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Editorial

Prokaryotic gene therapy to combat multidrug resistant bacterial infection

Gene therapy has multiple applications in human medicine. Its promise is enormous but its application requires refinement including development of more efficacious delivery systems. This editorial will discuss the use of a P1 bacteriophage lethal agent delivery system, LADS (Department of Microbiology/Immunology, Medical University of SC, Charleston, SC, USA), for the treatment of multidrug resistant bacterial infections. This system will use phage-targeting mechanisms to achieve delivery to the offending bacterial population.

In the face of widespread use, antibiotic resistant bacteria have arisen at an alarming rate. For example, *Staphylococcus aureus* is a pathogen prone to develop resistance, and is responsible for about 260000 nosocomial infections in the USA which causes between 60000 and 80000 deaths annually.[1] These infections result in an additional eight million hospital patient days per year, costing the USA healthcare system four billion dollars.[1] Vancomycin is the last effective antimicrobial available for the treatment of methicillin-resistant *S. aureus* infections. However, vancomycin-resistant clinical isolates have now emerged.[2] Like *S. aureus*, enterococcal infections no longer respond to a vast array of antimicrobials including vancomycin.[3] Enterococci account for approximately 110000 urinary tract infections, 25000 cases of bacteremia, 40000 wound infections and 1100 cases of endocarditis annually in the USA.[4] In contrast to the acquired resistance of Staphylococci and Enterococci species, *Pseudomonas aeruginosa* exhibits intrinsic resistance to many structurally unrelated antibiotics.[5] *P. aeruginosa* is the most common Gram-negative bacterium found in nosocomial infections and outbreaks in burns units are associated with high death rates (60%).

Treatment of resistant infections is increasingly hampered either by the prohibitive cost of existing 'new generation' agents or the total lack of effective antimicrobials on the market. Streptogamins, the first new class of antibiotic developed for human use in 30 years (Synercid, Rhône-Poulenc Rorer, Paris, France) has just been approved by the FDA for treatment of vancomycin-resistant *Enterococcus faecium* and *S. aureus* infections.[6] However, resistance related to horizontal transmission of *SatA* (confers resistance to Synercid) has already been documented in poultry which were fed a related streptogamin, virginiamycin.[7–10] The makers of Zyvox (Pharmacia & Upjohn, Peapack, NJ, USA), the first of a new category of drugs called oxazolidinones, plan to seek approval from the FDA by the end of the year.[11] Synercid and Zyvox are last resort drugs and are only effective against Gram-positive infections. Few companies have had the foresight to develop alternative therapies and a race now exists between the development of new effective antimicrobials and emerging drug resistant bacteria.

Currently two biological approaches are being studied as treatments for drug resistant bacterial infection. The oldest is based on administration of lytic bacteriophage. In 1915/1917 Frederick Twort and Felix D'Herelle independently discovered the principle of bacteriophage and attempted to exploit them clinically. Further advances in bacteriophage therapy were largely pre-empted following the discovery of penicillin in 1929 by Alexander Fleming and subsequent demonstration of its therapeutic potential in the 1940s. However, a resurgence of lytic phage therapy is now occurring (reviewed by Alisky *et al*).[12] In 1999 a young woman in Toronto with a *S. aureus* infection, resistant to extensive antibiotic therapy, was treated successfully with parentally administered bacteriophage.[13]

The second approach, LADS technology, utilizes a bacteriophage-based *in vivo* packaging system to create a targeted phagehead capable of delivering naturally occurring molecules with bacteriocidal activity to drug resistant bacteria (Figure 1). The delivery system consists of a transfer plasmid carrying the genes encoding the antimicrobial agents, a plasmid origin of replication, the P1 lytic origin of replication and a minimal PAC site. This plasmid is maintained in a bacteriophage P1 lysogen unable to package its own DNA. The defective lysogen provides all the replication factors needed to activate the P1 origin of replication on the transfer plasmid and all the structural components necessary to form mature virions. The lysogen also carries the c1.100 temperature-sensitive repressor mutation. C1 is responsible for the repression of functions leading to vegetative phage production. Induction of the lysogen by a temperature shift results in multiplication of DNA, packaging of the transfer plasmid into P1 phage heads and lysis of the production strain. Virions are harvested and used to deliver the transfer plasmid to the pathogen. The phagehead contains multiple copies of transfer vector DNA and is targeted to pathogenic bacteria by natural receptor mediated mechanisms. Upon delivery, plasmid DNA recircularises and expression of the lethal agent under the control of environmental, virulence-regulated or species-specific promoters results in rapid cell death. Similar strategies are under development for Gram-positive organisms. Lethal agents delivered by LADS are naturally occurring lethal genes associated with plasmids, bacteriophage, or bacterial chromosomes such as *doc*, *chpBK* and

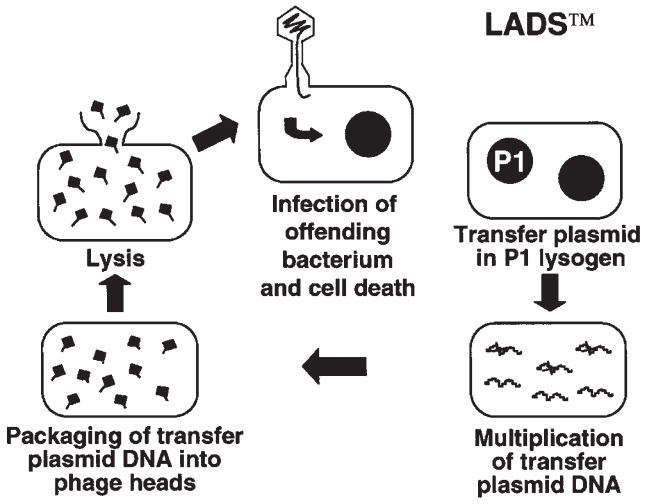

Figure 1 *Lethal agent delivery system.*

gef. A multitude of these genes exists (reviewed in 1997 by Holcik and Iver[14]). Our laboratory has tested a number of these lethality systems in *Escherichia coli*. At least one, *doc*, derived from bacteriophage P1[15] was experimentally determined to be lethal in *E. coli* and is either lethal or bacteriostatic in *P. aeruginosa*, *S. aureus* and *E. faecalis* (unpublished observations).

Potentially, phage therapy may encounter the same dilemma that arose from indiscriminate use of antibiotics, namely resistance. There are at least five ways resistance can occur: (1) interference with bacteriophage absorption; (2) prevention of bacteriophage DNA injection; (3) restriction/modification; (4) abortive infection; and (5) superinfection exclusion. One or more of these resistance mechanisms could, in the future, present problems for lytic phage or LADS therapy. However, it has been estimated that there are $4–6 \times 10^{30}$ prokaryotic cells in the biosphere with approximately 10-fold more tailed bacteriophage particles than cells.[16–18] Bacteriophage have been evolving with bacteria for 3.5 billion years and thus, although resistance can be demonstrated, evolution of existing phage seemingly continues to overcome resistance mechanisms. Such evolved bacteriophage are readily available to researchers, for example from sewage treatment plants, hospital effluents, or by laboratory manipulation (typically UV exposure) making modification of the LADS therapeutic system possible should resistance arise.

A frequently asked question relative to bacteriophage treatment of humans is the issue of the immune response. Using ϕX174, it was demonstrated that patients who are immunocompromised appear less likely to mount a response to bacteriophage administration.[19] A study performed in Poland confirms this observation.[20] From the clinical viewpoint, most septic patients are immunologically compromised. Since LADS therapy will usually be administered over a short time interval the likelihood of an acute immune response occurring is minimal as has been documented in practice for lytic therapy.[20]

Another concern is rapid clearance of circulating bacteriophage by the reticuloendothelial system. Merrill *et al*[21] developed an animal passage protocol to select bacteriophage able to evade clearance for long periods. Presently, a biotech company (Exponential Biotherapies, Port Washington, NY, USA) has been formed to exploit this observation.

There is a compromise faced by the biotechnology industry relative to the exploitation of lytic phage therapy and involves phage-mediated toxin production, horizontal transmission of unwanted genetic information, or development of resistant phage. First, the issue of toxin production includes: Shiga-like toxins of *E. coli*, cholera toxin of *Vibrio cholerae*, and cytotoxins of *P. aeruginosa*. For example, phage K139 confers to *V. cholera* a gene product that enhances enzymatic activity of cholera toxin. Horizontal exchange of virulence genes and/or antibiotic resistance will also occur and has been regularly observed during evolution of bacteria and their cognate phages.[22] These issues are less likely to be relevant for LADS because the agent acts rapidly to kill offending bacteria, is nonreplicating reducing the risk of resistance and essentially eliminates the issue of horizontal gene transfer. This contrasts with lytic therapy where the phage genome replicates and reinfects risking lysogenic conversion and development of resistance.

In the late 1990s, the market for antimicrobial therapeutics for human infectious disease was estimated at ten billion dollars per year in the USA and twenty-five billion worldwide. Thus, for the pharmaceutical industry the sales potential for drugs to treat infectious diseases is very large. The indiscriminate use of antibiotics over the last 70 years has lead to the emergence of resistant bacteria. Few new antibiotics in the developmental pipeline are predicted to overcome existing resistance mechanisms. The LADS therapeutic, based on sound bacteriophage principles, will be administered over a short duration to patients who fail antibiotics. Once the therapeutic is injected by the phagehead, it kills the bacterium quickly, limiting patient exposure thereby reducing development of bacterial resistance and deleterious horizontal gene transfer. We expect to have this prokaryotic gene therapy in clinical trials within 18 months.

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