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THE BIOLOGICAL EMPIRE OF THE BACTERIOPHAGE: A SUMMARY OF THE SEMINAR AND DISCUSSIONS AT THE 34TH OLD HERBORN UNIVERSITY SEMINAR

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INTRODUCTION

The 34th Old Herborn University Seminar focussed on the biological empire of the bacteriophage (phage), bacterial viruses that represent the most abundant biological entities on Earth. Phage are not only the most abundant and most diverse entities in biology but they occupy an unusual position (or no position) on the tree of life. Given that they have a genetic basis (they can have ssRNA, dsRNA, ssDNA to dsDNA genomes of lengths varying from 2kb to over 1Mb) they are subject to evolution and are therefore very much part of the ongoing process of life on Earth, but because they are not metabolically active they cannot strictly be considered to be living. Notwithstanding their status as being alive or dead, they play a very important role in bacterial life and therefore in life on Earth. It has been estimated that up to half of all bacteria meet their end through an encounter with a phage. Phage can practice either a lytic or lysogenic lifecycle but in either event the end result is a lysed bacterial cell releasing many copies of the intact phage particle (there are exceptions to this, as is the case for almost any statement about phage, in that some bacteria can release filamentous phage while remaining intact).

The purpose of the 34th Old Herborn University Seminar was to gather a group of experts to examine some of the recent findings about phage and the role they may play in human health through their relationships with their target bacteria. Phage can be used in medicine in a manner similar to how we use antibiotics to target pathogenic bacteria causing infections, an approach usually termed 'phage therapy', but it is also likely that the 'virome' (the term used to describe the collection of phages and viruses resident in the microbiome) also plays a role in human health by influencing the composition and functionality of the bacteriome. On the first day of the Seminars the invited speakers presented recent data on various aspects of phage biology while day two consisted of an in-depth discussion of the presentations, involving both speakers and members of the scientific advisory board. In the following short account I will summarise some of the presentations and discussions but I encourage the reader to read the individual papers produced by each author for a more definitive and appropriately referenced account of their work.

PHAGE THERAPY

Volker Rusch opened the Seminar by welcoming the participants before our first speaker introduced the topic of phage therapy and the historic role of

the Eliava Institute. We had an ideal speaker in Mzia Kutateladze, the Director of the G. Eliava Institute of Bacteriophages, Microbiology and Virology, headquartered in Tbilisi, Georgia. Almost a century ago George Eliava worked at the Pasteur Institute in Paris with Felix d'Hérelle before returning to Georgia and setting up the Institute that bears his name. He was later joined by d'Hérelle in Tbilisi and together they learned how to exploit phage to treat infectious disease. The Eliava Institute has spent decades deploying phage to combat infection and have built a knowledge base unmatched elsewhere in the world. The strategy has been not only to develop personalised therapies but to create cocktails of phage that can be used across multiple patients. Currently the Eliava produces up to six of these cocktails for different indications, for example the Intesti phage preparation targets *Shigella*, *Salmonella*, enteropathogenic *E. coli*, *Proteus*, *Enterococcus*, *Staphylococcus* and *Pseudomonas aeruginosa*. Mzia spoke with passion about the excellent safety record with the use of phage as therapeutics but was clear as to some of the drawbacks in terms of a lack of appropriate placebos and the use of different preparations making it difficult to build a clear picture of their efficacy. She shared some impressive data from a number of field trials, some in soldiers on campaign and some conducted in large numbers of people in Ukraine and Russia in the 1970's, where phage treatment led to a significant reduction in the burden of illness. She finished by offering a hopeful vision of future trials in a number of countries on phage therapy.

The topic of phage therapy was continued with our two subsequent speakers, Martha Clokie from the University of Leicester and Graham Hatfull from the University of Pittsburgh. Martha focussed on one aspect of her work involving the clinical development of phage for treating urinary tract infections (UTIs). She highlighted the scale of the problem in that 400 million UTIs are diagnosed and 236,786 UTI-related deaths globally every year, and clearly established the unmet need for novel approaches. She and her team are approaching the issue with rigour and a clear strategy designed to bring phage into the clinic. She was also clear about the hurdles facing phage therapy approaches, from finding the right phage to proper formulation, delivery, efficacy and potential immunogenicity. She highlighted the ability of phage to work against biofilms, an important aspect of UTIs. Martha's group are keen to deploy ecological strategies for phage selection to get the best outcomes for patients. To this end they are utilising their knowledge of phage genomes to predict and select the most appropriate phage to use in cocktails. She also shared some exciting data derived from pig and chicken models to demonstrate the ability of phage to traverse the gastrointestinal tract and reduce pathogen counts. A really exciting presentation from a scientist at the forefront of this area of research.

Another leading scientist in the field is Graham Hatfull, a scientist whose work has generated headlines globally for one particular study involving a child with a lung transplant and an untreatable *Mycobacterium abscessus* infection. But first Graham outlined the SEA-PHAGES platform. This is an absolutely inspiring programme to encourage schoolchildren to find phage in their environments. Over 5,500 students take part each year and they even get to name the phage that they help to discover and characterise. But the highlight of Graham's talk was the story of how he and his group developed a phage cocktail, using both natural and engineered phage, to treat a disseminated

infection with antibiotic resistant *M. abscessus* in a 15-year-old girl. Graham outlined how a phage cocktail was personalized for *M. abscessus* GD01 and was subsequently delivered by intravenous administration at a dose of 10^9 PFU (plaque forming units), twice daily. He confirmed the personalised nature of the treatment in that the same cocktail does not work for most other strains, but encouragingly they saw no adverse reactions, and no phage resistance was observed. Graham was determined to emphasise that while the patient made a complete recovery and the infection was resolved, this remains an observation and does not remove the need for properly controlled clinical trials. Graham and his lab have gone on to produce phage for compassionate use for another 35 patients, but he stressed the need for properly controlled trials if phage therapy is to meet the needs of patients. He also told the audience that phage engineering may well be necessary to increase the range and efficacy of mycobacterial phage.

This concluded the 'phage therapy session' and the talks and the discussion on the following day all illustrated the promise of this approach for the treatment of infections, particularly antibiotic resistant infections. However, no one was underestimating the challenges that lie ahead for phage therapy to become mainstream in 'Western' medicine. The specificity of phage, their ability to multiply and therefore evolve within patients, the possibility of resistance development, issues with sceptical regulators were all topics that were discussed at length. But as might be expected from a group of scientists working with phage, there was more optimism than pessimism that phage therapy can be, if not a replacement, then at the very least an important adjunct to antibiotic therapy in the years to come.

PHAGE-HOST INTERACTIONS

Normally when we talk about phage and their hosts, we mean their bacterial hosts, but this session was dedicated to role of phage in impacting on the mammalian, mainly human, host. Three excellent speakers participated in this session, Paul Bollyky of Stanford University, Jeremy Barr from Monash University and José-Manuel Fernández-Real from the University of Girona. Paul got us started by explaining how the Pf phage that infects *Pseudomonas aeruginosa* can be considered to be a novel human pathogen. *P. aeruginosa* is a Gram-negative bacterium that causes skin and lung infections and is considered a critical priority pathogen by the World Health Organisation (WHO). The cost of treating *P. aeruginosa* infections has been estimated at >$1 billion/per annum. Paul explained that Pf phage is a lysogenic filamentous phage, an Inovirus with a ssDNA genome. Pf phage are one of those unusual phages that do not lyse their bacterial hosts but are extruded from the living cell. Paul went on to demonstrate that Pf phage contributes to *Pseudomonas* biofilm formation and bacterial colonization, and that intracellular Pf phage trigger innate immune responses that antagonize bacterial clearance. The Pf phage aggregate outside of the cell and confer a number of properties on the resultant biofilms that are informed, including making the cells more resistant to antibiotics. They also make sputum more adherent and viscous. The presence of phage can lead to tangling of the cilia on the surface of human

epithelial cells, thus impacting on the ability of cilia to clear an infection. The phages are present in a high percentage of *P. aeruginosa* infections in the lungs of patients with cystic fibrosis, and those patients suffer a more rapid decline in lung function over time. Paul was able to add weight to these observations in a series of elegant animal models of disease that showed that Pf phage are required to establish robust infection in mice. In part at least this could be ascribed to the ability of Pf phage to inhibit the production of tumour necrosis factor (TNF) in response to LPS. Paul's group was also able to show that Pf phage are internalised by mammalian cells and both associated with and contribute to the progression of wound infections. Even in the absence of their bacterial hosts Pf phage can inhibit wound healing.

Next up was Jeremy Barr, who set out to show us that phage can also enter mammalian cells, leading to transcriptional and immune responses to these internalised phage particles. Using cell line models Jeremy's group showed that phage can be found inside cells, in a cell-type dependent manner. The size of the phage particles also influenced uptake. Jeremy followed a detailed research strategy to investigate the means by which phage were trafficked in the cell, and the detailed response of the cell to this phage 'invasion'. The transcriptional profiles revealed a number of pathways that are much too complex to detail here, but really emphasised the sophisticated interactions between phage and the mammalian host.

Last in this session was José-Manuel, who gave a fascinating talk on the gut microbiome, bacteriophages and cognitive function. One of the most fascinating parts of a comprehensive presentation concerned a recent paper authored by José-Manuel and his group entitled 'Caudovirales bacteriophages are associated with improved executive function and memory in flies, mice and humans'. It comes as a surprise to most phage biologists that phage could play a role in executive function and memory in humans but José-Manuel made a compelling case that this is indeed true. Once again, I will leave it to his dedicated paper in this monograph to make the case more convincingly than I can on his behalf, but it is an exciting prospect for phage biologists to consider going forward.

PHAGE GENOMICS

The final session was delivered by myself and José Penades of Imperial College London. In my presentation I focussed on the challenge of turning the *in silico* phage genomes that are being reconstructed in sequencing projects into 'real' phage that can be studied in the lab. I gave the example of the crAssphage, the most abundant phage in the human gut but which until recently had never been grown in the lab. Scientists in my group, led by the talented Andrey Shkoporov, managed to identify *Bacteroides intestinalis* as the host for crAss001. This phage is approximately 100kb in size and practices an unusual lifecycle in that it co-exists with its target host in culture, both in the lab and in animal models. This mimics what we see in human longitudinal studies where the phage and its host co-exist over 12 months in different individuals. The underlying mechanism involves switching between different capsular polysaccharides that act as a receptor for the phage, ensuring that a mix of both sensitive and resistant hosts are present at all times. This almost certainly impacts the

functionality of the host in the gut since these surface structures are also the interface between the bacterium and its neighbours, and its mammalian host. Being able to grow the phage also permitted the structural analysis of the phage using cryo-EM, which also revealed several novel features in this podovirus. The benefits of turning *in silico* phage into 'real' phage will have to be realised for many additional phage genomes in years to come, a significant challenge for phage scientists.

José Penades gave a fascinating talk on how phage can mobilise pathogenicity islands encoding immune systems as weapons to eradicate competitors. José works with *Staphylococcus aureus*, a talented pathogen that encodes a number of pathogenicity islands (SaPIs). Many of these virulence genes are located on phage inducible chromosomal islands (PICIs) that can be mobilised at high frequency by phage. While these were discovered in *S. aureus*, they are now believed to be widespread among across the bacterial Kingdom. José showed us some elegant work that demonstrated that some PICIs can encode phage resistance mechanisms that can even block prophage induction and horizontal gene transfer. This is an underappreciated aspect of phage biology and it was truly exciting to get a glimpse of this hidden world where bacterial competition is supported by phages.

DISCUSSION

It was a real pleasure to participate in the discussion session that was both lively and informative. It is rare that scientists get the chance to go back over a session with the speakers, exploring the synergies and points of difference in the previous days' seminars. We took full advantage of it, dissecting the finer points of the talks, and enjoying the debates that ensued. The objective was not to reach consensus but all were agreed that phage therapy offers an important tool for our clinical colleagues, but one that is faced with significant challenges to deliver on that promise. The role of phage in interacting with the mammalian host was also highlighted as an under-researched aspect of phage biology. This is an area that would have been regarded with scepticism until recently but is now becoming an accepted aspect of how phages can potentially impact on the health and even executive function of the mammalian host. Lastly, we were all agreed (when would scientists not agree on this point?) that more fundamental research in needed and that we are only scratching the surface of the extraordinary diversity and importance of the role of phage in shaping and influencing bacterial communities, and therefore human health.