USE OF PHAGES IN THERAPY AND BACTERIAL DETECTION

Michael McKinstry and Rotem Edgar

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> "Do you mean to say you think you've discovered an infectious disease of bacteria, and you haven't told me about it? My dear boy, I don't believe you quite realize that you may have hit on the supreme way to kill pathogenic bacteria . . . And you didn't tell me!"—Sinclair Lewis (25)

There is now a consensus that there is a need for new antimicrobial agents given the inevitable development of resistance to currently available antibiotics. However, numerous obstacles face major drug makers, not least of which is the considerable cost of bringing a new drug to market (23). A low rate of return from pharmaceuticals used to treat infectious diseases relative to the high profit from those used to treat chronic illnesses can make pharmaceutical companies reluctant to fund research into the development of new antibiotics (37) (Fig. 1).

The start of the antibiotic era in the late 1940s brought the promise of an end to bacterial infections; however, within 5 years after the introduction of penicillin therapy, 50% of *Staphylococcus aureus* isolates demonstrated resistance (44). Methicillin was introduced in 1959 and was highly effective against penicillin-resistant *S. aureus*, but by 1961, there were already reports of methicillin-resistant isolates of *S. aureus* from the United Kingdom (12) (Fig. 2). The continued emergence of drug-resistant bacteria combined with the threat of bioterrorism necessitates a renewed effort to develop rapid detection methods and novel treatment strategies for infectious diseases. Bacteriophages may provide a viable option.

FÉLIX D'HERELLE AND EARLY PHAGE THERAPY

The use of bacteriophages to treat infectious disease followed shortly after their discovery independently in 1915 by the English bacteriologist and physician Frederick Twort and in 1917 by the French-Canadian bacteriologist Félix d'Herelle. While Twort observed the phenomenon of bacterial lysis on agar plates, it was d'Herelle who strongly advanced the hypothesis that this phenomenon was due to a virus, which he termed bacteriophage. d'Herelle was quick to realize the potential for therapeutic applications of phages, and he championed the concept of using them to treat bacterial infec-

Michael McKinstry and Rotem Edgar, National Cancer Institute, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892.

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Edited by Matthew K. Waldor, David I. Friedman, and Sankar L. Adhya

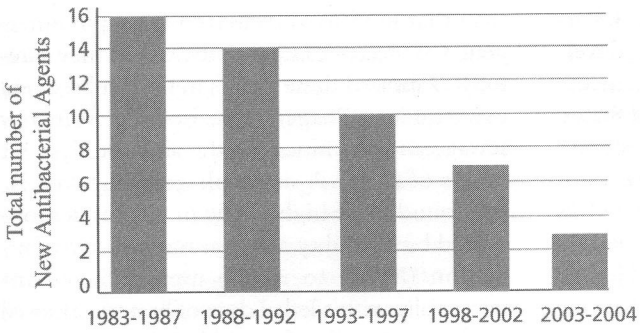

FIGURE 1 Approved antibacterial agents, 1983–2004. The graph shows the numbers of new antibacterial agents approved in the United States for the given time frames. Modified from The Infectious Diseases Society of America website (www.idsociety.org) with permission.

tions (38). The first effort to use phages therapeutically occurred in Paris in 1919 at the Hôpital des Enfants-Malades (39). d'Herelle, along with several interns and the hospital Chief of Pediatrics, ingested an antidysentery phage preparation to confirm its safety and subsequently administered the solution to a 12-year-old boy suffering from dysentery. The boy's symptoms ceased after one dose, and he fully recovered in a few days. Three additional patients were "cured" with the antidysentery phage shortly thereafter, and the experiment was deemed a success. However, despite its apparent clinical success, this type of ad hoc experimental design which is lacking in experimental rigor hampered phage therapy throughout its early days.

The potential use of phages to treat infectious disease attracted the attention of large pharmaceutical companies in the United States in the 1920s and 1930s (29). For example, Eli Lilly produced "Staphylojel" for the treatment of streptococcal infections, and E. R. Squibb & Sons sold a phage filtrate preparation for *Staphylococcus* sp. Phagoid Laboratories, Inc., marketed an array of phage-based products, including Phagoid-Staphylococcus, Phagoid-Gonococcus, and Phagoid-Arthritis. The American Medical Association's Council on Pharmacy and Chemistry felt that the advertising submitted by Phagoid Labs was "overenthusiastic and uncritical," and the council was unable to recommend that their products be included in the listing of New and Nonofficial Remedies due

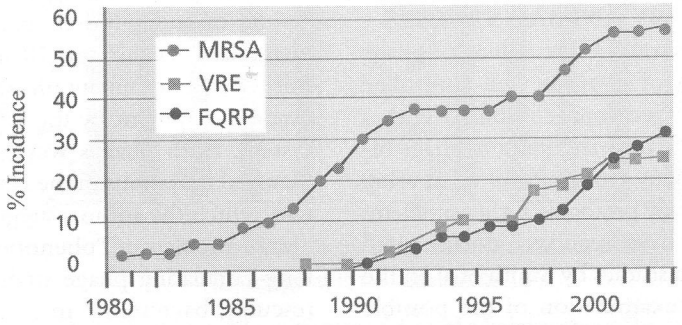

FIGURE 2 Bacterial resistance spread, 1980–2003. The chart shows increases in rates of resistance for three bacteria that are of concern to public health officials, i.e., methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and fluoroquinolone-resistant *P. aeruginosa* (FQRP). Data were collected from hospital intensive care units participating in the National Nosocomial Infections Surveillance System, a component of the Centers for Disease Control. Modified from The Infectious Diseases Society of America website (www.idsociety.org) with permission.

to "unscientific, unwarranted claims" (35). Overreaching claims, clinical failures, and the development of antibiotics all played a role in the downfall of phage therapy in the United States.

d'Herelle continued phage therapy studies and generated numerous journal publications and books, including *The Bacteriophage and Its Behavior* (1926) and *The Bacteriophage and Its Clinical Applications* (1930). In 1924, d'Herelle was appointed Director of Laboratory Service at the International Quarantine Council in Egypt (20), which was set up to monitor and prevent the spread of plague and cholera passing from Asia to Europe through the Suez Canal (39). While there, d'Herelle treated four patients suffering from bubonic plague by the direct injection of antiplague phage into their buboes. All of the patients recovered completely, and the results were published in the French medical journal *La Presse Medicale* (40). Following his experience in Egypt, d'Herelle continued to travel the world, examining the role of phage therapy to control cholera epidemics in India (1926) and accepting an appointment as Professor of Protobiology at Yale University (1928). He played a central role in the establishment of a phage institute in Tblisi, Soviet Georgia (1934), which continues to pursue phage therapy applications to this day.

CONTEMPORARY ANIMAL STUDIES

An often cited reason for the failure of phage therapy is the lack of rigorous, well-controlled experiments. This problem, combined with a fundamental lack of understanding of phage biology, doomed many early efforts to develop reliable phage therapy protocols. Other problems with early efforts have been well documented (3, 24). Controlled studies by Smith et al. in the 1980s led to a reexamination of the possible use of phages to treat infections caused by a variety of bacteria. Smith and Huggins set out to test the efficacy of a highly lytic phage against a K1 strain of *Escherichia coli* isolated from a case of human infection in an acute mouse infection model (36). They attributed the failure of the phage to eradicate experimentally induced infection to a lower degree of activity in vivo than that in vitro as well as to the rapid emergence of phage-resistant mutants, and they carefully examined these points in their subsequent experiments. Phages were isolated from raw sewage and examined for the ability to lyse K1 strains of *E. coli*. A single phage, designated R, was found to be highly lytic in vivo, as demonstrated by its ability at a low multiplicity of infection (MOI) to rescue mice infected intracranially with a lethal dose of bacteria. Buoyed by this success, Smith and Huggins went on to demonstrate that a single dose of phage R was superior to multiple doses of streptomycin, tetracycline, chloramphenicol, ampicillin, and trimethoprim plus sulfafurazole. Furthermore, any phage-resistant mutants were shown to have alterations in their capsules that were associated with decreased virulence in mice. Significantly, they were careful to quantify the levels of phage and bacteria in the blood and spleen during the course of infection.

Earlier studies demonstrated the highly effective role of the innate immune system in the rapid removal of phages from the circulatory system, thereby decreasing the efficacy of intravenously injected phages (16). Merril et al. were able to overcome this problem by the serial passage of phage introduced intraperitoneally in mice to select a mutant that was able to remain in circulation for long periods of time (28). The results of independent experiments passing phage lambda through 10 successive cycles in mice were two mutant phages that were able to evade entrapment by the reticuloendothelium system. Both phages were shown to contain identical mutations in the lambda capsid E protein, which the authors suggested conveyed the "long-circulating" phenotype. Each of these long-circulating phage strains was effective at rescuing bacteremic mice that were given a lethal dose of *E. coli*, with less morbidity than that achieved by the parental phage.

Antibiotic resistance in *S. aureus*, a common cause of serious wound and surgical infections, is well documented (13). By some estimates, >50% of clinical isolates in Japan exhibit resistance to several antibiotics (1). Recently, the first case of vancomycin-resistant *S. aureus* was

reported in the United States. Matsuzaki et al. were one of the first groups to examine the application of phages for the treatment of *S. aureus* infection by using a murine model (26). Mitomycin C was used to induce lysogens from 72 strains of *S. aureus*, consisting of 43 methicillin-sensitive and 29 methicillin-resistant strains. Supernatants were tested for phages with lytic activity, and one phage, designated ϕMR11, was selected based on its broad host range. The genome of ϕMR11 was sequenced to rule out the presence of any antibiotic resistance or toxin genes known to be associated with *S. aureus* phage strains, a critical step in the selection of a phage with therapeutic potential. The intraperitoneal (i.p.) injection of mice with 8×10^8 cells of *S. aureus* strain SA37 was shown to cause death in >80% of mice after 24 h and in 100% of mice after 7 days. This bacterial dose was deemed optimal for testing the efficacy of ϕMR11 in animal rescue experiments. Mice that were challenged with *S. aureus*, followed immediately by i.p. injections of various amounts (MOI of 0.1 to 200) of phage, demonstrated significant protection, with optimal phage protection in the MOI range of 1 to 200. An MOI of 200 was chosen for subsequent experiments. The protective effect of phage ϕMR11 was examined at several time points up to 1 h after the administration of a lethal dose of bacteria (Fig. 3). The majority of mice were still alive at any time point after 24 h, whereas 80 to 100% of the untreated controls were dead. There was evidence of phage efficacy in animals treated at 60 min post-i.p. injection of bacteria, when animals in the control group (bacteria only) already showed signs of physical deterioration.

Since the initial report of vancomycin-resistant *Enterococcus faecium* (VRE) in 1989, there has been a rapid increase in infection and colonization reported by U.S. hospitals (34). Biswas et al. recently examined the possible use of phages to treat VRE infections (4). A lytic phage was isolated from a municipal sewage treatment facility and was shown to be active against a large number of VRE clinical isolates (57%).

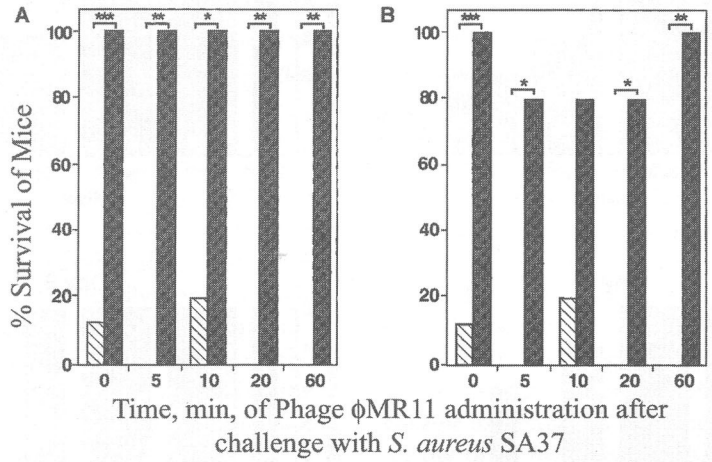

FIGURE 3 Protective effects in mice of delayed administration of bacteriophage ϕMR11. Purified ϕMR11 (MOI, 200) was administered to five mice at the various time intervals indicated, after a challenge with *S. aureus* SA37 (8×10^8 cells). One milliliter of phage-free brain heart infusion broth supplemented with 20 mM $MgCl_2$ and 20 mM $CaCl_2$ was injected into mice as a control. Survival rates were determined after 1 (A) and 7 (B) days. Shaded and hatched columns represent the phage-treated and untreated mouse groups, respectively. Asterisks signify statistically significant differences compared with the controls. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.002$. Modified from reference 26 with permission of the publisher.

This phage strain was used in subsequent animal studies. Forty-five minutes after the administration of a lethal dose of VRE, phage was delivered i.p. at various doses, and animals were scored for their state of health over time. At high MOIs (0.3 and 3), all animals survived, with only minimal symptoms of illness. A lower MOI resulted in lower survival rates, of 40 to 60%, on day 6. When phage treatment with a high MOI was delayed in bacteremic mice for 5 h, all of the animals recovered, and even when treatment was delayed 24 h after the mice were infected, the recovery rate was 50% (Fig. 4).

While it is clear that phages can provide beneficial effects, it is important to avoid phages encoding virulence factors. For example, structural components of some bacteriophages may be pathogenic. Additionally, some phages encode regulatory factors that increase the expression of bacterial virulence genes, whereas others encode enzymes that alter bacterial components related to virulence. Several studies have shown increased virulence of a bacterial pathogen after phage treatment (for a review, see reference 42). An additional concern is that in some phage therapy applications, phage-mediated lysis will lead to the release of harmful quantities of endotoxin, a problem that can be overcome by use of a genetically modified nonlytic phage. For example, a lysozyme-defective phage may prevent the release of endotoxins by killing bacteria without lysis. Hagens et al. compared the therapeutic efficiency of a genetically modified nonlytic, nonreplicating phage with that of a lytic phage in mice infected with *Pseudomonas aeruginosa* (19). In vitro experiments showed that when the modified phage was added at an MOI of 50 to *P. aeruginosa* cultures, there was no change in the optical density at 600 nm, indicating that the cells

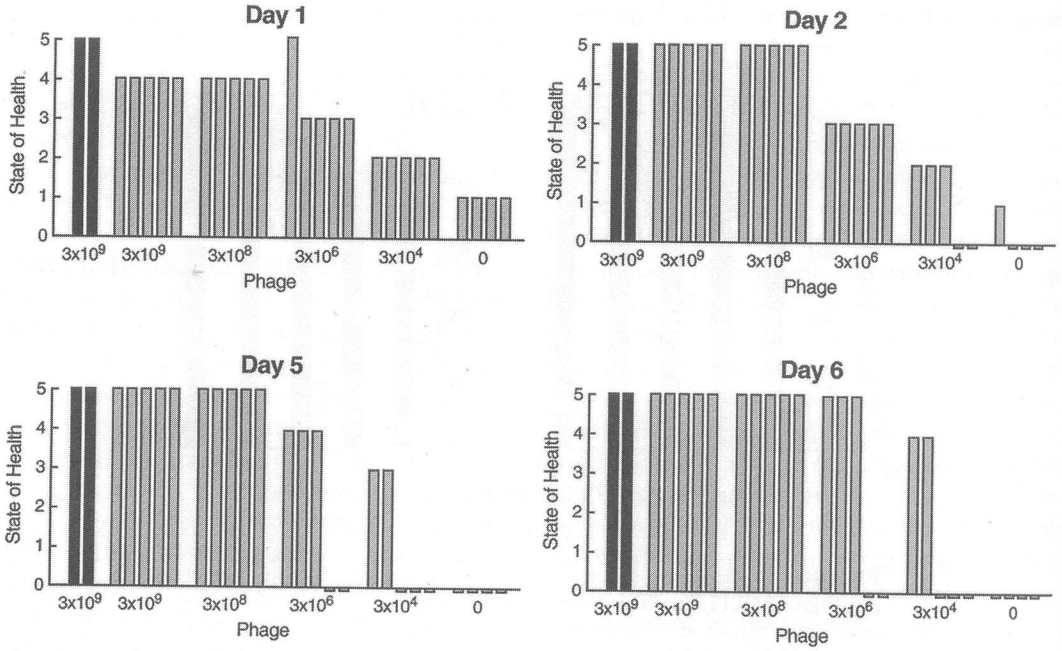

FIGURE 4 Dose effect of phage ENB6 on rescuing mice from lethal VRE bacteremia. Each bar indicates the state of health of mice after i.p. administration of the minimum lethal dose of VRE followed by a single dose of phage at the indicated concentration 45 min after the bacterial challenge. The group on the far right (four mice) was an untreated control group, given SM buffer i.p. instead of phage. The group on the far left (two animals) was a phage control group, injected only with phage (at the high dose) and not infected with bacteria in order to determine if the phage preparation itself produced adverse effects in mice (4).

remained intact. The same cultures were monitored for endotoxin release, which increased only sevenfold after 7 h, in contrast to 60-fold for cultures treated with lytic phage. In an acute infection model in mice, the levels of tumor necrosis factor alpha and interleukin-6, indicators of inflammatory responses in mice after the administration of endotoxin, were lower in a group treated with nonreplicating phage. In additional in vivo experiments, mice were infected i.p. with 3×10^5 CFU (three times the minimal lethal dose) and then challenged with phage at 45 min or 6 h postinfection at an MOI of 1,000. All members of the control group died, and 75 and 100% of the treated mice survived when the treatment was administered at 45 min and 6 h postinfection, respectively. When the nonlytic phage was administered to mice treated with five times the lethal dose of *P. aeruginosa*, 95% of the treated mice survived after 24 h and 73% survived after 48 h. However, when a lytic phage was used under the same conditions, only 60% survived after 24 h and 20% survived after 48 h. These experiments show that a nonreplicative phage can be as effective as, if not superior to, a replicating phage for the treatment of experimental infections by the *P. aeruginosa* strains used for this work.

When considering the use of phages for the treatment of gastrointestinal infections, it is important to recognize the complexity of bacterial colonization in the mammalian intestinal tract. Chibani-Chennoufi et al. studied the gastrointestinal passage of orally applied T4-like phage in mice and their activity on resident or orally administered *E. coli* (10). In these experiments, T4-like phage survived gastrointestinal transit in adult mice, with no major inactivation in the stomach, as fecal phage titers roughly correlated with the initial orally applied phage titers. Resident *E. coli* titers were determined by plating dilutions of mouse fecal material on a medium that allowed for the differentiation of lactose-fermenting *E. coli* from other intestinal flora. The *E. coli* gut flora did not have a significant change in titer after the oral administration of phage, despite demonstrating susceptibility to the test phage in vitro. Additionally, no evidence of intestinal phage replication was observed. Ampicillin-resistant strains of *E. coli* introduced into the gut were readily lysed by phage introduced into the supply of drinking water. From these and other observations, two possibilities were suggested. The intestinal *E. coli* cells may exist in a physiological state that does not allow phage infection, or alternatively, the *E. coli* gut flora may occupy a niche that is physically protected from phage infection.

OTHER APPLICATIONS OF THERAPEUTIC PHAGES

The concept of phage therapy extends to applications that do not involve infectious disease. For example, filamentous bacteriophages present an excellent platform for targeted gene delivery to mammalian cells (22). The genetic flexibility of filamentous phages supports the display of a wide variety of peptide ligands fused to viral capsid proteins, allowing phages to be targeted to a specific eukaryotic cell surface receptor.

The intranasal administration of such a genetically engineered filamentous phage has been used to present antibodies to a specific target in the brain. By this route of administration, bacteriophage appear to reach the brain in significant titers, presumably by entering the olfactory nerve endings and moving in a retrograde direction through or along the axons into the brain (31). This application was employed by Frenkel and Solomon, who used a filamentous phage as a delivery vector for antibodies to β-amyloid into the brains of mice as a potential early diagnostic of Alzheimer's disease (14).

Recently, several groups have demonstrated the efficacy of anticocaine antibodies that bind cocaine in circulation and block its ability to enter the brain (8); however, this approach is not able to exert its effect directly on the central nervous system. Carrera et al. (7) investigated the use of a phage engineered to express a cocaine-binding protein targeted to the brains of rats for the treatment of cocaine addiction. The activity of the protein delivered and displayed by the phage reduced cocaine-mediated behaviors in rats in a way that was similar to pharmacological antagonism.

PHAGES AS BACTERIAL DETECTION TOOLS

In the worlds of medicine and public health, it is necessary to have methods of detection for bacterial pathogens that are rapid, specific, and sensitive. Antibody-based assays offer sensitivity, but they can be laborious and in some cases yield results that cannot be confirmed by culturing (9). Rapid PCR assays increase the sensitivity and are available for many pathogens, but they can be expensive and technically difficult to perform. A phage-based approach may offer a convenient, rapid, and highly specific detection option, particularly in circumstances in which the assay cost is important.

Plaque Assays

Tuberculosis persists as a major public health problem in developing nations. The emergence of drug-resistant strains along with the human immunodeficiency virus pandemic further clouds an already bleak picture (47). Along with new antimicrobial agents, sensitive and cost-effective detection methods are needed. Although unlikely to supplant PCR-based detection methods (41), rapid phage-based assays may provide a viable low-cost alternative. McNerney et al. (27) reported the development of a phage plaque assay for the detection of *Mycobacterium tuberculosis*. After optimization by the use of *Mycobacterium bovis* BCG, the assay was applied in a routine diagnostic setting, in which bacteria from clinical samples were cultured in broth for 24 h prior to the addition of the mycobacteriophage D29. The assay proved to be slightly more sensitive than direct smear microscopy, and although no false-positive results were reported in the study, it should be noted that phage D29 is active against a broad range of mycobacteria. Consequently, specificity may suffer in settings where infections with other species of mycobacteria occur. Due to these limitations, McNerney et al. do not feel that this assay is ready for use as a routine diagnostic test.

Gali et al. reported the development of a high-throughput plaque assay for the identification of multidrug-resistant *M. tuberculosis* (15). Rifampin resistance was cited as a marker of multiple drug resistance in *M. tuberculosis*. Both rifampin-resistant and -sensitive clinical isolates were cultured in a microtiter plate for 24 h in the presence of rifampin prior to the addition of phage D29, and incubation was continued for an additional 90 min. Ferrous ammonium sulfate was used to inactivate unbound phage, while those inside the bacteria were protected and able to replicate. Progeny phage were detected by spotting small aliquots on lawns of the indicator strain, *Mycobacterium smegmatis*; the presence of plaques for any given sample indicated rifampin resistance. The total assay time was 48 h, and there was 100% agreement with the BACTEC 460 TB susceptibility testing method (Becton Dickinson Laboratory Systems, Sparks, Md.). The rapid identification of multidrug-resistant *M. tuberculosis* would aid physicians in planning appropriate treatment regimens.

Fluorescently Labeled Phages

E. coli O157:H7 is a cause of serious foodborne illness. Most often associated with contaminated undercooked beef, *E. coli* O157:H7 affects approximately 73,000 individuals each year in the United States (21). Oda et al. reported the use of a green fluorescent protein (GFP)-labeled T2-like bacteriophage to detect the presence of *E. coli* O157:H7 (32). Unlike conventional culture methods, a GFP-labeled phage is able to detect cells in a viable but nonculturable state, a common state for bacteria in natural environments (11, 45). This phage was developed through homologous recombination, which was used to fuse *gfp* to the phage major capsid protein gene *soc*. Upon adsorption of the labeled phage to a bacterial target, the bacteria were clearly visible under an epifluorescence microscope. Specificity was demonstrated by a lack of binding to *E. coli* strain K-12. Additionally, the reporter phage bound efficiently to dead cells, which were generated by gentle pasteurization.

Goodridge et al. (17, 18) reported the development of an immunomagnetic separation bacteriophage-based assay for the detection of

E. coli O157:H7. A fluorescent bacteriophage was prepared by staining with the nucleic acid dye YOYO-1 (Molecular Probes, Inc., Eugene, Oreg.). After binding of the cells to *E. coli* O157-specific immunomagnetic beads, the fluorescent bacteriophage was added. Detection was performed either by epifluorescence microscopy, flow cytometry (forward light scatter and side scatter fluorescence were measured simultaneously), or scanning electron microscopy. The method allowed for the detection of 100 CFU of *E. coli* cells/ml in ground beef and raw milk after a 10-h enrichment step. The use of a two-level detection system overcomes the problem of specificity when using immunomagnetic separation (due to the nonspecific attachment of other organisms to the magnetic beads).

Other Detection Methods

Neufeld et al. (30) described the use of a novel electrochemical method for the rapid identification and quantification of pathogenic bacteria. This system utilizes a bacteriophage specific for one bacterial species among a mixed population, thereby lysing only this species and releasing its intracellular enzymes, which can be monitored by amperometric measurement.

The amperometric measurement is performed by use of an electrochemical biosensor, an analytical device for detecting a target compound in solution. A specific biological recognition element (bioreceptor) is integrated within or intimately associated with an electrode that converts the recognition event to a measurable electrical signal. In a model system, the use of a lytic phage specific for *E. coli*, with β-D-galactosidase enzymatic activity as a marker, allowed for the detection of as little as 1 CFU/100 ml within 6 to 8 h. In theory, this electrochemical method can be applied to any combination of phage and bacteria by measuring the enzymatic marker released by the lytic growth of a specific phage.

Banaiee et al. described the use of luciferase reporter mycobacteriophages (LRPs) for the detection, identification, and antibiotic susceptibility testing of *M. tuberculosis* in a clinical microbiology laboratory (2). Viable mycobacteria emit light upon infection with LRPs, which, upon the addition of antibiotics, can be used to generate a susceptibility profile for any bacterial isolate. When tested against 523 sputum samples, the LRP method was found to compare favorably with other detection methods (92% accuracy versus 97% accuracy with BACTEC 460). However, the greatest advantage of the LRP method was a 2-day turnaround time for antibiotic sensitivity testing, in contrast to 10 days for the BACTEC 460 system (there was an overall agreement of 98.5% between the two methods).

Wu et al. (46) used the phage-mediated release of the enzyme adenylate kinase (AK) as a marker for *Salmonella* sp. and *E. coli*. Luciferase was used to measure the bioluminescence dependent on the AK-mediated conversion of ADP to ATP. Optimization of the conditions showed that maximum AK activity was observed for the cells in mid-log phase when the cell density approached 10^7 CFU/ml. Lag- and stationary-phase AK release was 10-fold lower. Additionally, an MOI of 10 to 100 gave an almost 100% release of AK, while only 30% of the intracellular AK was released by phages at a high MOI ($>10^4$). A reliable difference between samples and controls (noninfected cells) was observed at a cell density of approximately 10^3 CFU/ml for both *E. coli* and *Salmonella enterica* serovar Enteritidis when all the steps of the phage-mediated bioluminescent AK assay were optimized.

DEVELOPMENTS NEEDED FOR PHAGE THERAPY

Despite the optimism regarding the realization of phage therapy, both past and present, many obstacles remain. The effective use of phages to treat infectious disease will require a more thorough understanding of phage-bacterium-mammal interactions in vivo. Pharmacokinetic models may help to predict the optimal timing of inoculation for phage therapy applications (33). Using in vitro experiments, Weld et al. demonstrated an agreement of T4 growth with theoretical models; however, such models failed to predict the growth of T4 and phage K1-5 in

vivo (43). We believe that these findings, along with others cited previously in this chapter, demonstrate the need for a better understanding of the differences between phage-bacterium interactions in vivo versus in vitro.

Growth and purification standards need to be established for any phage to be selected for therapeutic use. In the case of gram-negative bacteria, the removal of endotoxin is critical for any phage preparation. Traditionally, CsCl density centrifugation of phages has been used to significantly reduce the endotoxin levels in phage preparations. In addition, Boratynski et al. reported the effective purification of phages from endotoxin by sequential ultrafiltration through a polysulfone membrane followed by chromatography on Sepharose 4B and Matrex Cellulofine sulfate (6). The resulting preparations contained endotoxin levels as low as 0.4 to 7 EU/ml, in contrast to the 150 to 2,500 EU/ml typically found after gel filtration chromatography alone.

The presence of toxin genes in some phages necessitates the DNA sequencing of any therapeutic phages as part of a rigorous characterization scheme. Although toxin genes are an important consideration, they are by no means the only virulence factor encoded by phages. Some phages are known to encode regulatory factors that increase the expression of bacterial virulence genes, while others contribute to the dissemination of antibiotic resistance genes through transduction (42). Recent advances in rapid DNA sequencing technology and the ability to search phage genomes against large databases will greatly enhance our ability to detect the presence of deleterious genes.

For a phage therapy to be effective in a clinical setting, a swift assessment of bacterial susceptibility will be necessary. This would require a large collection of phage stocks specific for given species of bacteria, preferably prepared on clinical isolates to overcome the problem of restriction modification. A microtiter plate format would facilitate the testing of large numbers of phages. Blasco et al. described a bioluminescence assay based on the phage-mediated release of AK, which converts ADP in the reaction mix to ATP (5). A luciferin or luciferase reagent added to the mix utilizes ATP for the emission of light, providing a rapid measure of bacterial susceptibility.

CONCLUSIONS

The potential for treating infectious diseases with phages has been pursued since their discovery, but for the reasons outlined in this chapter, phage therapy is not currently accepted in Western medicine. With the continuing threat of antibiotic resistance, it is time to reconsider developing phage therapy to the point where it can be useful in modern clinical practice. In fact, phages may actually have advantages over other treatments, such as high specificities for a given strain of bacteria, ease of production, and the ability to be adapted or engineered. Phages can also be used in the clinical setting as tools for detecting specific bacteria from patient samples and for the rapid identification of antibiotic-resistant bacterial strains. A better understanding of phage biology and phage-bacterium-host interactions and the ability to genetically manipulate these viruses may allow for the realization of the early promise of phage therapy.

> "I think it may now be time for you to use phage in practical healing. I want you to experiment with phage in pneumonia, plague, perhaps typhoid, and when your experiments get going, make some practical tests in collaboration with the hospitals. Enough of all this mere frittering and vanity. Let's really cure somebody!" —Sinclair Lewis (25)

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