from a randomized clinical trial for drug efficacy. 175 participants were included that had received either zidovudine + lamivudine, the same combination with indinavir added, or indinavir monotherapy. When adjusted for baseline CD4+ count, drug cohort and patient age, the analysis indicated with a significance of $p = 0.009$ a 3.16-fold decreased viral load at 48 weeks follow up in the GBV-C seropositive group in comparison to their seronegative counterparts (57). Another mutualistic relationship has been highlighted in the interaction between ART treatment and GBV-C titers by Björkman et al., who documented a substantial increase in GBC-V copies from 95 to 6000/ml with a significance of p

<0.001 after initiation of highly active ART, HAART. Patients who terminated the drug regiment displayed decreasing GBV-C counts and HIV progression (58). The study, though only 28 patients, indicates improved health outcomes with ART with GBV-C viremia, and again suggests a beneficial correlation between GBV-C seropositivity and HIV replication.
Correlation between GBV-C viremia and quality of life for HIV patients has also been elucidated by Tillman et al (59), who by retrospective analysis of quality of life questionnaires found elevated quality of life across all assessed parameters for GBV-C viremic patients compared with the non-viremic controls. Among the questionnaires was ED-5Q which assesses anxiety/depression, mobility, pain, self-care, and usual activities. Given that this finding is representative for the relevant population, this is strong evidence of viral contribution of human health (52), though subjectivity of questionnaire data must be taken into account.

The biology of GBV-Cs inhibition of HIV was investigated by Xiang et al in 2004, investigating the hypothesis that HIV-suppressive chemokines are upregulated in coinfected patients. CD4 is the main HIV receptor, but coreceptors CXCR4 and CCR5 were discovered after recognition of non-CD4+ T cell susceptibility to HIV infection was documented (60), serving as potential targets for pharmaceutical treatment.
Peripheral blood mononuclear cells were collected and coinfected. Assays were subsequently performed by quantification of HIV replication via antigen p24 and mRNA expression for the CCR5 ligands RANTES and macrophage inflammatory proteins 1α and 1β, and CXR5 ligand SDF. GBV-C infected cells presented upregulated HIV-receptor ligand mRNA compared to cells infected with a different virus. Concordantly, GBV-C coinfected cells displayed p24 antigen production inhibition in a manner dependent on timing and dose of infection (61), given the HIV strain utilized either coreceptor. If GBV-C coinfection confers human benefit solely towards CCR5 and/or CXC4 strains, this could partially explain the conflicting findings regarding the protective benefit of coinfection within the literature (54, 56) if assessment was performed on HIV strains utilizing e.g gp41 or gp120 for cellular entry to a significant degree.

Findings are not homogenized within the literature regarding the conferred benefit to HIV patients with GBV-C coinfection. However, with multiple papers demonstrating statistically significant benefit in the areas of HIV replication, AIDS progression, beneficial relationship with

ART and quality of life outcomes, there is strong indication for a mutualistic relationship between HIV and GBV-C coinfected patients, with possible benefits to other viral coinfections.

Bacteriophages – A role in natural functioning and potentials of clinical utility

Bacteriophages present a truly exciting venue for research in viral utility with regards to human health. Obligate to bacterial cells as host and malleability of expressed epitopes and capsid contents entail a wide range of applications. The phageome is the term for our innate human bacteriophages, with an estimated 35-2800 species with high proportions of Myoviridae, Siphoviridae and Podoviridae families of the Caudovirales order(62), and families Inoviridae and Microviridae.

Manrique et al. demonstrated in 2016 a potential link between phageome abnormalities and development of inflammatory bowel diseases (IBD) ulcerative colitis (UC) and Crohn's disease (CD). The researchers performed ultra deep sequence analysis of two healthy unrelated individuals for detection of sequence variants, and compared their findings with an existing dataset of 62 healthy and 102 IBD diseased individuals, and generated classifications describing the frequency of a bacteriophage across the sample population. 23 species forms the core of the phageome with > 50% prevalence, and this phage set prevalence was compared to the 102 patient population suffering from UC or CD. Core phage prevalence was significantly decreased ($p < 0.0001$) in CD and UC patients, by 52% and 42% respectively. The 62 healthy individuals presented on average 62% of the 23 core bacteriophages, decreased to 37% and 30% in UC and CD patients respectively ($p < 0.001$) (62). These findings demonstrate significant disturbance in phageome composition in IBD patients, complementing the known bacterial biome disturbance now well recognized in these conditions. With the current absence of determined underlying etiology and the quality of life impairment UC and CD confer, this presents an exciting avenue of research towards symptomatic alleviation, induction of disease remission and potential curative treatment via the avenues of microbiome manipulation and targeted therapeutic delivery. With a high probability of a multifactorial etiology of IBD, phageome disturbance may be an underappreciated component of IBD pathophysiology and remission-relapse dynamics.

Immune barrier compromise is another suspected etiology for IBD, and with the gastrointestinal mucus layer providing an excellent environment for bacterial growth, barrier integrity is crucial to gut homeostasis, and phages are found in significantly higher levels on mucin-producing cells.

The underlying mechanism lies in an affinity between immunoglobulin-like domains on phage capsids and the glycoprotein component of mucin (63), alongside the required bacterial hosts in near proximity. Barr et. al demonstrated a ~4.4-fold higher phage-to-bacteria ratio in surfaces with mucus production compared to without, across several animal species including humans (64).

Development of antibacterial properties within this mucus layer to prevent pathogenic developments reasons from an evolutionary perspective. Barr and colleagues supported this statement by cultivating mucus-producing A549 and T84 cells (human lung and colon cells

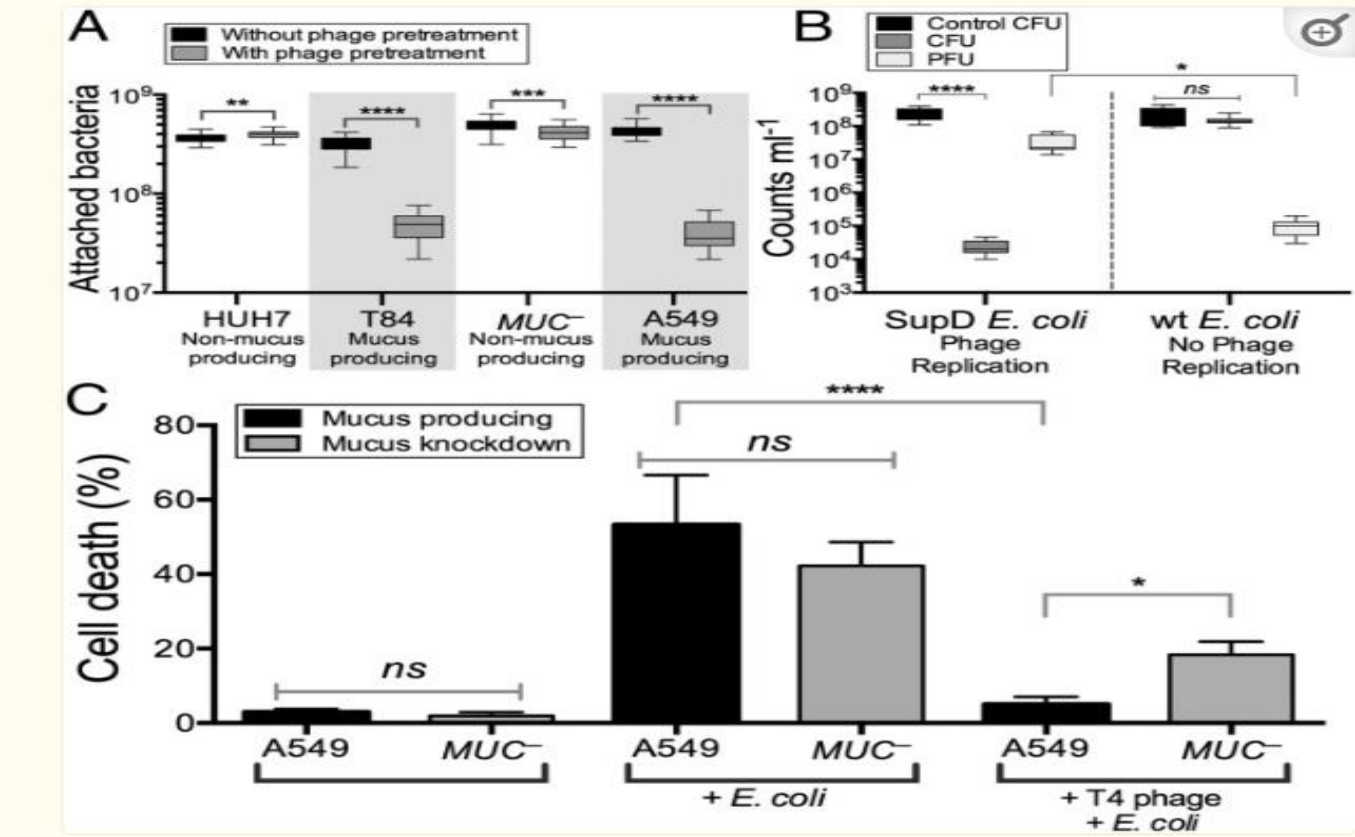


Figure 2: Graphic representation of Barr et al. findings on relationship between phage treatment and bacterial replication and pathogenesis, including dependency of replication competence. Barr et al. (2013)"Effect of phage adsorption on subsequent bacterial infection of epithelial cells". www.ncbi.nlm.nih.gov/pmc/articles/PMC3696810/ (64)

respectively) and non-mucus producing cells lines before incubation with E. coli for four hours after pre-treatment with the well-studied E. coli phage T4 for 30 minutes. Quantification of bacterial presence showed markedly decreased bacterial presence in both T4 pre-treated mucus-producing cell lines (t values > 30, p <0.0001) in comparison to non-mucus producers (64).

Decreased bacterial ligation was shown dependent on the lysogenic phage replication by utilizing the replication incompetent phage amber mutated T4, and comparing bacterial quantity in A549 cells incubated with either wild-type E. coli or a strain capable of suppressing the amber replication-inhibiting mutation. Results showed a $>10^4$ diminished bacterial colonization in the T4 replication competent cell batch in comparison with the wild-type ($p<0.001$) (64). The direct implication to human health was demonstrated by a 72.2% decrease in pathogenicity, measured by cell death, in cells pre-treated with T4 phage prior to an E. coli bacterial challenge ($p=0.0181$) (64). These findings directly demonstrate the protection conferred to human cells from E. coli invasion by the presence of the T4 bacteriophage. Although E. coli is a component of healthy human gut microbiome and T4 is not, these findings highlight the dense accumulation of phages in human gut mucosa and the ability to protect from bacterial proliferation and cellular death. These findings point towards the normal expression of phages as a component in maintaining human health, in this example by acting as a non-host derived component of the innate immune system providing anti-bacterial synergism with the host-derived mucus layer.

T4, alongside lambda and T7, are among the most numerous and well-studied phages to date, in part due to their potential as high-specificity treatment delivery systems. This is especially true for T4 due to a comparatively large capsid of 120 x 86 nm, allowing for more efficient drug or gene delivery than e.g. adenoviral vectors which are restricted to carrying one or two genes (65). Modification potential reaches beyond capsid content alteration. Surface protein expression enables production of ligand-receptor specific therapeutic substance delivery, opening an avenue for e.g. phage-mediated tumor cell injection of chemotherapy or metabolic function inhibitors, or as a vaccination vector. This is a function termed phage display. Tao et al. demonstrated the latter by inducing antibody and immune cell responses against Yersinia pestis in a murine model. This was achieved by constructing T4 phage capsids that either contained a recombinant gene for Y. pestis protein F1-V, expressed the protein on the capsid surface, or both. Also included was a conventional adjuvant-boosted protein-based vaccine. Results show all groups displayed robust Th2 responses, assessed via IgG quantitation. However, potent Th1 cellular responses, as measured by INFγ titers, were restricted to T4 capsids containing the FV-1 gene. The strongest cellular response was observed in capsids both containing the gene and expressing the FV-1 protein (66). This demonstrates an ability of phage-based vaccine delivery to overcome the historically difficult problem of inducing both Th1 and Th2 immune responses by vaccination

(67). Alongside the phage characteristic of obligate bacterial residency, this vector is inherently nontoxic and noninfective, can be readily cultivated in E. coli and retains both structural and functional integrity after at least two years of cold storage (66), and neither group eliciting cellular and humoral responses required adjuvant underscores the potential phage-based vaccination vector presents.

Phage therapy shows promise in targeting not only pathogenic bacteria but mechanisms of pathogenicity itself. Miernikiewicz et al demonstrated in 2016 in a murine model decreased lipopolysaccharide-mediated (LPS) inflammation after exposure to glycoprotein 12 (gp12), a tail adhesin located on the tail of the T4 phage utilized for bacterial adherence. The experiment was conducted by cultivating T4 phages and purifying the tail adhesin gp12 before incubating murine fibroblasts and human microvascular endothelial cells with different gp12 concentrations to confirm no endogenous cytotoxicity, and indeed cellular growth in gp12-incubated cells were no different from controls. Dynamic light scattering demonstrated the ability of purified recombinant gp12 to form dimers and trimers with LPS, prior to investigations of potential anti-inflammatory effects from this ligation.

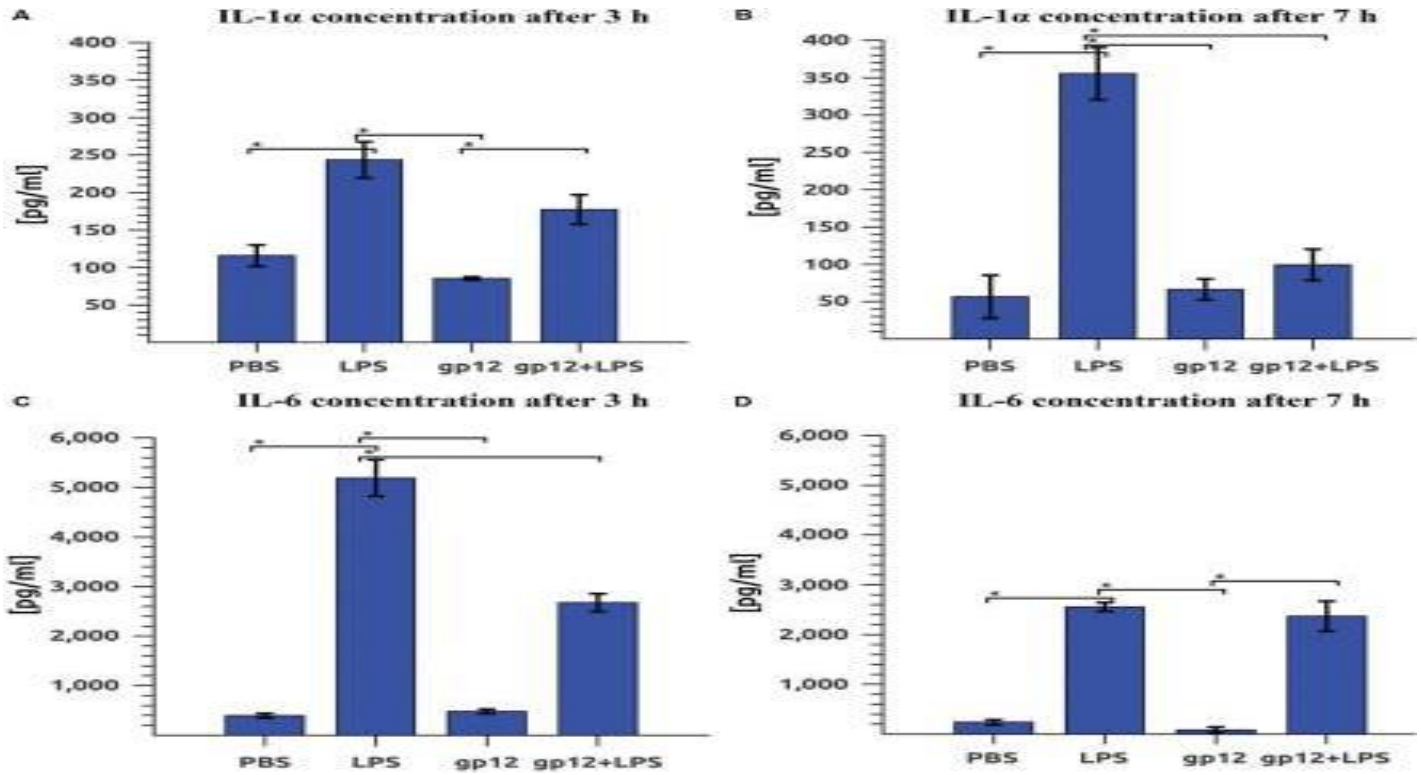


Figure 3: Decreased quantities of proinflammatory cytokines in cells treated with purified phage gp12 compared with untreated control samples at different time intervals. Miernikiewicz (2016) "T4 phage adhesin Gp12 counteracts LPS-induced inflammation in vivo (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4943950/) (68)

This effect was measured by quantification of IL-1α and IL6 levels after LPS exposure. Cells challenged with LPS exposure and administered gp12 saw a statistically significant ($p = 0.002$) 72% decreased IL-1α seven hours after exposure. IL6 assessments displayed a significant reduction after three hours of 48% ($p=0.001$) while no significant effect was observed after seven hours. Their findings were enhanced by histological demonstration of decreased leukocyte infiltration in visceral tissues in LPS + gp12 samples compared to strictly LPS-challenged cells (68). These findings indicate potential in bacteriophage components, in this case a T4 phage bacterial ligation mechanism, to combat infection-derived inflammation by neutralizing an offending molecule. If these findings are equivalently reproduced in a human model it could contribute to development of a bacteriophage-based treatment not solely for eradication of infecting bacteria but its mechanisms of pathogenicity directly. This further elucidates the clinical potential of phages and phage-derived therapies. The ability of phage structural components to display epitopes presents an exciting mechanism of pathologic molecule interception, thereby possibly expanding the utility of phage-based treatments to e.g treatment of cancer and autoimmune diseases. An exiting experiment could entail the synthesis of phage components expressing glutamic acid decarboxylase, one of the main targets of autoantibodies in diabetes type 1 patients, before assessing for differences in insulin production and beta islet cell survival between subject and control.

There is pre-existing evidence supporting this experiment within the literature as a similar approach has achieved clinical benefit in a murine multiple sclerosis model. Rakover et al. demonstrated that filamentous phages modified to express myelin oligodendrocyte glycoprotein (MOG), a target of autoantibodies in multiple sclerosis, were admissible to the central nervous system via intranasal injection, bypassing the blood brain barrier. This caused decreased anti-myelin antibodies, which normally provokes autoimmune encephalomyelitis. Phage-MOG injected mice maintained higher neural functioning with reduced proinflammatory cytokine levels, particularly when assessing IL-6, INFγ and monocyte chemoattract protein (69). Phage-based therapies show promise as safe and effective treatments for inflammatory and autoimmune conditions, offering potential for both affordability and accessibility. Conditions such as type 1

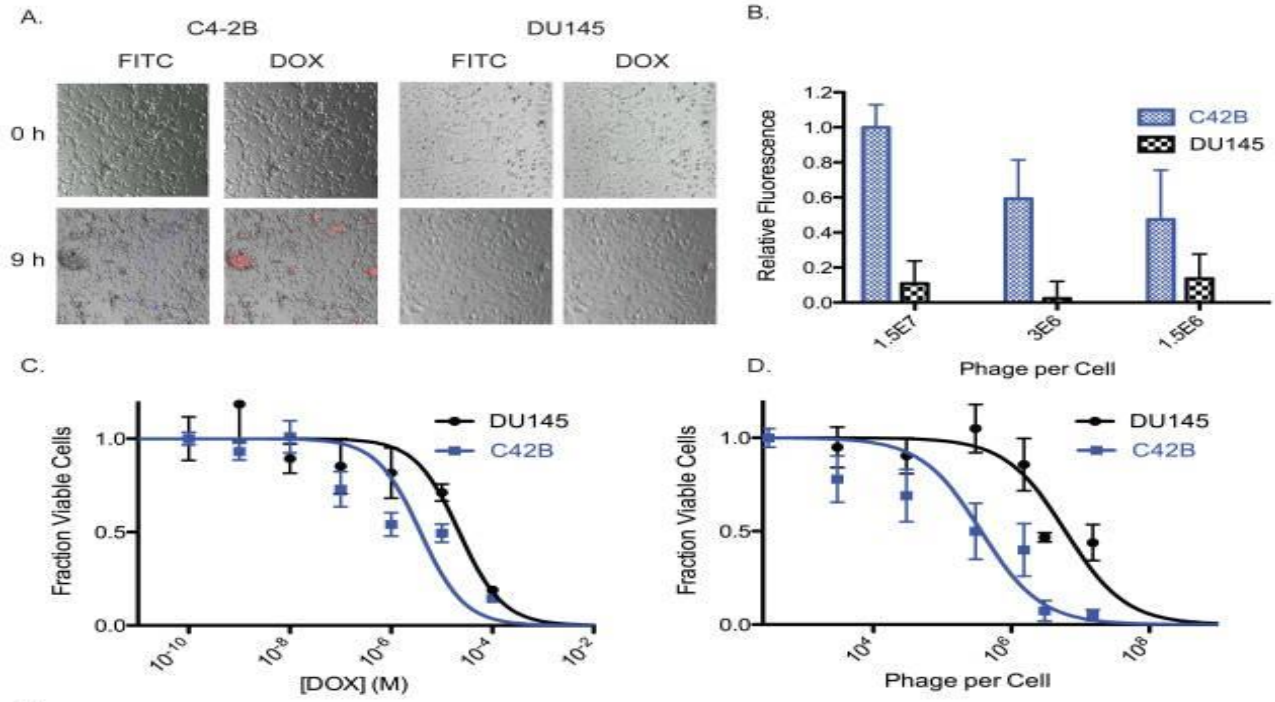
diabetes mellitus, LPS-mediated sepsis, and dermatological disorders with inflammatory components and a wide selection of other conditions could benefit from further investigation into the clinical application and feasibility of these therapies.

Frenkel and Solomon provided further evidence of the clinical potential of phage therapy by demonstrating in vivo visualization of amyloid β plaques using the filamentous phage M13. Through modification to express anti-β amyloid antibodies and labeling with a fluorescent marker prior to intranasal administration, they observed plaque accumulation in the hippocampus of murine Alzheimer's models, a brain region commonly affected in the early stages of the disease. The ability of M13 to traverse the blood-brain barrier and target specific molecules for diagnostic imaging underscores its clinical utility not only in therapy but also in diagnostic applications (70).

Expanding on M13 phage versatility, Ghosh et al demonstrated its potential for targeting, imaging and chemotherapeutic delivery to prostate cancer cells in vitro through gene refactoring and phage display technology. Nanostructure-mediated drug administration are in current clinical use and continues to evolve, but existing methodologies lack the ability to simultaneously permit imaging of target cells. Ghosh engineered M13 to display peptides p3 and p8, the former being fused with SPARC binding peptide (Secreted Protein and Rich in Cysteine), a molecule overexpressed in aggressive cancers of prostate, colon, lung, breast and brain cancers, as well as melanoma (71). All are among the most prevalent malignancies, imposing high annual socioeconomic burdens. Doxorubicin was loaded within the M13 capsid, and its release facilitated by the ligation between p8 and DKF, a peptide motif recognized by the lysosomal cysteine protease cathepsin B with frequent overexpression in prostate cancer cell lines (72). M13 was furthermore enabled for fluorescent detection by ligation to a green dye for the phage itself, and red dye to express doxorubicin deliverance.

Recombinant M13 was applied in different concentrations to two prostate cancer cell lines of high and low SPARC expression, C42B and DU145 respectively, to demonstrate phage capabilities, the latter acting as control. As seen in figure A of figure 4 below, where FITC and DOX columns represent phage and doxorubicin expression respectively, recombinant M13 facilitated detection of high SPARC-expressing cancer cells 9 hours post treatment, freeing it’s chemotherapeutic capsid contents, neither of which was seen in the control. The researchers

noted rounded cell morphology and surrounding cellular debris in the C42B cell line, indicating cell death, likely doxorubicin elicited. This is substantiated in figure B, demonstrating fluorescent intensity ~ one magnitude higher on SPARC positive cells at different M13 concentrations compared with the control. With cell-specific targeting capabilities confirmed, the intended impairment of cell functioning was evaluated by MTT assay, a colorimetric test assessing cell metabolic activity and hence viability, after a 14 hour recovery period in media. Represented in figure D, phage-mediated doxorubicin administration resulted in an approximately 20-fold decrease in cell viability of C42B cells compared with DU145, which again was ~ 10^2 times more effective than free doxorubicin controls as measured by IC_{50} represented in figures C and E, at impairing cell viability (86).



E.

	C42B IC_{50}	DU145 IC_{50}
Free DOX (μM DOX)	3.7 (.72 to 19.5)	22.0 (7.2 to 66.8)
M13-983-Alexa-DOX (Phage per cell)	3.6E5 (1.8E5 to 7.0E5)	6.1E6 (3.7E6 to 9.9E6)
M13-983-Alexa-DOX (μM DOX Equivalents)*	.05 (.02 to .1)	.86 (.52 to 1.4)

Figure 4: (A) Ligation of modified M13 phage as expressed by bioluminescence. (B) Relative levels of florescence (C) Cell viability 14 hours post free doxorubicin exposure. (D) Cell viability 14 hours post doxorubicin delivered via phage vector. (E) IC_{50} of free and phage-mediated doxorubicin. Figures A-E displays sample and control cells.

Ghosh (2012) “Refactored M13 bacteriophage as a platform for tumor cell imaging and drug delivery “ (72) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3905571/

This demonstrates the enhanced capabilities of chemotherapeutics by targeted delivery through a phage vector system. It would reason that results of cell lines C42B and DU145 could be reversed had the selected binding molecule been in favor of DU145 ligation. Hence, phage-mediated cancer cell treatment, imaging and monitoring holds potential as an effective protocol with lower levels of resulting systemic chemotherapeutic toxicity due to potential of high cellular specificity, depending on the presented epitopes of the target cell and its cellular specificity within the patient. Theoretically, phages and other viruses could facilitate cell epitope architecting by cellular genomic insertion of desired molecule prior to therapy initiation, potentially granting access to specialized homing therapeutics.

ESKAPE pathogens and viral treatment

Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and the Enterobacter species form the ESKAPE pathogens, known for their difficulty of clinical management due to high prevalence of antibiotic resistance. An observational study from 2019 by Marturano and Lowrey performed on a dataset of 1.1 million patients found ESKAPE pathogens representing 42.2% of all bloodstream infections, and were associated with a $5500 increased cost of care, partially explained by a ~3.3 day prolonged length of admission (73) compared with other bacteria. Alongside a 2.1% increase in absolute mortality, the socioeconomic toll of these bacteria grow clear, largely stemming from their tendency of resistance to the empiric antibiotics. The study found no higher incidence of antibiotic resistance among ESKAPE bacteria, but high percentages of resistance for frequently employed antibacterials like penicillin, ampicillin, cefazolin, ceftriaxone, vancomycin and piperacillin-tazobactam with exceptionally significant values depending on gram positive ($p < 1e-45$) or gram negative ($p < 0.000005$) status (73).

Phage- and phage-antibiotic combination therapy has emerged as a potential strategy to combat ESKAPE pathogens in the age of increasing antibiotic resistance. Kamal and Dennis demonstrated in 2015 that phages KS12 and KS14 when cultivated in the presence of different sublethal antibiotic concentrations develops into larger plaques, a mechanism termed phage-antibiotic synergy, or PAS, and possesses penetrative effect on biofilms. Tetracycline,

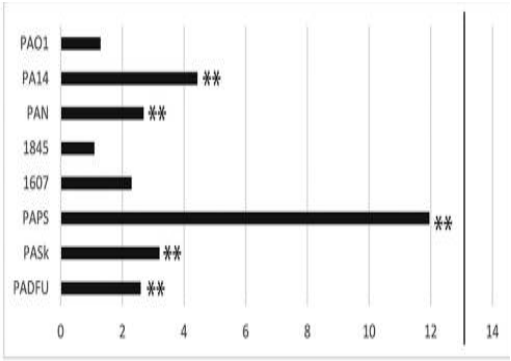
meropenem and ciprofloxacin trials saw the most pronounced effect on cellular morphology, and both phage plaques and titers increased alongside increasing antibiotic concentrations until a maximal threshold. Clinical utility was demonstrated in a larval model infected with *Burkholderia cenocepacia* biofilms*,* presenting increased survival in KS12 with low dose meropenem larvae compared with KS12 or meropenem alone (74). Synergism appears to be dependent upon the applied antibiotic concentration, likely due to either higher concentrations decreasing bacterial cell host concentration to suboptimal levels for phage replication, or the antibiotic negatively effecting phage replication itself. Chaudry et al. demonstrated this by assaying biofilm clearance by phages combined with different antibiotics at various concentrations, and finding e.g increased clearance of *P. aeruginosa* biofilms inoculated with tobramycin at MIC x 1, though not present at MIC x 8. Staggered application of phage and antibiotic also showed a synergistic effect, with tobramycin or gentamycin application 24 hours post phage administration yielding statistically significant ($p < 0.04$) bacterial cell reductions (75). Tobramycin and gentamycin were among the drugs with no significant effect when applied simultaneously with phage, possibly due to the synergism disruptions mentioned earlier. This highlights the importance of antibiotic, dosage and timing to achieve beneficial results, and would require extensive work prior to widespread clinical utilization in humans. The prospect of a new weapon against resistant bacteria and their biofilms is however both intriguing and important, given the global rise of resistant colonies and lacking developed additions to the antibiotic arsenal.

Phages are not restricted to direct lysis, proliferation inhibition or potentiation of antibiotics, but also show potential to restore antibiotic sensitivity in previously resistant strains. Chan et al. demonstrated in 2016 the ability of OMKO1, a lytic bacteriophage of *P. aeruginosa,* to restore antibiotic sensitivity to multidrug resistant cultures. OMKO1 utilizes the outer membrane porin M (OprM) of the multidrug efflux systems MexAB- and MexXY-OprM for bacterial ligation. Chan hypothesized OMKO1 to instigate evolutionary pressure, spawning development of phage resistance by alterations to the porin component to block phage binding, thereby selecting in favor of bacteria with an altered drug reflux system (76). The experiment was conducted on model *P. aeruginosa* strains and samples from environmental and clinical environments of both OMKO1 sensitive and resistant colonies, comparing the efficacies of ciprofloxacin, tetracycline, ceftriaxone and erythromycin as measured by MIC.

Seen in Figure 5, among 32 comparative analyses of MICs in OMKO1 sensitive and resistant isolates to the four antibiotics, 27 showed statistically significant or highly significant ($p < 0.05$ or $p < 0.01$) reductions of MIC in OMKO1-resistant strains. It is noteworthy that of the 12 analyses on clinical isolates PAPS, PASk and PADFU, 100% showed statistically significant reduced MIC with 2/3 being highly significant. Chan and colleagues concluded that OMKO1 was indeed capable of inducing the hypothesized and desired rebalancing of phage-drug resistance. Furthermore, 38 *P. aeruginosa* strain genomes available in the NCBI GenBank were analyzed to demonstrate MexAB and MexXY-related genes are strongly conserved across strains, indicating the ability of OMKO1 to infect a large selection of existing and emerging genotypes (76). This demonstrates clinical phage utility through an evolutionary mechanism. Focusing on combating antibiotic resistance, it is not unreasonable to envision additional pathways, for instance modifying capsid content to include nucleotides encoding a protein triggering reestablishment of antibiotic sensitivity or disrupting resistance, e.g. blocking part of the intracellular cascade leading to drug efflux.

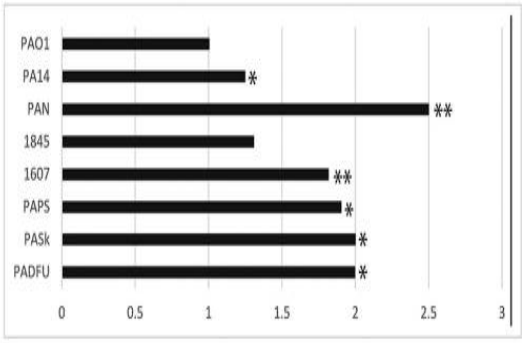
Ciprofloxacin

	OMKO1 Sensitive	OMKO1 Resistant
PAO1	0.073 ± 0.023	0.057 ± 0.021
PA14	0.210 ± 0.035	0.047 ± 0.011
PAN	0.064 ± 0.000	0.024 ± 0.008
1845	0.136 ± 0.049	0.126 ± 0.039
1607	0.104 ± 0.018	0.045 ± 0.057
PAPS	0.172 ± 0.031	0.014 ± 0.010
PASk	5.333 ± 0.288	1.666 ± 0.289
PADFU	3.667 ± 0.577	1.417 ± 0.144



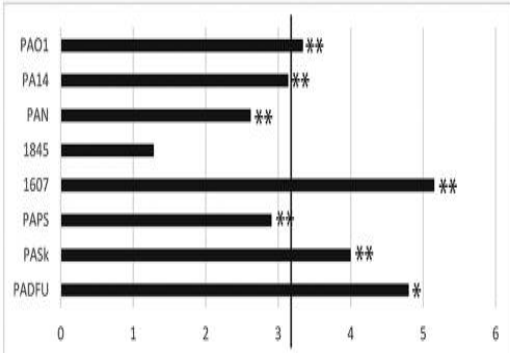
Ceftazidime

	OMKO1 Sensitive	OMKO1 Resistant
PAO1	0.667 ± 0.144	0.663 ± 0.352
PA14	1.000 ± 0.000	0.800 ± 0.112
PAN	1.500 ± 0.000	0.600 ± 0.000
1845	0.917 ± 0.144	0.700 ± 0.209
1607	1.000 ± 0.000	0.550 ± 0.112
PAPS	0.667 ± 0.144	0.350 ± 0.137
PASk	1.167 ± 0.144	0.583 ± 0.144
PADFU	1.333 ± 0.289	0.667 ± 0.144



Tetracycline

	OMKO1 Sensitive	OMKO1 Resistant
PAO1	6.333 ± 0.577	1.896 ± 0.200
PA14	10.67 ± 1.155	3.400 ± 0.894
PAN	7.333 ± 1.155	2.800 ± 0.837
1845	6.667 ± 2.309	5.200 ± 1.789
1607	11.33 ± 1.155	2.200 ± 0.447
PAPS	5.667 ± 0.577	1.950 ± 0.068
PASk	58.67 ± 9.238	14.67 ± 2.309
PADFU	48.00 ± 16.00	10.00 ± 2.000



Erythromycin

	OMKO1 Sensitive	OMKO1 Resistant
PAO1	256.0 ± 0.000	5.600 ± 1.497
PA14	34.67 ± 4.619	10.80 ± 1.789
PAN	31.33 ± 1.155	4.200 ± 0.447
1845	256.0 ± 0.000	15.60 ± 0.000
1607	61.33 ± 4.619	7.600 ± 0.894
PAPS	24.67 ± 7.024	4.400 ± 0.894
PASk	149.3 ± 3.464	22.00 ± 3.464
PADFU	256.0 ± 0.000	55.67 ± 8.021

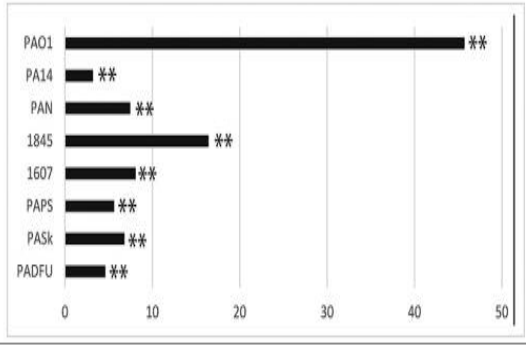


Figure 5: Numerical and graphic representation of minimal inhibitory concentrations of four antibiotics for eight different strains of *P. aeruginosa*, either resistant or sensitive to phage OMKO1. Black vertical line represents increased sensitivity of model strain PAO1 knockout for OprM, fully disrupting MexAB and MexXy efflux systems. Chan (2016) "Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa",* (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4880932/ (76)

The clinical and socioeconomic consequences of the ability to restore antibiotic sensitivity in resistant bacteria are difficult to overstate. With existing theoretic basis and experimental indications of this possibility, there is an argument to warrant significant economic support to the field of phage-mediated antibiotic resensitization. Chan demonstrated the clinical utility of their findings in 2018 when a single administration of OMKO1 and ceftazidime managed to resolve a chronic *P. aeruginosa*-infected aortic graft with associated aorto-cutaneous fistula. The patient, a 76 year old man had prior to the emergency use authorization of phage therapy seen recurrent hospital admissions with *P. aeruginosa* bacteremia, site of origin suspected and later confirmed as the aortic graft. Direct administration to the graft of OMKO1 and ceftazidime by CT-guided intramediastinal injection was aborted due to extensive regional scarring, and was instead administered through the fistula. Vital signs remained satisfactory 24 hours post procedure, with normalizing biochemical parameters. Antibiotics were discontinued and at four-week control there was no remaining sign of infection beyond a superficial *Candida* infection. At the time of publication, three years post-intervention, the patient had not represented with bacteremia or other complications (77).

As prosthetic graft infections presents an incidence range of 0.6 to ~10% (76), with potential for catastrophic medical events and consequently high socioeconomic burden, this direct demonstration of life-saving clinical utility of phage therapy may warrant larger scale clinical trials, especially considering the low to no associated side effects (78).

Conclusion

Humans coevolved with viruses since the origination of our species and arguably even earlier. Innate viruses contribute to the development of progeny and maintenance of homeostasis in various methods and environments. With scientific development, the ability to diagnose and sequence viral nucleotides has begun to unravel the extent of global viral presence. This literature review has utilized modern peer reviewed publications to portray multiple avenues where utilization of natural intraviral competition, by spontaneously occurring coinfections or in

vitro/vivo inoculation of decreased to non-pathogenic viruses can confer benefits to its human host. Modern techniques of viral modification has further demonstrated these long-standing sources of disease to serve as allies and tools, granting the ability to visualize and treat normal and complicated clinical problems with specificity and low toxicity, directly or indirectly. Whether through the natural intraviral relationships or by genetic and biochemical engineering, viruses are an exciting avenue of research for decades to come, promising novel discoveries and possibilities in service of human health.

Recommendations

With indications of utility in imaging, diagnostics, targeted therapeutic delivery systems and potentially low-cost treatments for conditions including but not limited to autoimmune and/or inflammatory conditions, cancer and (resistant) antibiotic infections, the author recommends further studies targeted at development of novel, safe and accessible technologies for clinical utilization. Due to the frightening prospect of multidrug resistance becoming increasingly dominant in non-clinical environments and a stressed antibiotic arsenal and supply, it would be an encouraging development if exploration in the utility of bacteriophages to aid against this issue.

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