Therapeutic monitoring of the host-bacterium-phage interaction in a child

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Abstract

Successful phage therapy for extensive chronic osteoarticular infection in a child resulted in an initial flush of bacterial contents into the bloodstream with an inflammatory response marked by fever, local pain and upregulation of genes associated with autophagy and innate immunity. Monitoring of phage and bacterial kinetics and the human host response allowed early dose adjustment and indicated a strong therapeutic effect within two weeks.

Main Text

Bacteriophages (phages), first used as antibacterial agents more than a century ago¹, offer new hope for highly resistant infections^{2,3} but clinicians remain uncertain about optimal dosing and treatment duration. Here we describe phage, bacterial and host responses to intravenous (IV) phage therapy for a chronic osteoarticular infection complicating K-wire insertion for traumatic fracture-dislocation in a 7-year-old girl. Previous surgical debridement and hardware removal and three months of intravenous antibiotics had failed to stop the progress of disease (Fig. 1) and no other medical or surgical options were available. Under informed consent, we administered 10^{11} plaque forming units (PFU)/mL of Pa14NPΦPASA16 (PASA16) once (days 1, 2, 4–7) or twice daily (days 3, 8–14; Fig. 2a) for two weeks, beginning three months into a twelve-month course of IV colistin and aztreonam. Strong lytic activity of PASA16 against a surgically obtained isolate of *P. aeruginosa* (Ppa2.1; efficiency of plating [EOP] ~ 100%) was unaffected by these antibiotics *in vitro* (**Supplementary Fig. 1**).

Transiently increased heel pain 8 hours after the first dose of PASA16 was followed by fever to 39.7°C several hours later. Low-grade temperatures (37.7–37.8°C) were also recorded ~ 6–12 hours after the second and third doses of phage but none thereafter. C-reactive protein (CRP) peaked on day 4 (20.1 mg/L) (Fig. 2a) and a slight increase in serum Ig-G levels from baseline to day 30 (14.7 g/L to 16.1 g/L, normal range 6.24–14.4 g/L) was noted, with no change in serum complement levels (C3/C4). Pain-free weight bearing on the affected leg was achieved for the first time since the initial injury, seven weeks after completion of phage therapy, and long-term follow-up demonstrated clear radiological improvement (Fig. 1).

Bacterial and phage genomes were quantified in DNA extracted from whole blood and serum respectively, as previously described³ (**Supplementary Text**). Standard curves were generated by spiking tenfold serial dilutions of log-phase *P. aeruginosa* and PASA16 into whole human blood (*P. aeruginosa*, $1.3–1.3 \times 10^6$ colony forming units [CFU]/mL) or serum (phage, $1 \times 10^{1-7}$ PFU/mL). Viable phage was recovered from serum onto a lawn of an aztreonam- and colistin-resistant strain of *P. aeruginosa* (JIP697; EOP ~ 100%), also as previously described³. All experiments were performed in duplicate.

Blood cultures yielded no bacteria at any stage, but viable phage was recovered from pre-dose samples up until day 5 consistent with productive infection of target bacteria throughout the dosing interval (Fig. 2a). Increased phage dosing in the second week was associated with further bacterial lysis but the

dynamics of bacterial and phage DNAemia (to $\sim 10^5$ genome equivalents/mL), similar to previous observations^{3,4} are most consistent overall with a classic “predator-prey” relationship (Fig. 2a), with predators (phage) thriving while prey (bacterial hosts) are plentiful but declining as prey populations are consumed.

Endotoxin levels in the administered phage dose were below the accepted human pyrogenic threshold of 5 EU/kg (see **Supplementary Text** for calculations). The expected amount of bacterial endotoxin accompanying the surges of *Pseudomonas* DNA (10^2 - 10^3 /mL) into the bloodstream is orders of magnitude higher at between 0.05 and 0.5 ng/mL^{5,6} (~ 40 – 400 EU/kg) and expected to produce a strong inflammatory response. Significant differences in the expression of 18 genes associated with the innate immune response (Gene Ontology: 0045087, $p = 1.03 \times 10^{-11}$) between D0 (immediately prior to phage therapy) and D2-4 (24–72 hours after initiation of phage therapy) were demonstrated by principal component analysis (**Supplementary Fig. 2**; $p < 0.05$) and coincided with the fever, localised pain and spike in *Pseudomonas* DNA and CRP (Fig. 2a). Hierarchical clustering analysis (**Supplementary Text**) confirmed enrichment of human innate immune response-associated genes on D2 vs. D0 (Fig. 2b). The relative enrichment of seven of these (*ATG5*, *CD180*, *CD244*, *PEZ1*, *IL5RA*, *PTGDR2*, *SMPD3*) persisted for at least two weeks after completing phage therapy (D29), but were no longer significant beyond D5-7 after *post-hoc* adjustment (Benjamini-Hochberg). Gene Set Variation Analysis (**Supplementary Text**) further demonstrated early immediate upregulation of genes enriched for innate immune response in periods 1 (D2, D4) and 3 (D9, D11) in synch with the kinetics of *Pseudomonas* DNAemia, followed by upregulation in genes enriched for adaptive immune response from D5 onwards (Fig. 2c), reflected at the systems level by the hierarchical clustering analysis.

We note the differential expression of genes linked to autophagy such as *ATG5*, that may be beneficial in clearance of *P. aeruginosa* infection⁷, and others (*CD180* and *CD244*), which may dampen the inflammatory response⁸. The kinetics of significant changes in toll-like receptor signalling, autophagy and JAK-STAT signalling pathways (**Supplementary Fig. 3a-c**) align temporally with the clinical signs and symptoms and phage-bacterial kinetics described above. Taken together, these data indicate an innate immune response to phage-mediated bacterial lysis. It appears that clinical signs and widely available measures such as C-reactive protein, together with simple quantitation of phage and bacterial kinetics, can be used to guide the therapeutic interaction of the three main actors: phage, bacteria, and human host. The kinetics of the response are similar to previous descriptions and indicate that most benefit accrues within the first ten days of treatment. Such multi-modal therapeutic phage monitoring is important to help us understand this intervention better.

Declarations

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Sydney children's Hospital Network Human Research Ethics Committee. We would also like to thank Jessica Sacher and Jan Zheng from Phage Directory, Atlanta, GA, United States for helping our team source phage therapy for this patient and Brian Gloss, from the Westmead Institute for Medical Research core facility (Bioinformatics), supported by the Westmead Research Hub, the Cancer Institute New South Wales, the National Health and Medical Research Council and the Ian Potter Foundation, for his assistance in gene networks and pathway visualisations.

Author contributions

AK conceived the project, collated the data and prepared the manuscript. AK and JI directed phage treatment of the patient and interpreted the clinical and laboratory data. RCYL analysed and interpreted the transcriptomic data. APF analysed and interpreted the phage and bacterial kinetics data. AK, JI, RCYL and APF critically revised the manuscript. SAO, RH and RNP isolated and characterised the phage used to treat the patient. SA, PNB and QD oversaw overall clinical and orthopaedic management of the patient. MB, JF and BA provided guidance and advice during phage treatment. All authors reviewed the manuscript, contributed to revisions and approved the final version.

Potential conflicts of interest and financial disclosures

Bacteriophage Pa14NPΦPASA16 was isolated and characterized by the Hazan laboratory (Hebrew University, Jerusalem, Israel) and prepared for the treatment of this patient by Adaptive Phage Therapeutics (Maryland, USA) on compassionate grounds with no commercial value to The Children's Hospital at Westmead. MB, JF and BH are employees of Adaptive Phage Therapeutics. No other authors have any conflicts of interest to disclose. This work was supported by grants to JI from the Australian National Health and Medical Research Council.

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Figures

Figure 1

Magnetic resonance imaging of the lower leg of a paediatric patient with chronic *Pseudomonas aeruginosa* infection following K-wire insertion (removed), over 12 months of treatment and follow-up 1a. One week prior to intravenous (IV) antibiotics: extensive phlegmonous material along prior internal fixation device tracts within the calcaneus, talus and distal tibia with bone marrow inflammation; marked ankle joint synovial thickening and enhancement with phlegmonous material; tenosynovitis and myositis.

1b. Two months after surgical debridement and initiation of IV colistin and aztreonam, four weeks prior to phage therapy: new focus of infection in anterior process of the talus; worsening marrow oedema and enhancement in the distal tibial epiphysis, talus, navicular, and cuneiforms bones; slight reduction of enhancement and marrow oedema in the calcaneus and posterior subtalar recess. 1c. After five months of IV colistin and aztreonam, 6 weeks after completion of a two-week course of IV phage: marked reduction in the marrow oedema adjacent to surgical tracks and in the anterolateral talus; persistent ankle synovitis and enhancement along the surgical tract; prominent distal tibial epiphyseal and tibiofibular joint enhancement; new marrow oedema and enhancement in the first metatarsal. 1d. After 12 months of IV colistin and aztreonam, eight months after completion of phage therapy: marked reduction in the marrow oedema adjacent to the surgical tracts along the tibiotalar and subtalar joints and resolution of first metatarsal marrow oedema and enhancement.

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Figure 2

Pseudomonas aeruginosa and bacteriophage kinetics in blood over 2 weeks of therapy and 7 weeks of follow-up (2a) with corresponding gene expression profile (2b) and gene set variation analysis (2c) 2a. PFU: plaque forming units; CRP: C-reactive protein; phage doses given once (short) or twice (long vertical arrows) daily; shaded temperature boxes represent brief episodes of high-grade (dark) or low-grade (light) fever; blood samples obtained prior to morning phage dose. 2b. Hierarchical clustering analysis of gene

expression profile for a paediatric patient receiving intravenous phage. D0: pre-phage, D2-D11: during phage administration, D15: one day post-phage therapy, D29: 15 days post-phage therapy. Significantly up-regulated genes (n=58, horizontal bar at bottom, yellow end) in D2-4 were enriched for innate immune response (adjusted $p = 1.03 \times 10^{-11}$) and genes enriched for adaptive immune responses were expressed from D5-7 onwards (unadjusted $p = 0.004$, adjusted $p = 0.20$), with D15-29 showing a similar profile to D0 (linkages on top). 2c. Gene Set Variation Analysis of genes enriched for Innate Immune Response and Adaptive Immune Response from D0 to D29 was based on REACTOME pathways accessed using MSigDBR. Overlapping genes from both lists were extracted as a separate gene set prior to GSVA analysis. This visualisation reflected the dichotomy of upregulation in genes enriched for innate immune response in period 1 (D2, D4) and then upregulation in genes enriched for adaptive immune response from D5 onwards.

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