Pseudomonas Biofilms, Cystic Fibrosis, and Phage: a Silver Lining?

Harald Brüssow

Nestlé Research Center, BioAnalytical Department, Food and Health Microbiology Group, Lausanne, Switzerland

ABSTRACT In contrast to usual laboratory conditions, most bacteria in the human body grow in biofilms. Encased in a structured matrix, many pathogens display heightened resistance to antibiotics. *Pseudomonas aeruginosa* lung infections in cystic fibrosis patients represent a prime example of the clinical challenges that antibiotic resistance in biofilms can represent. In the March 6, 2012 issue of *mBio*, Colin Hill and his colleagues report on experiments that add to the evidence that *Pseudomonas* phages are a potential treatment option for these infections.

With an incidence of 1 out of 3,500 live births, cystic fibrosis (CF) is the most common life-threatening autosomal recessive genetic disease in Caucasian children. The causative mutations are located within a single gene, the CF transmembrane regulator. Half of the affected patients show a deletion of a specific phenylalanine residue (ΔF508), but the genotype-phenotype relationship is complex. It has been proposed that the strikingly high prevalence of this deleterious mutation could be due to a higher resistance to toxin-induced diarrhea or tuberculosis among heterozygotes.

CF affects the gut and the pancreas (exocrine pancreas insufficiency leads to malabsorption and failure to thrive), but for most CF patients, life expectancy is determined by the development of lung disease. The failure to clear thick and abundant mucous secretions from the lung leads to chronic coughing at a young age, followed by frequent lung infections, including repetitive episodes of pneumonia. Chest percussion to remove the secretions and antibiotics constitute a mainstay of treatment. However, for most CF patients, recurring lung infections finally lead to end-stage lung disease, which can be treated only with lung transplantation. The median survival age for CF patients is currently >32 and 29 years for men and women, respectively.

The microbiology of CF is complex. *Staphylococcus aureus* colonizes the lung in 30% of CF patients early in life. Small, slow-growing colony variants displaying antibiotic resistance, including methicillin-resistant *S. aureus* (MRSA) strains, are characteristically found. In early adolescence, the lungs typically become chronically infected with *Pseudomonas aeruginosa*; up to 80% of adult CF patients are colonized with this pathogen. Nonmotile, anaerobic, mucoid variants of *P. aeruginosa* grow in a biofilm within the lungs, a structure that confers resistance to antimicrobials.

Since today's CF patients reach adulthood, still other facultative pathogens have a chance to enter the lung. Bacteria of the *Burkholderia cepacia* complex, for instance, are now recovered from 10% of the lungs in adult CF patients. *Burkholderia* infections can transform a mild pulmonary case into a rapidly deteriorating lung infection associated with bacteremia and death within 6 months. Even among patients with a less dramatic course, some surgeons consider *Burkholderia* infections to be a contraindication for lung transplantation. In hospital settings, *Burkholderia*-infected patients should be segregated from other CF patients to prevent nosocomial spread of the pathogen.

Since lung infections are the life-limiting factor for CF patients and since the major CF pathogens are increasingly difficult to treat with antibiotics, alternatives or adjuncts to antibiotics are urgently needed. The *mBio* article by Colin Hill and his colleagues (1) provides an encouraging outlook in this regard. The authors explored the possibility of treating *P. aeruginosa* infections with bacteriophages, an attractive potential therapeutic option for several reasons.

First, *S. aureus* and *P. aeruginosa* are classical targets of the Soviet phage therapy approach. The Russian company Microgen currently sells phage preparations against both pathogens, either singly or in combination, for treatment of pyogenic infections. Frequently used for treating pulmonary infections, these products are registered medicines in Russia. Second, it is relatively easy to isolate "virulent" (as opposed to "temperate") phages against the three major pathogens of CF patients. Large collections of phages of *S. aureus*, *P. aeruginosa*, and *B. cepacia* exist, many of which are fully sequenced. This is not a trivial statement, since phages are not isolated easily against any bacterial species.

Third, the aforementioned phages have a broad host range. Unlike antibiotics, most phages are strain specific, making them targeted to the pathogen of interest and preventing collateral damage to commensal bacteria. However, this specificity can be a problem for treatment, and often a cocktail of different phages is needed to achieve a reasonable coverage of a given pathogenic species. Fortunately, phages against CF pathogens have a good, if not an excellent, pathogen coverage. Phage K, for instance, infects nearly half of *S. aureus* isolates from large collections, and phage Stau2 infects 80% (2).

Fourth, the efficacy of phage therapy approaches has been demonstrated in animal infection models with CF pathogens. For example, *B. cenocepacia* bacterial density in the lung was reduced after intraperitoneal but not intranasal phage application to mice. The lytic effect was specific to the replication-competent phages, since no lytic effect was seen with UV-inactivated phages or a phage that had no *in vitro* lytic activity against the infecting *Burkholderia* strain. Phages showed amplification in the lung and reduced pathogen-induced inflammation signs. In treated mice, phages were colocalized with degraded bacteria in perivascular lung areas and alveolar septa (3).

The recent article by Alemayehu et al. (1) provides suggestive evidence that bacteriophages might also be of use against *P. aeruginosa* infection. They showed that suitable phages can be isolated from sewage, a common starting material for phage isolation. By sequencing

Published 3 April 2012

Citation Brüssow H. 2012. *Pseudomonas* biofilms, cystic fibrosis, and phage: a silver lining? mBio 3(2):e00061-12. doi:10.1128/mBio.00061-12.

Address correspondence to harald.bruessow@rdls.nestle.com.

the phage, they demonstrated the absence of undesired genes in their therapeutic phages, an essential condition for later phage application in human subjects. They then reported that the phages infect *Pseudomonas* growing in a biofilm. Finally, the authors demonstrated the efficacy of intranasally applied phages in a *P. aeruginosa* lung infection model using state-of-the-art imaging techniques (although it should be noted that mice do not get chronically infected with *P. aeruginosa*, so biofilms are thus not formed in this transient infection model). Mice treated with intranasal phage at a phage-to-bacterium ratio of 1:1, but not with lower ratios, survived a lethal *P. aeruginosa* infection.

The data reported by Alemayehu et al. (1) confirm previous observations from other groups and therefore represent important steps toward developing effective CF phage therapy.

Fittingly, work on phage therapy for *P. aeruginosa* infection continues at the Pasteur Institute in Paris, where phages and phage therapy were first described by Felix d'Herelle nearly 100 years ago. Using bioluminescent *P. aeruginosa*, Debarbieux et al. (4) demonstrated a rapid killing of bacteria in phage-treated animals and a reduction in inflammatory markers. The researchers selected phages which grew well on clinical *P. aeruginosa* isolates from CF patients and showed that they achieved dose-dependent protection in a mouse lung infection model (5). Phages showed *in vivo* replication, decreased the alveolar pathogen load, and prevented histopathologic lesions. Phage treatment could be delayed for a few hours with the same results, and administering the phages a day before pathogen challenge also prevented the infection. When phages were administered to uninfected mice, they persisted for 5 days in the lung, suggesting the potential for preventive phage use.

Similarly, the biofilm activity of *Pseudomonas* phages reported in the Alemayehu et al. article confirms earlier work. British researchers found that phages had bacteriocidal effects on *Pseudomonas* in biofilms (6). When using excess phages, a 100-fold bacterial titer decrease was observed. *Pseudomonas* phages could also diffuse through 8% alginate gels, which mimic the conditions in *Pseudomonas* biofilms. Digestion fragments from the alginate were released from phage-treated polymers. Phage researchers from the Republic of Georgia and structural biologists subsequently showed that the depolymerizing enzyme is part of the phage particles (7, 8).

Pretreatment with phages may also eventually be feasible. Researchers at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, demonstrated that pretreating catheters with a cocktail of *Pseudomonas* phages prevented biofilm formation. Intact but not heat-killed phages achieved a 1,000-fold titer reduction of the bacteria in the biofilm grown on a catheter in the presence of human serum (9). The latter observation is important, since in the transition from bench to bed, the devil is in the details. The lead authors of the Alemayehu article observed this for themselves in earlier work, in which they explored the potential of *S. aureus* phages for treating bovine mastitis. In that study, they found that milk contained an inhibitory activity that inactivated the broad-host-range *S. aureus* phage K (10), a fact that explains the observations from Canadian researchers who failed to detect an *in vivo* inhibitory effect of phages on bovine mastitis.

Despite the setbacks with *S. aureus* phage use in farm animals, the prospects for applying *Pseudomonas* phages in humans are encouraging. Three clinical trials that address *Pseudomonas* infections in venous leg ulcers (Intralytix), burn patients (Belgian military) (11), and the outer ear canal (causing diseases ranging from mild swimmer's ear to necrotizing otitis externa in diabetics and AIDS) (Biocontrol, United Kingdom) have been registered. So far, no adverse effects of phage treatment have been observed. In a controlled phase II clinical trial, 24 otitis externa patients received either a single small dose of *Pseudomonas* phage cocktail or a placebo. The researchers found evidence for *in vivo* phage replication, a decrease in *Pseudomonas* counts in some patients, and symptom amelioration in most of the treated patients (12). One might criticize the study for its small size and for the fact that only patients with *Pseudomonas* ear infection susceptible to the applied phage were enrolled, but the study set a standard for a detailed combined clinical, microbiological, and statistical evaluation of phage therapy approaches.

The three senior authors of the *mBio* article (1) are members of PhageWorks, a phage solution company recently founded in Cork, Ireland, by scientists working in two important research organizations of the Irish government: the Teagasc Food Research Centre and the Alimentary Pharmabiotic Centre. The current study relies on years of research in the dairy field, but one hopes that the knowledge acquired in the food sector on practical aspects of phage research can eventually serve the biomedical research community and the development of therapeutic applications for phages as well. This reminds me of the words of Louis Pasteur, who wrote, "There are similarities between the diseases of animals and humans and the diseases of beer and wine." For the Cork researchers, he might have spoken of "cheese and yogurt." Looking beyond the fences of one's "own professional specialization" can lead to important cross-fertilization.

ACKNOWLEDGMENTS

The author is a senior research scientist at the Nestlé Research Center in Lausanne, Switzerland, and one of his research projects is on phage therapy of bacterial diarrhea.

REFERENCES

1. **Alemayehu D, et al.** 2012. Bacteriophages ϕMR299-2 and ϕNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. mBio **3**(2):e00029-12.
2. **Hsieh SE, et al.** 2011. Wide host range and strong lytic activity of *Staphylococcus aureus* lytic phage Stau2. Appl. Environ. Microbiol. **77**:756–761.
3. **Carmody LA, et al.** 2010. Efficacy of bacteriophage therapy in a model of *Burkholderia cenocepacia* pulmonary infection. J. Infect. Dis. **201**:264–271.
4. **Debarbieux L, et al.** 2010. Bacteriophage can treat and prevent *Pseudomonas aeruginosa* lung infection. J. Infect. Dis. **201**:1096–1104.
5. **Morello E, et al.** 2011. Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. PLoS One **6**:e16963.
6. **Hanlon GW, et al.** 2001. Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol. **67**:2746–2753.
7. **Glonti T, et al.** 2010. Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. J. Appl. Microbiol. **108**:695–702.
8. **Leiman PG, et al.** 2007. The structures of bacteriophages K1E and K1-5 explain processive degradation of polysaccharide capsules and evolution of new host specificities. J. Mol. Biol. **371**:836–849.
9. **Fu W, et al.** 2010. Bacteriophage cocktail for the prevention of biofilm formation by *Pseudomonas aeruginosa* on catheters in an *in vitro* model system. Antimicrob. Agents Chemother. **54**:397–404.
10. **O'Flaherty S, et al.** 2005. Inhibition of bacteriophage K proliferation on *Staphylococcus aureus* in raw bovine milk. Lett. Appl. Microbiol. **41**:274–279.
11. **Merabishvili M, et al.** 2009. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One **4**:e4944.
12. **Wright A, et al.** 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin. Otolaryngol. **34**:349–357.