ANNUAL | 2024 CONFERENCE

#Microbio24

EDINBURGH, UK
08 – 11 APRIL 2024

Image credit: iStock/bnoragitt

INVITED AND OFFERED TALKS

MICROBIOLOGY SOCIETY

Biofilm Prevention and Control

Invited talk: Discovering biofilm resistant materials to prevent medical device-associated infections

Paul Williams

University of Nottingham, Nottingham, United Kingdom

Abstract

Strategies for reducing bacterial biofilm formation traditionally focus on incorporation of antimicrobial agents into biomaterials, an approach that increases the challenges associated with the emergence of multi-antibiotic resistance. Greater success could be achieved through the discovery of materials that are inherently resistant to biofilm formation. To address this 'materials gap' we developed a combinatorial microarray methodology that enabled us to screen diverse homo- and co-polymers in high throughput for novel multi-pathogen biofilm-resistant materials. The most effective chemistries identified through structure function analysis could not have been predicted as biofilm-resistant from our current understanding of bacterial surface interactions. The mechanism(s) by which these materials resist biofilm formation *in vitro* and *in vivo* cannot be explained in simple physicochemical terms or without considering the dynamic adaptive behaviour of bacterial cells. Single cell tracking, measurements of surface adhesion strength, cell aggregation and evaluation of biofilm maturation by motility and surface sensing mutants in comparison with the wild type suggest that biofilm development stalls during the reversible-irreversible attachment phase. An optimized resistant polymer that emerged from the high throughput screen has now been approved for human use and is currently undergoing clinical trials for the prevention of catheter-associated urinary tract infections.

Invited talk: New ways of looking at biofilms and biofilm-related infections

Tom Coenye ORCID iD

Ghent University, Ghent, Belgium

Abstract

There is a growing awareness that the behaviour of bacteria (whether it be single cells, small agregates or mature biofilms) is to a large extent determined by the microenvironment at the site of the infection. Important microenvironmental factors include oxygen levels, the presence/absence of various carbon and nitrogen sources, as well as the presence/absence of certain host-derived factors. Considering the importance of the microenvironment in determining antimicrobial susceptibility and the outcome of antimicrobial treatments, especially for biofilm-related infections, there is an urgent need for novel tools and approaches that allow us to study susceptibility in conditions that mimick what happens in a patient, before and during treatment. In addition, the profound differences in microenvironmental conditions found in a patient and the conditions organisms are exposed to *in vitro* in the lab may have a profound effect on recovery and may contribute to diagnostic failures (e.g. culture results come back negative, even if there are clear clinical signs of infection). In this presentation I will talk about the work we are doing regarding the optimisation of biofilm-based susceptibility testing in the context of respiratory tract infections in cystic fibrosis, and prosthetic joint infections. In addition, I will discuss the use of microcalorimetry in combination with disease-specific media to improve diagnosis of biofilm-related infections.

Invited talk: *Pseudomonas aeruginosa* increases the susceptibility of *Candida albicans* to amphotericin B in dual species biofilms

Farhana Alam[1], Sarah Blackburn[2], Jack Davis[2], keely Massar[2], Hung-Ji Tsai[1], Jessica Blair[3], Rebecca Hall[2]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, United Kingdom. [2]Division of Natual Sciences, School of Biosciences, University of Kent, Canterbury, United Kingdom. [3]Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom

Abstract

Biofilms are the leading cause of nosocomial infections and are hard to eradicate due to their inherent antimicrobial resistance. *Candida albicans* is the leading cause of nosocomial fungal infections and is frequently co-isolated with the bacterium *Pseudomonas aeruginosa* from biofilms in the Cystic Fibrosis lung and severe burn wounds. The presence of *C. albicans* in multi-species biofilms is associated with enhanced antibacterial resistance, which is largely mediated through fungal extracellular carbohydrates sequestering the antibiotics. However, significantly less is known regarding the impact of polymicrobial biofilms on antifungal resistance.Here we show that, in dual species biofilms, *P. aeruginosa* enhances the susceptibility of *C. albicans* to amphotericin B, an effect that was biofilm specific. Transcriptional analysis combined with gene ontology enrichment analysis identified several *C. albicans* processes associated with oxidative stress to be differentially regulated in dual species biofilms, suggesting that *P. aeruginosa* exerts oxidative stress on *C. albicans*, likely through the secretion of phenazines. However, the mitochondrial superoxide dismutase *SOD2* was significantly downregulated in the presence of *P. aeruginosa*. Mono-species biofilms of the *sod2*D mutant were more susceptible to amphotericin B, and the susceptibility of these biofilms was further enhanced by exogenous phenazines. We propose that in dual species biofilms, *P. aeruginosa* simultaneously induces mitochondrial oxidative stress, whilst downregulating key detoxification enzymes, which prevent *C. albicans* mounting an appropriate oxidative stress response to amphotericin B, leading to fungal cell death. This work highlights the importance of understanding the impact of polymicrobial interactions on antimicrobial susceptibility.

Invited talk: Managing biofilms by disrupting bacterial signalling mechanisms

Miguel Camara ORCID iD

National Biofilms Innovation Centre, Biodiscovery Institute, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

Abstract

The treatment of bacterial biofilm infections has primarily relied on using antibiotics to inhibit crucial biological functions. This has resulted in several issues including the natural resilience of some bacteria to these antimicrobials and the fast emergence of resistance when targeting cell viability. An alternative approach involves the inhibition of virulence without impacting growth and one way to achieve this is through the interference with quorum sensing (QS)-mediated signalling processes.

We have developed QS inhibitors with the ability to target the Pseudomonas Quinolone System as a model system and have shown that they can attenuate virulence and sensitise biofilms to the action of antibiotics. However, we have encountered some challenges when using these inhibitors such as strain differences, growth conditions and biofilm penetration issues that we have been addressing and will be discussed in this presentation. Despite these challenges, interference with QS-mediated signalling processes remains a promising strategy to manage biofilms.

Invited talk: Insights into Mechanisms of Staphylococcus aureus Biofilm Formation and Prevention

Joan Geoghegan

University of Birmingham, Birmingham, United Kingdom

Abstract

Staphylococcus aureus frequently adopts a biofilm phenotype in host tissues and on indwelling medical devices. In the biofilm state, bacteria can resist immune and physical clearance mechanisms leading to persistent, difficult to treat infections. Our understanding of the molecular interactions underlying biofilm development in staphylococci has advanced remarkably over the past fifteen years. Surface-located cell wall anchored (CWA) proteins tether *S. aureus* to host tissues or to indwelling medical devices that become conditioned with blood plasma proteins. The cell to cell interactions that occur during biofilm accumulation in *S. aureus* rely either on the production of a matrix of poly-N-acetyl-β-(1–6)-glucosamine or on CWA proteins forming interactions with identical partner proteins on a different cell. Additionally, CWA proteins can promote biofilm accumulation through their interactions with soluble host-derived factors such as fibrinogen. Preventing the formation of or disrupting *S. aureus* biofilms is an attractive strategy to control infection. This talk will focus on recent insights into biofilm development in *S. aureus* and our research on developing methods to prevent and disrupt biofilm formation.

Invited talk: Beneficial biofilms for bioremediation of persistent organic pollutants

Birthe Kjellerup ORCID iD

University of Maryland, College Park, USA

Abstract

Biofilms are often looked at as a nuisance or they are detrimental due to the harm that these complex communities can inflict on engineered systems in the environment. However, biofilms have a long and evolutionary advantage that we can benefit from instead of trying to defeat them. Examples of this are applications of biofilms for recycling of nutrients and bioremediation of contaminants in wastewater, soil and sediments. In this presentation, examples of beneficial biofilms for bioremediation of persistent organic pollutants such as polychlorinated biphenyls (PCBs) in sediments and chlorinated solvents in groundwater will be discussed. The removal of these pollutants is a priority because of their ability to enter the food chain and due to their toxicity thus impacting public health. Activated carbon (AC) and other sorptive substrates can adsorb PCBs from sediments and simultaneously function as substratum for co-localizing PCB-degrading bacteria in biofilms. The AC-biofilm particles can then be utilized as a delivery system to address PCB contamination. The direction of biofilm formation on sorptive materials has become an efficient way for bioremediation in contaminated environments.

Invited talk: Bacteriophage-biofilm dynamics in *Pseudomonas aeruginosa*

Jeremy Webb ORCID iD

National Biofilms Innovation Centre (NBIC), Southampton, United Kingdom. University of Southampton, Southampton, United Kingdom

Abstract

There is increasing recognition that bacteriophage, as specialised viruses of bacteria, can influence biofilm development, dynamics and function and that this in turn can impact human health and disease states. We have identified and studied filmentous bacteriophage, which have a single-stranded DNA genome, and shown that they can promote bacterial aggregation, biofilm formation and chronic infections. We have also studied the dynamics of how filamentous phage contribute to the extracellular DNA (eDNA) within the biofilm matrix of *Pseudomonas aeruginosa*, a critical component influencing biofilm stability and antimicrobial resistance. By employing long-read DNA sequencing techniques, we have examined the composition of eDNA, revealing a significant overlap with host genomic DNA, but with a marked enrichment of sequences related to (but not always entirely homologous to) filamentous phage. These findings provide new insight into eDNA origins within biofilms, and suggest the novel possibility of biofilm reinforcement through phage-derived eDNA. This research not only expands our understanding of biofilm structural dynamics but also underscores the potential of phage-based interventions in combating biofilm-related diseases or for controlling or engineering biofilm function.

Sticking together: Evolution of multiple biofilm mechanisms in isolates of Non-aureus Staphylococci causing Prosthetic Joint Infection

Lisa Crossman[1,2,3], Leanne Sims[3], Rachael Dean[4], Heather Felgate ORCID iD[3], Iain McNamara[4], Teresa Diaz-Calvo ORCID iD[3], Claire Hill[3], Mark Webber ORCID iD[3], John Wain ORCID iD[3]

[1]SequenceAnalysis.co.uk, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Quadram Institute Bioscience, Norwich, United Kingdom. [4]Norfolk and Norwich University Hospital, Norwich, United Kingdom

Abstract

Non aureus Staphylococci (NAS) are a known important cause of prosthetic joint infection (PJI), however, there are difficulties ascribing pathogenicity to members of the genus since they are avid skin commensals and contaminants. Biofilm is a known pathogenic trait in PJI and may be an enabling factor in infection. Although biofilm has been relatively extensively studied in *S. aureus*, far less is known about how biofilms are formed and maintained amongst the several different species of the genus generally termed coagulase negative staphylocci (CoNS).

In this study, we characterised a large clinical collection of different species of NAS using whole genome sequencing and biofilm assays. Using statistical analyses, we showed that the ability to form biofilm can be mediated by alternative mechanisms. We built a machine learning model to identify sequences of interest between different species and capabilities of biofilm formation. Biomarker sequences classified by the ML model suggested sequences to distinguish biofilm capability in different genomes as indicated experimentally by RT-qPCR across strains grown in alternative culture conditions.

Taken together, these data indicate that biofilm formation amongst the NAS is complex and has arisen multiple times. Further investigations are needed to fully elucidate the different mechanisms employed and to ascertain the importance of biofilm in PJI itself.

Conditional evolution and novel mechanism of biocide tolerance in two nosocomial Gram-positive pathogens

Maria Solsona Gaya ORCID iD[1,2], Heather Felgate ORCID iD[1,2], Harsha Siani ORCID iD[3], Cynthia B. Whitchurch ORCID iD[1,4], Mark A. Webber ORCID iD[1,2]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [3]GAMA Healthcare Ltd., Hemel Hempstead, United Kingdom. [4]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom

Abstract

Biocides are critical in the control and prevention of healthcare-associated infections. The emergence of bacterial tolerance/resistance to biocides and cross-resistance to antibiotics is therefore a potential concern and understanding how nosocomial pathogens respond to biocidal agents will inform infection prevention and control.

We used a biofilm evolution model and RNAseq to study how *Staphylococcus aureus* and *Enterococcus faecalis* responded to challenge with two important biocides. Biofilm and planktonic lineages were exposed to sub-lethal concentrations of Chlorhexidine digluconate (CHG) and Octenidine dihydrochloride (OCT) in parallel for ≈250 generations.

Both pathogens could adapt to both biocides with planktonic lineages able to survive higher concentrations of CHG and OCT before growth was inhibited. Evolved isolates had no major fitness deficit and low-level changes to susceptibility to various other antimicrobials were observed after biocide exposure.

Sequencing of *S. aureus* mutants from independent lineages identified repeated loss of function changes within fatty acid kinase (*fakA*) after exposure to both biocides in all conditions. Analogous changes were observed within the homologous gene in parallel experiments with *E. faecalis*. Significantly lower ethidium bromide accumulation was observed in *fakA* mutants when compared to the WT, suggesting differences in membrane permeability and fluidity. TEM revealed differences in the cell wall between the WT and the evolved isolates and RNAseq identified multiple pathways involved in envelope homeostasis after biocide exposure. This work strongly suggests a novel role in biocide tolerance for FakA and current work is studying the mechanistic basis for this and relationship to phospholipid production.

Activity of urinary extracellular vesicles against *E. coli* biofilms

Ashraf Zarkan ORCID iD, Janusha Jeevananthan, Felix Craig-McFeely, Timothy Williams

University of Cambridge, Cambridge, United Kingdom

Abstract

Urinary Tract Infections (UTIs) are the most common bacterial infections and up to 80% of infections are caused by *E. coli*. Repeated infection (recurrent UTIs) is common and linked to the formation of biofilms. Urinary extracellular vesicles (uEVs) are small 'packages' released from all cells in the urinary tract. uEVs have been previously shown to exert antimicrobial activity against *E. coli,* but their effect on *E. coli* biofilms is less understood. To investigate the activity of uEVs against *E. coli* biofilms, uEVs were isolated from human urine by ultracentrifugation, characterised by transmission electron microscopy and proteomic analysis, and quantified by nanoparticle tracking analysis. Predetermined quantities of uEVs were then incubated with *E. coli* biofilms, and biofilm inhibition was monitored. We show that uEVs are capable of inhibiting *E. coli* biofilms in a dose-dependant manner, thereby highlighting the importance of uEVs in combatting biofilm-associated UTIs caused by *E. coli*.

Haemoglobin is a potent driver of biofilm formation and virulence in *Candida albicans*

Ed Deshmukh-Reeves, Campbell Gourlay

University of Kent, Canterbury, United Kingdom

Abstract

C. albicans is the most common human fungal pathogen and the causative agent of candidiasis. Systemic candidiasis is associated with very poor patient outcomes, with mortality rates reported between 30-60%. Antifungal drugs are often ineffective in treating these infections despite susceptibility testing showing efficacy *in vitro.* This is due to *C. albicans* ability to form biofilms, creating a barrier of extracellular matrix to prevent the penetration of antifungal agents. Here we show that haemoglobin released from blood is a potent driver of biofilm formation. Using confocal microscopy we demonstrate that *C. albicans* is able to adhere to and invade extracellular clusters of haemoglobin which are then stitched together rapidly to form a comprehensive biofilm. Using a *G. mellonella* infection model we also show that haemoglobin can significantly increase virulence in *C. albicans* further highlighting its importance in candidiasis cases.

Our data suggest an evolved mechanism by which *C. albicans* utilises haemoglobin released from lysed blood cells for structural purposes, rather than as an iron source, to evade environmental stresses and persist in the host. Based on our findings we investigate the disruption of aggregated and deposited proteins as a mechanism of biofilm control that does not rely on the use of traditional antimicrobials.

Screening combinations of antibiotics and matrix degrading enzymes using an *in-vitro* biofilm model for ventilator associated pneumonia

Dean Walsh[1], Trevor Lithgow[2], Ana Traven[2], Freya Harrison[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Monash University, Melbourne, Australia

Abstract

Numerous pathogens are capable of causing ventilator associated pneumonia (VAP), including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*. Currently there is a lack of accurate *in-vitro* models of the VAP environment. This greatly limits our understanding of how the VAP environment alters pathogen physiology and the efficacy of VAP therapies. Here, we showcase a reproducible model that simulates biofilm formation in the VAP environment.

Defined as a pneumonia occurring after more than 48 hours of mechanical ventilation via an endotracheal tube (ETT), VAP results from biofilm forming on the ETT and seeding the lower airways with pathogenic microbes. The prevalence of VAP varies between 9-65% with mortality rates as high as 76%. In our biofilm model each of the critical pathogens are grown on ETT segments in the presence of a novel synthetic airway surface liquid (ASL) growth medium to simulate the VAP environment. Using matrix-degrading enzymes, confocal microscopy, and cryo-SEM we were able to determine that the VAP environment greatly alters biofilm matrix composition and structure of VAP pathogens compared to standard laboratory growth medium. Furthermore, when grown in our model, the biofilms of VAP pathogens required very high concentrations of antimicrobials to eradicate, if they could be eradicated at all. However, combining matrix-degrading enzymes with antimicrobials greatly improved biofilm eradication of all VAP pathogens. Our model can not only inform on fundamental microbiology in the VAP environment, but also act as a screening platform for promising antibiofilm therapies such as the use of matrix-degrading enzymes.

A New Channel for Biofilm Treatment: Exploring the Potential of Biofilm Transport Channels for Drug Delivery

Liam Rooney ORCID iD[1], Beatrice Bottura[1], Lindsey Florek[2], Ross Martin[1], Paul Hoskisson[1], Lars Dietrich[2], Gail McConnell[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]Columbia University, New York City, USA

Abstract

The socioclinical burden of biofilms demands new prevention, control, and treatment methods to improve remediation efforts. New routes of drug delivery are one avenue that shows promise for the development of new anti-biofilm therapies. However, new routes for drug delivery necessitate new understandings of biofilm physiology. We developed a suite of cross-scale imaging methods to identify and investigate nutrient transport channels in mature *Escherichia coli* biofilms. These channels permeate the entire biofilm, have a mean diameter of 15 µm, exhibit differing fractality and structure depending on nutrient availability and physical environment, are scaffolded by a proteinaceous matrix and, most importantly, facilitate the uptake of small particles from the external milieu. These factors position biofilm transport channels as excellent candidates for targeted drug delivery to improve biofilm eradication. We demonstrate a multi-faceted approach to understanding the chemical microenvironment of these channels, specifically the oxygen profile which contributes to antimicrobial degradation. We combine microscopic fluorescent nanosensing, electrochemical profiling, and biosensing to measure the oxygen content of biofilm transport channels. In turn, these data inform the design of delivery methods for channel-targeted antimicrobial therapies. Moreover, we show the exploitation of biofilm transport channels for drug uptake using fluorescent antibiotic analogues and fluorescent viability assays to visualise antimicrobial activity in live biofilms at unprecedented scales. Our findings describe the chemical microenvironment of biofilm transport channels and demonstrate a feasible new delivery method for improved penetration of antibiotics.

Deciphering microbial dynamics in a Ready-to-Eat Food production facility: Insights into Listeria monocytogenes persistence

Maria Diaz ORCID iD[1], Heather Aird[2], Thanh Le Viet[1], Ana Victoria Gutierrez[3], Oleksii Omelchenko[1], Nasmille Larke-Mejía[1], Lluis Moragues-Solanas[1], Joachim Fritscher[1], Nicolle Som[1], Falk Hildebrand[1], Jim McLauchlin[1], Frieda Jørgensen[4], Mathew Gilmour[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]UK Health Security Agency, Food, Water and Environmental Laboratories, York, United Kingdom. [3]Quadram Insitute Bioscience, Norwich, United Kingdom. [4]UK Health Security Agency, Food, Water and Environmental Laboratories, London, United Kingdom

Abstract

Listeria monocytogenes is a foodborne pathogen that poses significant concern for the food industry due to its ability to persist through food safety control efforts such as cleaning and disinfection. An understanding of a more comprehensive set of factors that contribute to its survival, including the resident microbiota coexisting in relevant environments, is critical for preventing and eradicating this pathogen.

We used metagenomics to track the resident microbiota on non-food contact surfaces in a facility producing ready-to-eat foods, characterized by persistent contamination with a CC121 *L. monocytogenes* strain. Swabs were collected during regular operations and before and after cleaning procedures in two areas (production and preparation) within the high-care zone of the factory.

Despite cleaning efforts, the microbiota exhibited a consistent composition, dominated by *Pseudomonas fluorescens*. While shared populations were observed between production and preparation areas, differential members were identified in both areas, such as an increased relative abundance of *Sphingomonas aerolata* in the production zone. Notably, *Listeria* spp. eluded metagenomic detection but were confirmed using the standard culture method, suggesting that *Listeria* spp. existed at very low abundance.

Over the sampling period, a stable resident microbiota was observed on non-contact surfaces, indicating adaptation to cleaning procedures. *Listeria* spp., albeit at low abundance, were part of this community, likely benefiting from the overall microbial mutualism under stresses imposed in environmental conditions. Understanding the interactions of microbial communities in food processing environments is crucial for devising effective control strategies against persistent pathogens like *L. monocytogenes*.

Reactive Oxygen®- An Antimicrobial System for the Prevention and Management of Biofilm Infection

Eleanor Hill[1], Raymond Allan[2], Zakia Alhareth[2], Charlotte Hind[3], Mark Sutton[3], Nicole Wood[4], Riina Rautemaa-Richardson[4], Jeremy Crane[5], Joanne James[1], Thomas Hall ORCID iD[1]

[1]Matoke Holdings Limited, Abingdon, United Kingdom. [2]De Montfort University, Leicester, United Kingdom. [3]United Kingdom Health Security Agency, Salisbury, United Kingdom. [4]University of Manchester, Manchester, United Kingdom. [5]Hammersmith Hospital Imperial College Healthcare NHS Trust, London, United Kingdom

Abstract

Antimicrobial resistance in biofilm communities contribute to chronic infections, requiring the development of novel biofilm-targeting antimicrobials. Reactive oxygen species (ROS) broad-spectrum activity disrupts critical pathogen pathways, including biofilm formation. RO® technology utilises stabilised enzymes to enable the sustained release of antimicrobial concentrations of ROS *in-situ*, at the target site.

RO® products were tested against wound organisms in planktonic and biofilm state. Time-kill curves were performed using a modified Miles-Misra assay to determine the cell counts. Single and dual species biofilms were established over a 48-hour period before treatment with RO®. Biofilm viability and biomass were established by colony enumeration methodology and crystal violet staining respectively. Biofilms were imaged with a Zeiss-LSM900 confocal microscope. Fungal susceptibility testing followed the EUCAST_E.DEF_7.3.2 method.

Rapid bactericidal kill was achieved within 4, 6 and 24-hours of RO® treated clinical isolates of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Acinetobacter baumannii* respectively. No recovery of viable organisms occurred after 24-hours, suggesting the absence of resistant bacteria. RO® treated biofilms exhibited a decrease in cell viability of 3-log, 4-log and 6-log for *S. aureus*, *P. aeruginosa* and *A. baumannii* respectively. *P. aeruginosa* and *S. aureus* isolates demonstrated a decrease in biofilm biomass. Additionally, RO® technology is effective against Candida species of fungi, demonstrating complete inhibition of growth in all species tested.

RO® demonstrates strong antimicrobial activity against WHO and ESKAPE pathogens. Biofilm disruption with RO® technology is associated with microbial death and biofilm clearing, providing a novel method to manage infection and could aid in reducing the global antibiotic burden.

Isolation and Characterisation of Targeted Phage Cocktail for Phage Therapy of Biofilm Associated Prosthetic Joint Infections.

Nathan Burton ORCID iD[1], Luís Daniel Rodrigues Melo ORCID iD[2], Bethany Pearce[1], Michaël D Tadesse[1], Evangelos Vryonis[3], Antonia Sagona[1]

[1]University of Warwick, Coventry, United Kingdom. [2]University of Minho, Braga, Portugal. [3]University Hospital Coventry, Coventry, United Kingdom

Abstract

Prosthetic joint infections are a devastating complication post joint arthroplasties, which without effective management leads to limb amputation and/or death. A significant proportion of Prosthetic joint infections is caused by the primarily commensal Coagulase negative Staphylococci family of bacteria, which result in thick highly antibiotic resistant biofilms at the site of infection. Combinatorial therapy of antibiotics and bacteriophages may represent a strategy to overcome resistance with previous research suggesting that when resistance develops for antibiotics, susceptibility to bacteriophages increases. Here, we isolate a novel phage cocktail and assess the viability of this phage cocktail therapy to eradicate nosocomial Staphylococcal biofilms. Using clinical isolates isolated from prosthetic joint infections, supplied by University Hospitals Coventry and Warwickshire, we isolate four new bacteriophages towards these difficult-to-treat pathogens from sewage effluent for the intention of treating infections, caused by these organisms. We characterize these new bacteriophages by electron microscopy and sequencing and have assessed whether combinatorial therapy of antibiotics and bacteriophages is more effective than currently applied antibiotic only therapies. We also estimate the safety of our phage cocktail to in vitro to human epithelial cells when used as an adjuvant to antibiotic treatments, finding our phage cocktail to be a potentially safe and effect adjuvant to be used alongside antibiotics such as vancomycin, flucloxacillin and rifampicin when treating prosthetic joint infections.

Endodontic Infections: A Comprehensive Exploration from Patient Samples to Biofilm Models via Metagenomics, Culture Analysis, and Treatment Assessment

Rania Nassar[1,2], Mohannad Nassar[3], Lobna Mohamed[1], Abiola Senok[1,2], David Williams[2]

[1]College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai Health, Dubai, UAE. [2]School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom. [3]Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, UAE

Abstract

Endodontic infections are biofilm-mediated with a polymicrobial nature, and its composition determines pathogenesis and associated host-responses. Studies from different regions of the world have investigated microbiota of such infections, yet none have employed shotgun metagenomic sequencing nor have they been done for patients in the Arabian Gulf region. This study therefore addressed these research gaps. Further, this research sought to address another gap in endodontics which lies in the lack of biofilm models that replicate microbiome found *in vivo* in such infections. To this end, patients' samples were used in their natural state to develop biofilms on hydroxyapatite coupons. The developed model was then employed to investigate the antibiofilm effectiveness of a potential endodontic irrigant, namely phytic acid (IP6). Samples from root canals of necrotic pulp were collected (n=32). Shotgun metagenomic sequencing coupled with culturomics analysis were used to characterise collected samples and developed biofilms and to evaluate the models' reproducibility. Assessment of IP6's antibiofilm activity against developed biofilms was based on confocal microscopy and recovered colony forming units. The analysed microbiota showed high bacterial diversity with no significant differences between symptomatic and asymptomatic cohorts. Resistome, virulome, and functional analysis revealed no distinct cohort signatures. Analysis showed polymicrobial-nature of generated biofilms that maintained substantial reproducibility in their architectural characteristics. Additionally, IP6 exhibited antibiofilm action against developed biofilms. Using metagenomics sequencing complemented with culturomics analysis, this research addressed the knowledge gap concerning endodontic infections. The developed biofilm models reflect the polymicrobial-nature of these infections with maintained reproducibility hence it could be exploited in antibacterial assessment assays.

Revolutionising Meat Packaging: Exploring the Microbial Implications of a Reusable Solution

Alison Jane Reid ORCID iD, Yvonne Cortese, Romina Pezzoli, Andy Fogarty, Declan Devine

Technological University of the Shannon: Midlands Midwest, Athlone, Ireland

Abstract

Packaging is crucial in the food industry; however, this packaging increases the overall environmental impact of the entire food industry. The mass majority of the food packaging placed on the Irish market is single-use with only 1% of packaging being reusable. To reduce the environmental impact of food packaging, there is increasing interest in sustainable alternatives, including reusable packaging. While reusable packaging use is increasing in various industries across Europe, it is currently non-existent in the context of fresh meat packaging.

The aim of this study is to investigate the effects of washing and reuse on the surface characteristics of reusable plastic trays and their microbial colonisation. To do this, plastic materials were repeatedly weathered at high-temperatures to determine the impact of washing on the surface properties of polymers. Using food pathogens, *E. coli* and *B. cereus,* the level of bacterial adhesion could be characterised through biofilm formation assays. This study demonstrated that the level of bacterial adhesion was not influenced by the changes in material or the adverse effects of weathering. Biofilm formation indicated that viable bacteria were growing on the surface but there were no notable changes after weathering. A method will be developed to determine the impact of shelf life, reuse and novel decontamination strategies on the physical properties of plastic materials and how these surface changes affect microbial growth trends.

As a result, this study's goal is to guide the design of novel reusable meat packaging by identifying microbial critical control points.

In vitro modelling of polymicrobial infections of the catheterised urinary tract shows the effectiveness of the novel efflux pump inhibitor thioridazine against P*roteus mirabilis*

Isobel Garratt[1], Maryam Yazdizadeh Ravari[1], Ocean Clarke[1,2], Matthew Wand[3], Mark Sutton[3], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]UKHSA, Salisbury, United Kingdom

Abstract

Proteus mirabilis crystalline biofilm formation frequently blocks catheters, complicating patient care. Previous studies have demonstrated the potential for existing licensed drugs to inhibit crystalline biofilm formation through inhibition of the bcr/CflA efflux system. However, catheter associated urinary tract infections (CAUTI) are typically polymicrobial and it is unclear if these approaches are effective against *P. mirabilis* in these communities.

This study describes the development of a reproducible *in vitro* model of polymicrobial CAUTIs, and its application evaluating the impact of the efflux pump inhibitor thioridazine on catheter blockage. This model provides a reproducible community of common uropathogens: *P. mirabilis, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus*. Selective medias were developed and validated to facilitate specific enumeration of community members. In a polymicrobial community, *P. mirabilis* crystalline biofilm catheter blockage was increased 1.45-fold (versus models with *P. mirabilis* alone). We show polymicrobial communities reproducibly modelled using *in vitro* bladder models and stable for at least 72 hours. The application of thioridazine to *P. mirabilis* models significantly extends the time taken for catheters to block (2.4 fold increase) and is equally effective in monoculture and polymicrobial community experiments without affecting population dynamics in polymicrobial models. The polymicrobial CAUTI model provides a valuable tool for both basic and applied research in this area. This model facilitates more robust pre-clinical evaluation of approaches to control catheter blockage and biofilm formation, as well as helping to address more fundamental questions relating to evolution of antimicrobial resistance and other important traits in these pathogens.

Uncovering the role of second messenger signalling in *Acinetobacter baumannii* virulence

Lyuboslava Harkova, Rubén de Dios ORCID iD, Ronan McCarthy ORCID iD

Brunel University London, Uxbridge, United Kingdom

Abstract

Second messengers signalling systems play crucial role in bacterial capability to respond to environmental changes by mediating alternations in transcription, translation and enzyme activity. Second messengers are central regulators of various aspects of bacterial life including metabolism, pathogenicity, sessility and cell morphology. Indeed, these molecules are widely recognised mediators of virulence and antibiotic tolerance in well-studied pathogens like *Pseudomonas aeruginosa* where over 40 enzymes contain domains with the predicted potential to influence second messenger levels. The multidrug resistant nosocomial pathogen *Acinetobacter baumannii* has become increasingly prevalent over the last 20 years with carbapenem strains surpassing *P. aeruginosa* to become the World Health Organisation top priority pathogen. Despite this, relatively little is known about the role of second messengers in *A. baumannii* pathogenicity. In this work, we used high throughput screening of the *A. baumannii* AB5075 transposon mutant library to identify novel regulators of biofilm formation. Amongst the hits were genes predicted to influence second messenger levels. We further characterised the candidate with the strongest biofilm phenotype and uncovered a range of other phenotypes such as motility, exopolysaccharide production, virulence, antibiotic resistance and link with other signalling systems. RNA-Seq analysis of a clean deletion mutant compared to a complemented strain showed that operons and genes linked to the observed phenotypes such as *pgaABCD*, *csuA/BABCDE* and type IV pili genes were differentially expressed. Overall, we demonstrate the important role of second messenger signalling in *A. baumannii* and confirm that it modulates key phenotypes linked to virulence and antimicrobials resistance.

Celebration of Virology

Invited talk: Evolution of enhanced innate immune escape by SARS-CoV-2

Lucy Thorne[1,2], Ann-Kathrin Reuschl[2], Mehdi Bouhaddou[3], Ben Polaco[4], Matthew Whelan[2], Wilhelm Furnon[5], Vanessa Cowton[5], Andrew Davidson[6], Arvind Patel[5], Massimo Palmarini[5], Lorena Zuliani-Alvarez[4], Nevan Krogan[4], Clare Jolly[2], Greg Towers[2]

[1]Imperial College London, London, United Kingdom. [2]UCL, London, United Kingdom. [3]UCLA, Los Angeles, USA. [4]Quantitative Biosciences Institute (QBI), UCSF, San Francisco, USA. [5]CVR, Glasgow, United Kingdom. [6]University of Bristol, Bristol, United Kingdom

Abstract

Viruses are emerging at an increasing rate with climate change. A key question in preparedness is what gives a virus pandemic potential? The innate immune system presents a universal barrier to viral emergence and transmission. All viruses must evolve to manage this frontline defence, making it a powerful lens through which to understand what makes a pandemic virus special. We have followed evolution of SARS-CoV-2-host interactions in real-time. We first discovered that, despite recently emerging, wave 1 (W1) SARS-CoV-2 isolates could effectively manipulate the innate immune system in human airway epithelial cells. Emergence of variants-of-concern (VOCs) reflects selective pressure to adapt to a new host and escape immune defences for enhanced transmission. To understand the selective forces driving SARS-CoV-2 evolution, we coupled molecular virology with global proteomics and transcriptomics to compare VOC replication and host responses to W1. We discovered that VOCs Alpha to Delta, and recent Omicron subvariants independently evolved enhanced innate immune suppression. Strikingly, we found convergent VOC evolution to upregulate key innate immune antagonists ORF6, ORF9b, nucleocapsid, and a novel protein N*. We mapped the underlying genetic changes to regulatory regions governing subgenomic RNA synthesis and translation. We discovered that N* interacts with the transcription regulatory complex PAF1C, involved in innate immune regulation, suggesting VOCs have evolved a novel protein function to further manipulate the host transcriptional response. Altogether our findings implicate the innate immune system as a key selective force during transmission, and lay the groundwork to further understand mechanisms of pandemic virus emergence and evolution.

Invited talk: Influenza virus RNA polymerase – lessons in multifunctionality

Ervin Fodor ORCID iD

University of Oxford, Oxford, United Kingdom

Abstract

Influenza viruses contain a segmented negative-sense RNA genome. Each viral RNA (vRNA) segment assembles with viral RNA-dependent RNA polymerase and oligomeric nucleoprotein (NP) into a viral ribonucleoprotein (vRNP) complex. The viral RNA polymerase is responsible for both transcribing and replicating the vRNA segments in the context of vRNPs in the nucleus of the infected cell. We employ an interdisciplinary approach to dissect the structure and function of the viral polymerase, aiming to understand how it operates as both a transcriptase and a replicase, and how it participates in the nuclear export of the viral genome. To function as a transcriptase, the viral polymerase binds to the C-terminal domain (CTD) of host RNA polymerase II (Pol II) to produce capped and polyadenylated viral mRNA. As a replicase, the viral polymerase associates with a second viral polymerase bound to host acid nuclear phosphoprotein 32 (ANP32), forming a replication platform for the co-replicational assembly of the nascent viral RNA into an RNP complex. Late in infection, vRNPs are exported from the nucleus for assembly into virions at the cell membrane, with the viral nuclear export protein (NEP) mediating vRNP association with the host nuclear export protein Exportin 1. Our data show that the binding sites of Pol II CTD, ANP32 and NEP on the surface of the viral polymerase overlap, supporting a model in which the relative abundance and affinities of these host and viral factors temporally regulate the polymerase switch between transcription, replication, and nuclear export throughout infection.

Invited talk: Unravelling the mechanisms of virus-driven cellular transformation

Andrew Macdonald ORCID iD

University of Leeds, Leeds, United Kingdom

Abstract

Human papillomaviruses (HPVs) have evolved to parasitise the epithelial niches of their hosts to a remarkable extent and have effectively synchronised their replication cycle to the differentiation events of the infected keratinocyte. As a widespread pathogen, infection with HPVs is associated with significant global mortality. Sadly, HPVs are also associated with approximately 5% of all human cancers, which often arise because of the manipulation of host cell signalling processes by the virus. Understanding which cellular factors, pathways and processes are targeted by HPV increases our knowledge of the virus lifecycle and disease progression. As a master manipulator of the cell, HPV also serves as an excellent tool to uncover fundamental biological mechanisms and can be used to aid in our understanding of carcinogenesis in its broadest sense. In this seminar I will provide an overview of the key pathways associated with HPV-mediated transformation and highlight how our studies into HPV-associated disease has revealed new oncogenes, crucial in a range of highly aggressive human cancers.

Invited talk: Respiratory syncytial virus and human airway epithelium interactions – keys to understanding RSV pathogenesis.

Ultan Power

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom

Abstract

Many human respiratory viruses, like respiratory syncytial virus (RSV), target the airway epithelium as the principal site of infection and replication. Indeed, productive infection is largely restricted to apical ciliated cells, although other cells, such as goblet cells, pneumocytes, dendritic cells, macrophages, and neurons, may also be infected. Therefore, exploring the consequences of respiratory virus infection of human airway epithelium is critical to understanding their pathogenesis. We and others developed in-vitro/ex-vivo models of RSV infection based on well-differentiated primary human airway epithelial cell (WD-PAEC) cultures. We found that RSV infection of WD-PAECs replicated several hall marks of RSV infection *in vivo*, including infection restricted to apical ciliated cells, apical cell sloughing but limited cytopathogenesis, virus growth kinetics, and cytokine/chemokine responses. Importantly, RSV infection of WD-PAECs derived from nasal or bronchial brushes resulted in similar outcomes, indicating the suitability of nasal epithelium as surrogates for lower lung infections. As nasal epithelium is much more accessible, we used WD-PAECs derived from nasal brushes to explore the consequences of RSV infection of airway epithelium derived from cohorts of infants with histories of severe or mild RSV disease, newborns vs 1 year olds, and wheezers vs healthy children. Data from these studies will be presented and general concepts derived therefrom relating to RSV pathogenesis discussed.

Invited talk: Research investigation into the outbreak of unexplained non A-E hepatitis in Scottish Children

Antonia Ho ORCID iD[1], Richard Orton ORCID iD[1], Rachel Tayler[2], Patawee Asamaphan ORCID iD[1], Vanessa Herder ORCID iD[1], Chris Davis ORCID iD[1], Lily Tong[1], Katherine Smollett ORCID iD[1], Sarah McDonald ORCID iD[1], Louisa Pollock[2], Jim McMenamin[3], Kirsty Roy[3], Kimberly Marsh[3], Samantha Shepherd[4], Celia Jackson[4], Paul Henderson ORCID iD[5], Miranda Odam[6], Michael Levin[7], Massimo Palmarini ORCID iD[1], Surajit Ray ORCID iD[8], David Robertson ORCID iD[1], Ana da Silva Filipe ORCID iD[1], Brian Willett ORCID iD[1], Judith Breuer ORCID iD[9], Malcolm Semple ORCID iD[10], David Turner[11], Kenneth Baillie ORCID iD[6], Emma Thomson ORCID iD[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]Department of Paediatrics, Royal Hospital for Children, Glasgow, United Kingdom. [3]Public Health Scotland, Glasgow, United Kingdom. [4]West of Scotland Specialist Virology Centre, Glasgow, United Kingdom. [5]Child Life and Health, University of Edinburgh, Edinburgh, United Kingdom. [6]Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [7]Department of Infectious Disease, Imperial College London, London, United Kingdom. [8]School of Mathematics and Statistics, University of Glasgow, Glasgow, United Kingdom. [9]Division of Infection and Immunity, University College London, London, United Kingdom. [10]University of Liverpool, Liverpool, United Kingdom. [11]Scottish National Blood Transfusion Service, Edinburgh, United Kingdom

Abstract

Background. An outbreak of acute hepatitis of unknown aetiology in children was reported in Scotland in April 2022, and was subsequently been identified in 35 countries. Several studies have suggested an association with human adenovirus (HAdV), a virus not commonly associated with hepatitis.

Methods. In collaboration with Public Health Scotland, we performed a detailed case-control investigation of 32 cases recruited to the International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) WHO Clinical Characterisation Protocol UK (CCP-UK) and 74 control subjects. Next-generation sequencing (NGS), reverse transcription-polymerase chain reaction (RT-PCR), serology, and in situ hybridisation (ISH) were performed on clinical samples obtained from the cases and controls. Additionally, Human Leucocyte Antigen (HLA) typing was performed to identify associated host genetic factors.

Results. NGS performed on clinical samples from the early cases identified adeno-associated virus (AAV2) in 9/9 plasma and 4/4 liver samples, but not in sera/plasma of age-matched healthy controls or children with HAdV infection and normal liver function. Subsequently, 26/32 (81%) hepatitis cases versus 5/74 (7%) of controls were AAV2 RT-PCR positive. Using ISH, AAV2 was detected within ballooned hepatocytes alongside a prominent T cell infiltrate in liver biopsies. Furthermore, the HLA class II DRB1*04:01 allele was identified in 25/27 cases (93%), compared with a background frequency of 10/64 (15.6%; $p=5.49x10^{-12}$).

Conclusion. We report an outbreak of acute paediatric hepatitis associated with AAV2 infection, most likely acquired as a coinfection with HAdV which is required as a "helper virus" to support AAV2 replication, and HLA class II-related disease susceptibility.

Invited talk: Controlling the RNA world of a DNA virus

Gill Elliott ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Herpes simplex virus is a large DNA virus that transcribes and replicates its genome in the nucleus, expressing its genes via a classical cascade of expression. The virus encodes over eighty proteins, many of which are accessory proteins that are not essential for virus growth in culture but are important in the host. Over recent years it has become clear that a number of these accessory proteins form a finely balanced network that regulates the infected cell RNA environment and counteracts host responses. At the heart of this network is a protein called virion host shutoff protein or VHS, an endoribonuclease that regulates the translational environment of the infected cell, controls the temporal availability of transcripts and limits double-stranded RNA to inhibit stress responses. We have shown that when expressed in isolation, VHS induces an extreme translational blockade and a profound change to the cellular transcriptome by hijacking the mRNA decay machinery and causing the nuclear retention of mRNA. Intriguingly, viruses mutated for other proteins in the VHS network can similarly affect the virus transcriptome during infection, resulting in highly attenuated viruses that exhibit extreme shutoff. This suggests that these factors are required to counteract VHS activity and work in concert with it to optimise the cellular environment for virus production. These studies now enable us to tease apart not only the virus components of the network around VHS, but also the detail of how VHS interacts with the cellular mRNA decay machinery.

IFIT1 regulation in cells and its implications on antiviral response

Renata Fleith[1], Xin Yun Leong[2], Harriet Mears[3], Jessica Coomber[1], Edward Emmott[4], Daniel Mansur[5], Trevor Sweeney[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]The Francis Crick Institute, London, United Kingdom. [4]University of Liverpool, Liverpool, United Kingdom. [5]Universidade Federal de Santa Catarina, Florianopolis, Brazil

Abstract

Interferon Induced protein with Tetratricopeptide repeats 1 (IFIT1) is an antiviral protein that binds the 5´ends of RNAs lacking 2´-O-methylation of the first and second cap-proximal nucleotides (cap0). By doing this it acts as a sensor distinguishing self (Cap1 or Cap2) from non-self mRNAs, and as an effector molecule preventing the translation of its targets. Besides its direct antiviral effect, IFIT1 can regulate the expression of certain interferon stimulated genes, was shown to inhibit host cell translation, and is involved in other cellular processes such as apoptosis and inflammation. Due to its broad impacts, we hypothesise that cells have evolved mechanisms to modulate the levels of IFIT1 through IFIT3 interaction, that could control not only the antiviral response but also the cellular fate. We and others have shown that the oligomerization with IFIT3 is particularly important to stabilise IFIT1 and enhance its activity in cells. We have further investigated IFIT1 stabilisation mechanisms and found that IFIT3 protects IFIT1 from proteasome-dependent degradation. We present a screen to identify mechanisms of IFIT1 degradation and how these are impacted by IFIT3. By examining how IFIT3 regulates IFIT1 affinity to Cap0 versus Cap1 RNAs and how this impacts virus and host translation we will shed light on aspects of IFIT1-virus co-evolution and the requirement for IFIT1 fine tuning after induction.

Imaging of the intercellular spread of influenza A virus infection *in vitro* and *in vivo* reveals the importance of direct cell-to-cell spread.

Daniel Weir ORCID iD[1], Calum Bentley-Abbot[1], Colin Loney[1], Jack McCowan[2], Edward Roberts[2], Edward Hutchinson[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]CRUK Scotland Institute, Glasgow, United Kingdom

Abstract

Intercellular spread of influenza A viruses (IAVs) can occur by the release of virus particles or by the transfer of viral genomes directly between cells (direct cell-to-cell spread). Infection by extracellular viruses is well-studied, but the importance of direct cell-to-cell spread in IAV replication is unclear. To investigate this, we first established tissue culture models in which we could quantify the frequency of direct cell-to-cell spread. We showed that, even in the presence of drugs that completely inhibit extracellular virus spread, approximately 40% of infected cells are able to infect their neighbours, an effect that was consistent between IAV strains with different virion morphologies. We also showed that, regardless of their morphology, IAV strains induce the production of intercellular membrane connections called tunneling nanotubes (TNTs). Recent studies showed that TNTs can mediate the direct cell-to-cell spread of IAV *in vitro* but, as TNTs are extremely thin and fragile, identifying them *in vivo* is challenging and their relevance for replication within a host is unknown. To assess this, we infected mTmG mice with a reporter IAV allowing us to visualise infected cell membranes within tissues. Using super-resolution confocal imaging of lung sections, we showed that within an infected animal, IAV-infected cells extend TNT-like structures across the respiratory epithelium. Together, these results indicate that direct cell-to-cell spread is an important factor in IAV replication and that TNTs could enable IAVs to spread within an infected host.

Deep tissue oxygen levels reactivate human cytomegalovirus (HCMV) from latency

Elizabeth Elder ORCID iD[1], Ian Groves ORCID iD[2], John Sinclair ORCID iD[3], James Nathan ORCID iD[3], Benjamin Krishna ORCID iD[3]

[1]Public Health Agency of Sweden, Stockholm, Sweden. [2]Cleveland Clinic, Cleveland, USA. [3]University of Cambridge, Cambridge, United Kingdom

Abstract

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus which, after primary infection, establishes lifelong persistent infection, underpinned by viral latency. Latently infected cells maintain viral genomes without virus production and regularly reactivate. This reactivation is a major threat in the immunocompromised, immunonaïve and immunosuppressed, including patients receiving transplants.

The hypoxia-inducible factor (HIF) proteins regulate cellular changes to adjust to low-oxygen environments. The HCMV genome has abundant HIF response elements (HREs) including in the major immediate early promoter/enhancer region (MIEP/E), which controls latency/reactivation. This suggests that HCMV has evolved to reactivate under low oxygen. We cultured latently infected blood monocytes in various oxygen concentrations and observed hypoxia-induced reactivation of HCMV, but we did not see the same phenotype in CD34+ haematopoietic stem cells. CRISPR knockout of hypoxia-inducible factors in myelomonocytic THP-1 cells showed that reactivation requires the HIF-1α-HIF-1β complex but not HIF-2. While activation of the MIEP/E occurs after only 1 hour at 5% oxygen, after days of this treatment, monocytes differentiate into M2 macrophages, allowing full viral reactivation. This combination of rapid MIEP/E activation followed by differentiation allows timely reactivation of HCMV from latent infection.

This mechanism helps rationalise the high rates of reactivation in organ transplants, which often experience hypoxic conditions, as well as potential mechanisms for HCMV reactivation in hypoxic niches in the placenta and kidneys. The work also elucidates HIF-1α activity as a new avenue for antiviral drug development to target reactivation from latency.

TRIM21 effects intracellular neutralisation of incoming viruses via selective autophagy

Anna Albecka-Moreau ORCID iD, Tyler Rhinesmith, Leo James

MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Abstract

Antibodies patrol the extracellular space but access the cytosol during infection - for example after uptake of immune complexes by antigen presenting cells or attached to non-enveloped viruses or bacteria that have escaped from endosomes. Cytosolic antibody complexes are detected by TRIM21, an interferon-inducible ubiquitin E3 ligase and cytosolic antibody receptor, leading to complex ubiquitination and degradation. In the context of viral entry, this results in 'antibody-dependent intracellular neutralisation' wherein ubiquitination and degradation of the virus complex prevents infection. Neutralization is dependent upon 26S proteasome activity, but the mechanism of virion degradation is unknown. We performed a whole-genome CRISPR knock-out screen for cofactors of this process during intracellular neutralisation of human adenovirus serotype 5 (Ad5). We uncovered a requirement for multiple cofactors of selective macroautophagy in this process. Further validation by genetic and pharmacological experiments were consistent with the hypothesis that virions intercepted by TRIM21 are directed into the auto-lysosomal pathway of degradation. Using live cell microscopy, we observe rapid and specific recruitment of TRIM21 to antibody coated virions that enter the cytosol. This is followed by co-recruitment of selective autophagy adaptor proteins, in a process that is dependent on TRIM21 catalytic activity. Interception of incoming virions by TRIM21 prevents virus localisation to the nucleus, consistent with the block to adenovirus infection. Our study reveals a novel way in which intracellular catabolic processes link humoral and cell-mediated immunity.

A complex of three HSV-1 proteins antagonises cellular antiviral mechanisms by recruiting a cellular E3 ubiquitin ligase

Navneet Boparan[1], Marianne Perera[1], Jo Kite[2], Michael Weekes[2], Stephen Graham[1], Colin Crump[1]

[1]Division of Virology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom

Abstract

Herpes simplex virus-1 (HSV-1) is a double stranded DNA virus that is a highly prevalent human pathogen. Commonly known as the cause of cold sores, symptoms of HSV-1 infection can range widely, from asymptomatic to rare life-threatening diseases such as encephalitis. Infection is for life: HSV-1 establishes a latent infection in neurons and can periodically reactivate and cause disease throughout the host's lifetime.

A broad range of intrinsic and innate immune mechanisms are in place in cells to defend against invading pathogens. Herpesviruses such as HSV-1 have evolved multiple mechanisms to antagonise such cellular defences. For example, promyelocytic leukaemia nuclear bodies (PML-NBs) are multiprotein complexes that can silence viral DNA, activate type I and II interferons and induce apoptosis. HSV-1 encodes ICP0, an E3 ubiquitin ligase which has been known for many years to mediate proteasomal degradation of PML-NBs. We recently found that pUL55, a poorly characterised tegument protein can also cause the disruption of PML-NBs in ICP0-null viruses. Using immunoprecipitation-mass spectrometry, we show that pUL55 forms a complex with two other viral tegument proteins, including the protein kinase pUL13. Mutations preventing interactions between these viral proteins, or inhibiting pUL13 kinase activity, prevent PML-NB disruption. Furthermore, we show that, in concert, these three tegument proteins recruit a cellular E3 ubiquitin ligase and that inhibition of this E3 ubiquitin ligase prevents PML-NB disruption. Our study elucidates the function of this newly identified tripartite tegument protein complex and reveals a novel way in which herpesviruses antagonise intrinsic and innate immunity.

Viral and cellular RNA degradation mediated by the zinc finger antiviral protein (ZAP)

Clement Bouton ORCID iD[1], María José Lista[1], Grega Gimpelj Domjanic[2,3], Thomas Courty ORCID iD[1], Harry Wilson ORCID iD[1], Adam A Witney ORCID iD[4], Blair L Strang ORCID iD[4], Jernej Ule ORCID iD[2,5], Stuart J D Neil ORCID iD[1], Chad M Swanson ORCID iD[1]

[1]Department of Infectious Diseases, School of Immunology and Microbial Sciences, King's College London, London, United Kingdom. [2]National Institute of Chemistry, Hajdrihova 19, SI-1001, Ljubljana, Slovenia. [3]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. [4]Institute of Infection & Immunity, St George's, University of London, London, United Kingdom. [5]UK Dementia Research Institute, King's College London, London, United Kingdom

Abstract

ZAP is an antiviral protein that restricts a diverse range of viruses including retroviruses, alphaviruses, Ebola virus, and human cytomegalovirus. It binds CpG dinucleotides in viral RNA to target it for degradation or inhibit its translation. ZAP also inhibits expression of cellular genes and endogenous retroelements. Importantly, the cellular co-factors that ZAP requires to inhibit viral or cellular gene expression are unclear.

ZAP has been reported to interact with hundreds of proteins. To determine which of these are required for ZAP to inhibit viral gene expression, we did an RNAi mini-screen of proteins that regulate RNA degradation using HIV with a synthetic ZAP-response element containing CpGs (HIV-CpG). Depleting components of 5'-3' and 3'-5' degradation pathways or other proteins such as UPF1 did not increase HIV-CpG infectious virus production. However, knocking out the endoribonuclease KHNYN rescues HIV-CpG gene expression and virion production. ZAP does not induce an antiviral state so its mechanism of RNA degradation should not lead to intermediates that are sensed by pattern recognition receptors. Therefore, we mapped KHNYN cleavage sites using RACE and Nanopore sequencing and have identified enzymes degrading the cleaved HIV-CpG RNA fragments. The 3'-cleavage fragment is degraded by XRN1 and the 5'-fragment is degraded by an exosome-independent pathway that we are characterising.

ZAP targets a small number of cellular mRNAs but these control specific responses, including apoptosis. Using iCLIP, we have identified that ZAP often binds cellular mRNA targets in the 3' UTR and are analysing how ZAP regulates their expression.

Cultivating Diversity: Practical Ways to Make Microbiology More Inclusive

Decolonising the Microbiology Curricula at the University of Glasgow

Leighann Sherry ORCID iD, Georgia Sutcliffe, Lidia Errico, Zara Gatt, Nicola Veitch ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Movements such as the Rhodes Must Fall and Black Lives Matter have highlighted the need for 'decolonising the curriculum' efforts in academia. Decolonising the curriculum seeks to acknowledge and dismantle the way that imperialism and colonialism have shaped education, with a focus on inclusion and visibility of a wider range of viewpoints which have been historically excluded. To create awareness of the decolonising the curriculum movement and increase the diversity of content and recommended reading, teaching material was developed by final year project students, in the form of a workshop, and delivered to undergraduate students undertaking Life Sciences courses. Within science, there are clear links between knowledge progression and the exploitation of historically marginalised groups, with various key discoveries made to the detriment of people of colour. Examples including the Tuskegee Syphilis Study that investigated the progression of the disease and Koch's medical concentration camps in East Africa to treat trypanosomiasis, can be used to discuss where knowledge comes from, how it is derived and disseminated. These workshops acted as an introduction to the movement, focusing on contextualising the material linked to existing topics within infection biology, through group discussions and case studies. Throughout the workshop, students were receptive to and engaged with the subject material, taking active roles in group discussions. With ever-growing pressures to address issues of inequality and colonial histories, universities in the UK are slowly beginning to work with students to act. Our work showcases the relevance and scope of these issues within Life Sciences subjects.

Supporting staff on long-term leave through a 'roving researcher' scheme

Elizabeth Wynn

The Babraham Institute, Cambridge, United Kingdom

Abstract

Significant time away from the bench has an impact: on the research, on the team, but most of all on the career progression of the person away from the lab. There are many reasons for long-term leave but the most common type is maternity leave meaning female scientists are disproportionately affected by this issue. In 2020, the Babraham Institute piloted our Roving Researcher scheme not to replace those on leave but to support their science so that research momentum is maintained during the absence. This has since turned into a permanent position which has supported 17 projects over the last four years and the scheme has been replicated at other organisations like The Pirbright Institute and MRC Laboratory of Medical Sciences.

Developing a culture of equity in STEM - failures, successes and lessons learned

Emmanuel Adukwu

UWE Bristol, Bristol, United Kingdom

Abstract

Globally, science is a transformative discipline yet it remains largely exclusive and often described as elitist. Access to learning resources, quality of learning experiences and graduate outcomes continues to impact minoritised communities negatively. In the UK, the higher education sector is facing challenges to tackle barriers experienced by disadvantaged communities and in STEM, this remains an urgent priority. Whilst efforts are being made to be more inclusive in the UK HE sector, these remain slow and often tokenistic. Cultivating diversity requires intention and direction. This seminar will present approaches to develop a culture of inclusion and equity in the school of applied sciences at UWE Bristol.

Education and Outreach Symposium

Invited talk: Antimicrobial Resistance Awareness Adventures in Ibadan, Nigeria

Anderson Osemuahu Oaikhena ORCID iD, Dorothy U Cyril-Okoh, Chinenye L Ekemezie, Olufunmilayo Adewuyi, Elizabeth I Akande, Gabriel T Sunmonu, Faith I Oni, Oluwasegun Koleoso, Olukayode T Egbon, Ayorinde O Afolayan, Iruka N Okeke

University of Ibadan, Ibadan, Nigeria

Abstract

Antimicrobial resistance (AMR) poses a significant global health challenge, reputed to be responsible for one out of every 10,000 deaths in West Africa. Addressing AMR requires expanding awareness among healthcare professionals and the general public. While the Global Action and Nigerian National Action Plans prioritize AMR awareness alongside surveillance, stewardship, infection prevention, and making the economic case for AMR intervention, resources to support awareness initiatives remain limited. In Nigeria, particularly among those without a scientific background, many individuals remain unaware of the AMR crisis and its implications. Our group at the University of Ibadan contributes to AMR surveillance, stewardship, infection prevention and research. We additionally raise public awareness about AMR, through regular and tailored outreach programs led by volunteer postgraduate students in pharmaceutical microbiology. Beyond equipping these students with skills to act as anti-AMR advocates, these initiatives, targeting audiences with diverse educational and socio-economic backgrounds, have provided AMR containment information to high school and university students, market sellers, healthcare workers, taxi-cab operators, and the general public. Despite limited resources, our approach utilizes audience-appropriate language, tailoring communication to literacy levels and leveraging social media platforms. We communicate the meaning and significance of AMR as well as the importance of getting everyone involved in the collective effort against it. We have learnt new misconceptions about antimicrobial use among respondents, and these feedback into our strategies to dispel such fallacies. Our commitment to AMR transcends research to actively educating diverse communities, striving to empower individuals with knowledge necessary for collective action against AMR.

Invited talk: Degrees of Change: The Double-Edged Sword of Generative AI in Higher Education Assessments

Pam Birtill ORCID iD

University of Leeds, Leeds, United Kingdom

Abstract

Generative AI (GenAI) has rapidly evolved, with tools now creating original content, including images and text. In the previous year alone, we have witnessed an exponential growth in the ability of GenAI to perform tasks that were once the sole preserve of human intellect. These developments pose unprecedented challenges and opportunities for higher education.

The emergence of GenAI has sparked widespread debate over academic assessments. There is a clear tension between assessment validity, whereby we can be sure that students have acquired specific learning outcomes, and preparing students for a future where GenAI tools are integral to their professional lives.

In this talk, I will discuss the implications of GenAI for assessment design, focusing on inclusive, authentic, and fair assessment. I will discuss the sector response to GenAI, and how institutions can navigate the ensuing challenges. I will also explore how GenAI might offer ways to improve the way we assess learning, potentially moving us away from a grade-centric mindset to a more holistic understanding of student achievement. I aim to prompt discussion on the future of higher education, where we embrace GenAI, while upholding ethical and educational standards.

This abstract was created using chatGPT 4.0 as a writing partner.

Invited talk: Response and Responsibility: Creating a sustainable future through higher education

Zoe Robinson ORCID iD

Keele University, Staffordshire, United Kingdom

Abstract

Awareness of the need for urgent action to ensure a more sustainable future has reached a tipping point, seen through declarations of climate emergencies, increasing focus on the UN's Sustainable Development Goals, and the magnification of climate change in the public and government eye with the global acceleration of climate change impacts.

Higher Education Institutions have long been recognised as a major contributor to society's efforts to achieve sustainability, in part through the skills and knowledge that its graduates put into practice. 'Education for Sustainability' can take many forms, from the formal curriculum we teach, the co-curriculum and the opportunities we provide for students, and the 'hidden curriculum', in the learning that occurs from the settings in which we live, work and study. But how do we as educators navigate the tensions and synergies between the drive to ensure employable high earning graduates, metrics driven league tables, and the responsibility to ensure our students - society's future leaders - are equipped to become knowledgeable and engaged actors with the skills to educate and influence others, and the agency to enact change?

What is a scientist? Smashing stereotypes in science

Emma Waters ORCID iD, Gemma Langridge ORCID iD

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Background: Norwich is home to one of the country's largest research parks, yet still grapples with above average deprivation, with Norfolk being the most deprived county in the region. For Norwich Science Festival 2023, I organised an exhibition titled "What is a scientist?" aiming to: celebrate diversity in science, redefine the stereotypical vision of a scientist as an old, eccentric, white man playing with chemicals and inspire the youth to consider and explore science careers.

Methods: Supported by a Biochemical Society's Diversity in Science Grant, a captivating double-sided photo wall featuring 45 scientists from the Norwich Research Park was created. On one side, their everyday non-science profiles were showcased, whilst their science-related identities were revealed on the other. Members of the public interacted with volunteers who posed them the question "Who on this board do you think is a scientist?" with the ultimate revelation being that they were all scientists.

Results: This exhibition surprised and changed mind sets of individuals about scientists, by conveying the powerful message that anyone, regardless of their background, race, age, sex, disability, etc can be a scientist. This exhibition challenged the major stereotypes which remain in public perception, and also revealed many micro stereotypes for open discussion. Since the science festival, the exhibition has been displayed at multiple local and regional events, including schools.

Outcome: The far-reaching potential and use of alternative delivery formats of this exhibition has inspired me to work towards packaging this as an interactive online resource for schools.

Promoting Microbial Awareness Among School Children: A Multifaceted Educational Approach

Moe Kyaw Thu, James Woon, Madihah Rushaidhi

Newcastle University Medicine Malaysia, Johor, Malaysia

Abstract

The 'Promoting Microbial Awareness Among School Children' project in Johor state, Malaysia, targets children aged 10-12. This innovative educational initiative aims to deepen their understanding of microbiology and promote good hygiene. Through interactive workshops, which include fun activities like Microbe Bingo and Charades, and the involvement of Year 2 medical and biomedical science students as mentors, the project encourages scientific curiosity in young learners. Prior to the workshops, we conduct surveys to gauge the children's existing knowledge, allowing us to tailor the content effectively. This approach not only makes learning more relevant and engaging but also provides a unique teaching opportunity for the student educators, enhancing their skills and fostering a role model relationship with the children. Our methodology includes assessing the needs in local primary schools, designing custom workshops, developing educational games, and conducting post-workshop evaluations. These evaluations help us track improvements in the children's microbiological knowledge and attitudes towards hygiene. Ultimately, the project's success lies in its ability to engage and inspire, setting a foundation for a well-informed, science-enthusiastic future generation. It highlights the importance of early scientific education and the impact of peer-to-peer learning.

Enterprise in the Life Sciences Challenge: A resource efficient intervention which enhances Black, Asian and Minority Ethnic student employability and success.

Gemma Wattret ORCID iD, Jennifer Delaney, Elizabeth Golding

University of Liverpool, Liverpool, United Kingdom

Abstract

ELSC was designed to help address the employment attainment gap for Black, Asian and Minority Ethnic (BAME) students in the School. Over 40 students worked in inter-disciplinary teams to address real world challenges facing employers across the Life Sciences sector. Students were introduced to key concepts of entrepreneurial thinking, including activities for creative thinking, user-research and business modelling and were provided with support to develop a digital pitch to showcase their proposed solutions to address the challenge. Data from our research evaluating the initiative highlights the positive impact this authentic and innovative activity had on the development of students' employability skills, professional networks and awareness of career opportunities. Students' self-assessment of skill development showed an average increase across seven skills in high-demand within the Life Sciences sector and wider graduate labour market, including problem-solving, leadership and commercial awareness. 93% of participants stated the activity developed their confidence and enhanced their student experience. In addition to the impact on student employability and experience, this initiative supported wider institutional aims for student success in the areas of inclusion and support for entrepreneurship. The initiative itself directly aligns to the UN SDG Reduced Inequalities and students developed solutions to challenges facing the Life Sciences sector which link to wider sustainability challenges, including Responsible Consumption and Production and Good Health and Well-being.

Assessment of the efficacy of ChatGPT responses to bacterial species-specific questions in microbiology.

Manushi Dinasha, Gayan Gunatilake ORCID iD, Tanuri Ayanga, Pasindu Damsara, Chamudhi Prabashi

International Institute of Health Sciences, Colombo, Sri Lanka

Abstract

ChatGPT, an OpenAI chatbot, serves as a valuable tool for microbiological learning, offering information on bacteria, fungi, and viruses. However, assessing the accuracy of ChatGPT's responses is essential due to the potential for "hallucinations" in large language models (LLMs). This study focused on evaluating ChatGPT's accuracy in responding to species-specific questions, aiming to enhance microbiological learning. Questions were designed to simulate interactions at three proficiency levels. A clinical microbiologist finalized a list of 20 bacterial organisms, each with 18 specific questions. The ChatGPT 3.5 model was prompted at three proficiency levels for each question mimicking interactions from actual users. Analysis, based on David Greenwood's "Medical Microbiology: A guide to microbial infections" 18th edition, used a scale of accurate, mixed, incomplete, or inaccurate responses. Results revealed mean accuracy values of 64.81%, 66.66%, and 81.48% for low, moderate, and high proficiency, respectively. While high proficiency demonstrated a higher percentage of accurate responses, all other results were either mixed or incomplete, with no observed inaccuracies. The findings suggest that well-phrased questions yield higher accuracy in ChatGPT responses. Precise formulation led to a higher percentage of accurate answers, but partially accurate responses occurred with imprecise questions. The study emphasizes the influence of question formulation on answer accuracy, recommending further research to explore additional factors and comparing results with newer textbook iterations and also more advanced LLMs like ChatGPT-4.

Culturing marine bacteria from UK sediment samples to design co-created student research projects and promote microbiology and genomics education

Linda Percy ORCID iD, Caroline Smith ORCID iD

University of Westminster, London, United Kingdom

Abstract

In 2017-2018 we were involved in the Microbiology Society project "Antibiotics Unearthed" with approximately 50 undergraduate student volunteers (from Levels 3 to 6). In this extracurricular project, over 30 cultures of both Gram positive and Gram negative marine bacteria were isolated from UK sediments. Data was collected from student reflections about their contribution to the project.

This marine bacteria culture collection has gone on to become the backbone for the co-creation of extracurricular projects, the development of learning exercises and module practicals as well as over 40 undergraduate and 12 postgraduate projects. Additional University funding led to a two-year project to optimise extraction of DNA from marine bacteria for preservation of high-molecular-weight DNA with high yield for long read sequencing, and resulted in full genome sequencing of four of these cultures *Bacillus altitudinis*, *Bacillus hwajinpoensis*, *Marinobacter similis* and *Halomonas titanicae*. This has led to further genomics projects and use of bioinformatic tools including antiSMASH to predict secondary metabolites. The combination of microbiological and genomic approaches has enabled projects in the fields of sustainability and novel antimicrobials, as well as exploring pigments, and biopolymer production and screening enzymes for biotechnological applications. Students have contributed to outreach activities on antimicrobial discoveries. Here we present a synopsis of student projects and extracurricular learning activities since the creation of this marine bacteria culture collection.

Virtual Industrial Site Access: The use of 360 mapping and artificial intelligence to digitally and conceptually redefine field trips for microbiology undergraduates.

Samuel Waldron[1], Jerry Reen[1], Niall O'Leary[1,2]

[1]School of Microbiology, University College Cork, Cork, Ireland. [2]Centre for the Integration of Research Teaching and Learning, University College Cork, Cork, Ireland

Abstract

While much of the recent debate around AI in education has focused on the need to re-evaluate traditional assessment, it also offers exciting new avenues for student engagement and performance-based learning. The pedagogical alchemy of transforming disciplinary information into systematic, disciplinary knowledge is complex, but experiential learning and contextual grounding are essential elements for student development. In this regard, field trips have always had a unique and valuable impact in terms of showcasing scientific theory and/or laboratory aspects in their natural or applied context, at amplified operational scales. However, such approaches are not without their own logistical, financial and pedagogical challenges/limitations.

Technological advances in the capability, availability, and affordability of *non-expert user* 360 cameras, image manipulation software with embedded artificial intelligence, and virtual tour creation/cloud hosting have the potential to transform both the concept and the experience of field trips for students. By combining 360 mapping with AI manipulation, it is now possible to tailor student experiences within digital industrial surrounds and to introduce performative aspects allowing real time evaluations of learning outcomes. The VISTA project, funded via the Higher Education Authority of Ireland SATLE fund, explored this potential with undergraduate students in the School of Microbiology at University College Cork. Herein we report the design, development and deployment of this novel approach with various student cohorts to provide photorealistic, interactive, multiplatform, virtual access to industrial sites, together with AI based manipulations to create unique experiential learning opportunities. Aspects of scalability, sustainability and adaptability are also highlighted.

Environmental and Applied Microbiology Forum

DMSOP-cleaving enzymes are diverse and widely distributed in marine microorganisms

Ornella Carrion ORCID iD

University of East Anglia, Norwich, United Kingdom

Abstract

Dimethylsulfoxonium propionate (DMSOP) is a recently identified and abundant marine organosulfur compound with roles in oxidative stress protection, global carbon and sulfur cycling and, as shown here, potentially in osmotolerance. Microbial DMSOP cleavage yields dimethyl sulfoxide (DMSO), a ubiquitous marine metabolite and acrylate, but the enzymes responsible and their environmental importance were unknown. Here, we report DMSOP cleavage mechanisms in diverse heterotrophic bacteria, fungi and phototrophic algae not previously known to have this activity, and highlight the unappreciated importance of this process in marine sediment environments. These diverse organisms, including *Roseobacter* and SAR11 bacteria and *Emiliania huxleyi,* utilised their dimethylsulfoniopropionate (DMSP) lyase 'Ddd' or 'Alma' enzymes to cleave DMSOP via similar catalytic mechanisms to those for DMSP. Given the predicted teragram DMSOP production budget and its prevalence in marine sediments, our results highlight that DMSOP cleavage is likely a globally significant process influencing carbon and sulfur fluxes and ecological interactions.

The plastisphere protects *Salmonella* Typhimurium from UV stress under simulated environmental conditions and enhances its pathogenic potential

Michael Ormsby ORCID iD, Luke Woodford, Hannah White, Rosie Fellows, Richard Quilliam

University of Stirling, Stirling, United Kingdom

Abstract

In low- and middle-income countries (LMICs), plastic waste is ubiquitous in the environment, and has increased concurrently with both economic development and rapid urbanisation; this has amplified the effects of inadequate waste management and sanitation infrastructure. Distinct microbial populations can quickly colonise such debris in what is collectively known as the 'plastisphere'. There is increasing evidence that the plastisphere is a reservoir for human pathogenic bacteria, including *Salmonella enterica sp.* (such as *S.* Typhimurium), which can persist for long periods of time, retain pathogenicity, and pose an increased public health risk. In this study, we have shown that the plastisphere provides enhanced protection to *S.* Typhimurium against extreme environmental pressures in the form of ultraviolet (UV) radiation, allowing it to persist at concentrations capable of causing human infection, for up to 28 days. Additionally, using a *Galleria mellonella* model of infection, our results have indicated that *S.* Typhimurium has greater pathogenicity following its recovery from the UV-exposed plastisphere, suggesting that the plastisphere may select for more virulent variants within a population. This study demonstrates that the plastisphere can provide protection from UV stress and provides further evidence of environmental plastic waste acting as a reservoir for dangerous clinical pathogens. Quantifying the role of the plastisphere for facilitating the survival, persistence, and dissemination of human pathogens in the environment is critical for a more holistic understanding of the potential public health risk associated with plastic pollution.

Global ecosystem structure of atmospheric microorganisms

Elena S. Gusareva ORCID iD[1,2], Kutmutia Shruti Ketan[1], Lennard Wittekindt[1], Akira Uchida[1], Vineeth Kodengil Vettath[1], Ngu War Aung[1], Cassie Elizabeth Heinle[1], Elaine L. Oliveira[1], Balakrishnan N. V. Premkrishnan[1], Irvan Irvan Luhung[1], Lakshmi Chandrasekaran[1], Nicolas E. Gaultier[1], Kenny J. X. Lau[1], Yee Hui Lim[1], Anthony Wong[1], Wen Jia Phung[1], Samuel D. Spence[1], Anjali Bansal Gupta[1], Aishwarya Devakumar[1], Alexander Putra[1], Changsook Park[1], Choou Fook Lee[1], Deepa Panicker[1], Linwan Zhang[1], Poh Nee Ang[1], Sachin R. Lohar[1], See Ting Leong[1], Soo Guek Ng[1], Yanqing Koh[1], Zhei Hwee Yap[1], Tan Fang Yi[3], Justine J.A. Dacanay[1], Ana C. M. Junqueira[4], Benjamin P. Horton[3], Hie Lim Kim[2,1], Matthew C. Fisher[5], Daniela I. Drautz-Moses[1], Stephan C. Schuster[1]

[1]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore. [2]The Asian School of the Environment, Nanyang Technological University, Singapore, Singapore. [3]Earth Observatory of Singapore, AXA-Nanyang Chair in Natural Hazards, Asian School of the Environment, Nanyang Technological University, Singapore, Singapore. [4]Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. [5]School of Public Health, Imperial College London, London, United Kingdom

Abstract

Microbial life flourishes in astonishing diversity within various planetary ecosystems, encompassing aquatic, terrestrial, and atmospheric realms. The presence of liquid, solid, and gaseous phases characteristic to these ecosystems contributes to the wide-ranging abundance and dispersion of microbial biomass. Among these environments, the gaseous atmosphere stands out as one of the most challenging to investigate due to its extremely low biomass concentrations. However, by harnessing recent breakthroughs in ecological microbial research, we embarked on a comprehensive metagenomic analysis of over a thousand air samples collected from diverse locations worldwide. Our investigation unveiled distinct ecological traits exhibited by airborne microorganisms. Bacteria often displayed location-specific patterns, while fungi demonstrated a more extensive global distribution across a variety of geographic locations and climate zones. We also pinpointed core taxa that are globally present, appearing in at least 95% of the sampled locations in our study, along with their respective abundance levels. Furthermore, our analysis delved into the habitats and functional characteristics of these globally identified taxa. These findings represent significant progress in unravelling the intricacies of airborne microbial communities, contributing to a deeper understanding of the rich biodiversity present on our planet Earth.

Exploiting the algal phycosphere for co-production of bio-polymers

Zongting Cai, Jagroop Pandhal

Department of Chemical and Biological Engineering, the University of Sheffield, Sheffield, United Kingdom

Abstract

Algae-bacteria consortia biotechnology is an emerging field in biological research, leveraging synergy between different microbes and insights into the 'phycosphere' to optimize bioprocesses like bioproduct production. Focusing on novel, eco-friendly, third-generation bioplastics, bacterial polyhydroxyalkanoates (PHA) and microalgal cellulose, this study showcases the production of both materials using an engineered phycosphere around the microalgae *Nannochloropsis oculata*. The study first explores the feasibility of cultivating *N.oculata* in filtered industrial leachate, demonstrating its growth in 100% leachate with adjusted 3.3% salinity without additional nutrient source. Cultivation in 100% leachate leads to faster growth rate and biomass yield compared to standard F/2 media. However, diluting the leachate to 20%-40% using F/2 media (v/v) enhances growth rate. A relatively low light intensity of 40 μmol/m²/s was found ideal to avoid stress. Then, 5 bacteria naturally associated with the alga, and 16 bacteria from the leachate were isolated, with 10 confirmed as PHA producers through selective culturing, Nile blue A staining, PhaC gene PCR, and GC-FID analysis. These bacteria primarily belong to the genera *Microbacterium*, *Rhodococcus*, and *Pseudoalteromonas*. Co-culturing of *N.oculata* with selected strains, *Pseudomonas stutzeri* and *Rhodococcus rhodochrous*, achieved up to 5.4% bacterial PHA and 14% cellulose production (% dry cell mass). GC-FID analysis identified these PHAs as short and medium-chain-length polymers, characterised as 3HB, 3HO, and 3HD. SEM scanning characterised the spherical structure of algal cellulose extract, indicating high crystallinity. This study demonstrated how the phycosphere of *N.oculata* can enable the co-production of industrially valuable bioproducts, highlighting advancements in sustainable biotechnology.

THE DBBACT KNOWLEDGE BASE - ACHIEVING PAN-MICROBIOME BIOLOGICAL INSIGHTS, PERFORMING META-ANALYSIS AND ANSWERING ECOLOGICAL QUESTIONS

Noam Shental ORCID iD[1], Amnon Amir[2]

[1]The Open University of Israel, Raanana, Israel. [2]Sheba Medical Center, Ramat Gan, Israel

Abstract

Microbiome 16S rRNA studies have examined diverse habitats, including oceans, soil, plants, animals, and large cross-sectional human studies. However, finding commonalities between different experiments, habitats, or conditions is very difficult due to technical issues related to the usage of different primer pairs and relying on taxonomy for scientific reporting. Hence, grosso modo, the 16S rRNA microbiome literature has not been integrated into a collaborative body of knowledge. It often comprises effectively isolated studies, where researchers may be completely unaware of commonalities between their findings and those in other studies, especially across different niches or habitats. In the past five years, we have created dbBact, a knowledge base, to bridge this gap and lay the groundwork for gaining core pan-microbiome insights. Briefly, amplicon sequence variants (ASVs) from datasets of published papers are manually analyzed, and bacterial sequences associated with experimental conditions are uploaded to dbBact, together with ontology-based characterizations (e.g., "the abundance of sequence ACTGGA… was higher in fecal samples of horses with colitis compared to healthy controls in California"). **To date, dbBact contains data from more than 1000 published studies across diverse habitats, spanning ~370,000 unique 16S rRNA ASVs, with approximately 1,500,000 sequence-to-phenotype associations**. We demonstrated how dbBact provides many types of novel hypotheses that cannot be formulated by standard methods (Amir et al., Nucleic Acids Research, 2023). We will show how dbBact may be (1) applied as part of microbiome research in any niche; (2) allow a new type of meta-analysis; and (3) be harnessed to answer ecological questions.

Fluvial flooding and microplastics – the delivery of human pathogens into agricultural fields.

Chloe Pow, Hannah White, Rosie Fellows, Luke Woodford, Richard S Quilliam

University of Stirling, Stirling, United Kingdom

Abstract

Fluvial flooding can cause physical destruction to crops, and introduce waterborne pollutants, including microplastics, into arable and food-producing land. Microbial biofilm colonising the surfaces of environmental plastics (known as the 'plastisphere') provides an ecological niche for the increased survival of microorganisms, and often includes pathogenic bacteria capable of causing foodborne disease. Therefore, this study aimed to quantify potentially pathogenic bacterial species on the surface of plastic pollution at flood-prone agricultural sites next to three Scottish rivers. Visible plastic debris were collected along 100m transects from the river into adjacent agricultural fields, and selective media used to screen for*, Escherichia coli*, intestinal enterococci, *Salmonella* spp., *Campylobacter* spp., and *Klebsiella* spp. Isolated colonies were confirmed by PCR, screened for antimicrobial resistance, and tested for virulence using the *Galleria mellonella* model of infection. Our study has demonstrated that plastic debris in agricultural fields is frequently colonised by human pathogenic bacteria, which express varying levels of antimicrobial resistance. Therefore, growing crops, or keeping livestock, in fields that are contaminated with plastics from fluvial floodwater could pose a significant risk of introducing human pathogens into the food chain.

Exploiting microbial interactions for Food security

Emma Holden[1], Justin Abi-Assaf[1], Haider Al-Khanaq[1], Noemie Vimont[1], Mark Webber[1,2], Eleftheria Trampari[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

Fresh produce is implicated in over 30% of outbreaks of bacterial foodborne infection. *Salmonella* contaminates a wide range of food products with recent outbreaks including leafy greens, cucumbers, and alfalfa sprouts. The need for effective control strategies is urgent, given the ongoing threat to food security and public health.

Plants host diverse microbiota, pivotal for plant health and disease resistance. Some commensal microorganisms exhibit "biocontrol" properties against plant and human pathogens. However, limited understanding exists regarding how biocontrol strains can shield plants from human pathogen colonisation and how they exert their activity as part of a community.

To gain a better understanding of how *Salmonella* establishes successful colonisation of alfalfa seedlings, we used a genome-wide screen (Transposon Directed Insertion Sequencing-TraDIS). This screening unveiled key strategies employed by the pathogen at distinct phases of plant colonisation. Additionally, we investigated the role of biocontrol agents in suppressing *Salmonella in planta* and identified specific members of the native plant microbiota with strong suppressive activity. We employed RNA sequencing to characterise the mechanisms responsible for the suppressive activity against *Salmonella*.

This research provides valuable insights into the mechanisms *Salmonella* uses to establish communities in plants and explores how the use of biocontrol agents can mitigate foodborne pathogen colonisation in fresh produce ecosystems. Understanding these mechanisms will contribute to a broader understanding of community functions, with impacts that extend beyond their plant hosts and into human health.

Environmental field sampling supports clade 2.3.4.4 H5N1 high pathogenicity avian influenza investigations at seabird colonies, commercial poultry sites, and safari parks.

Caroline J. Warren[1], Joe James[1], Marco Falchieri[1], Thomas Lewis[1], Dilhani de Silva[1], Robert W. Furness[2,3], Ian H. Brown[1], Ashley C. Banyard[1]

[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]MacArthur Green, Glasgow, United Kingdom. [3]University of Glasgow, Glasgow, United Kingdom

Abstract

Since autumn 2021, H5Nx high pathogenicity avian influenza virus (HPAIV) has decimated wild bird populations, caused global poultry outbreaks and sporadic mammalian detections. Here we report on environmental sampling to assess virus transmission risk routes in various settings, seabird colonies, poultry production units and safari enclosures. Alongside clinical material, environmental samples; water, silt, dust, feather, and air were investigated for the presence or absence of viral RNA (vRNA) and infectious virus. Where wild bird mass mortality events were observed, no virus or vRNA was recoverable from freshwater or silt samples taken from underneath seabird carcases four months after death. This suggests that survival of virus and viral products in the environment is not a high risk in the longer term. In contrast, water samples at pre-cull chicken and turkey premises were positive for vRNA. Further, dust sampled from extract vents at each poultry production site tested positive for vRNA and viable virus, whilst feather samples were also vRNA positive. Airborne particles sampled inside and near (<10m) pre-cull poultry houses were positive for infectious HPAIV demonstrating that live virus may travel short distances through air. At greater distances (80m) no virus or viral products were detected. Following the detection of HPAIV in captive bush dogs at a safari centre, vRNA was found in water samples within the enclosure. Taken together, whilst environmental contamination may act as a source of infection, assessment of virus survival in different matrices and under different environmental conditions is required to fully understand risk from environmental contamination.

A structural and functional characterisation of the BREX bacteriophage resistance system

Sam Went[1], David Picton[1], Richard Morgan[2], Andrew Nelson[3], David Dryden[1], Darren Smith[3], Nicolas Wenner[4], Jay Hinton[5], Tim Blower[1]

[1]Durham University, Durham, United Kingdom. [2]New England Biolabs, Boston, USA. [3]Northumbria University, Newcastle, United Kingdom. [4]University of Liverpool, Liverpool, United Kingdom. [5]Liverpool University, Liverpool, United Kingdom

Abstract

The interminable arms race between phages and bacteria has produced myriad phage defence system modalities. Recently, systematic approaches to defence system discovery have unearthed a plethora of new systems that await characterisation. This study aimed to provide valuable insight into the mechanism of Bacteriophage Exclusion (BREX) phage defence through structural and functional characterisation. Utilising the Durham Phage Collection alongside BREX systems from *Salmonella, E. coli* and *E. fergusonii*, it was shown that phage defence varies between systems against a given phage and does not correlate with the number of recognition motifs. Further, phages encode mechanisms of inhibiting species-specific BREX systems. The generation of gene deletions then demonstrated essential genes for host methylation and phage defence. Next, the structure of the methyltransferase, PglX, was solved to a resolution of 3.4 Å. PglX displays two distinct domains joined by a central hinge, with conserved methyltransferase regions. To shed light on mechanisms of phage escape from BREX systems, the structure of PglX bound to the BREX inhibitor, Ocr, was solved to a resolution of 3.5 Å. Ocr binds along the C-terminal domain of PglX and provides insight on potential DNA binding positions. Finally, PglX was rationally mutated to alter the BREX recognition motif, both changing host methylation patterns and allowing defence against a previously resistant phage. Thus, PglX is the sole specificity factor of BREX defence, despite other components encoding DNA binding functionalities. Together, these results will guide further studies into the molecular mechanisms of BREX phage defence.

FORENSIC APPLICATIONS OF HUMAN RELATED MICROBIOMES

Nengi Ogbanga ORCID iD[1], Andrew Nelson[1], Noemi Procopio[2], Darren Smith[1]

[1]Northumbria University, Newcastle, United Kingdom. [2]University of Central Lancashire, Preston, United Kingdom

Abstract

The microbiome refers to the encompassing microbial community in a sample. The ubiquitous nature of microorganisms, and the ability of microbial communities to colonise specific environments in a reproducible manner, are of particular interest for forensic research. This is because microbial communities have the potential to answer forensically important questions regarding human profiling and death investigations.

This research focuses on the utilisation of human and soil associated microbes for two purposes. First, we aimed to understand whether soil microbiome associated with human decomposition can give indicate the presence of a mass grave. For this, six human cadavers were buried in an experimental mass grave, alongside 3 single graves with 1 donor each. Soil samples were collected at various depths before burial and 18months after burial. Classification prediction using random forest model showed 95% accuracy in distinguishing mass grave to single grave samples.

Secondly, we explored the oral microbiota of an understudied population – Nigerians. Oral swab samples collected from Nigerians were compared against other populations to understand microbial community across populations. We also conducted a longitudinal study to assess any changes to the Nigerian oral microbiome due to migration. Analysis for this study is ongoing and would be completed before the conference.

Our results from both studies illustrate that the microbiome can potentially be used in tracking communities between countries, which can then be used for human profiling, and in identifying mass grave sites. Overall, these results show the potential of microbiome research for forensic applications.

Increased hydrogen production from genetically modified Escherichia coli and their use within biocoatings

Kathleen Dunbar[1], Alexia Beale[1], Emily Brogden[2], Stefan Bon[2], Suzie Hingley-Wilson[1], Joseph Keddie[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Warwick, Coventry, United Kingdom

Abstract

Hydrogen offers a source of energy that does not produce any greenhouse gases when combusted, and hence the global demand for hydrogen fuel is growing. The production of hydrogen by bacteria is an attractive alternative because it does not require fossil fuel feedstocks, however, the yield needs to be increased for biohydrogen to be commercially competitive.

In this work, hydrogen was produced using genetically modified *Escherichia coli* in liquid cultures via dark fermentation. Bacteria were cultured anaerobically (in a hydrogen free environment) in Balch tubes for 24 h, and the headspace gas was measured using a residual gas analyser. Single knockouts of genes from the mixed acid fermentation pathway were initially investigated with the best performing single gene knockouts being combined to produce double knockouts. The best performing double knockout produced significantly more hydrogen than the single gene knockouts and produced four times more hydrogen than the wildtype strain. Interestingly, this double knockout also produced two times less carbon dioxide compared to the wildtype strain, making biohydrogen an attractive alternative to brown and grey hydrogen production methods.

Confining *E. coli* in a waterborne polymeric coating (to make a biocoating) was found to keep the bacilli metabolically active, but non-dividing. In ongoing work, the GM strains are confined in a biocoating to contain them in bioreactors and to protect them from external contaminants that can affect hydrogen production. Biocoatings have the potential to overcome many of the limitations present in traditional bioreactors.

Exploring the evolutionary ecology of microbes associated with the common garden snail

Andrew Matthews ORCID iD, Rachel O'Brien, Kelly Jones, Elze Hesse, Angus Buckling, Tatiana Dimitriu

University of Exeter, Penryn, United Kingdom

Abstract

The common garden snail *Cornu aspersum* is a cosmopolitan agricultural pest. A snail's body wall presents to its environment a mucosa rich in specialised glandular cells, which are believed to perform essential nutritional and defensive functions. Though overlooked, this mucosa plays a central role mediating microbial interactions. Here, we explored *C. aspersum*'s cuticular microbiome.

Snails sampled across Europe were characterised by nanopore 16S rRNA sequencing cuticular microbiomes. This revealed that *C. aspersum*'s cuticular-associated microbiota is rich in *Erwinia sp.* and in particular *Pseudomonas sp.*. Isolate-level variation in *Pseudomonas* showed that species identity varied with geographical location.

Confocal fluorescent microscopy confirmed that bacterial symbionts are resident in cuticular bacteriocytes, arrayed as club-shaped glandular cells throughout the cuticle, and are especially abundant in the anterior portion of the foot and in the mantle.

Symbionts could be cured using artificial diet containing high doses of antibiotics, but this was associated with high snail mortality. Provision of antibiotic-resistant *Pseudomonas* symbionts rescued hosts from death, however the extent of colonization was negatively correlated with host growth in surviving individuals. In these gnotobiotic colonisation experiments, we also observed a significant effect of the symbiont strain on snail growth, and on symbiont colonisation ability.

Using RFP-transformed antibiotic-resistant *Pseudomonas* to visualize invasion, we observed competitive exclusion by resident strains even under strong antibiotic selection favouring the invader.

Our results suggest that symbiosis in *Cornu aspersum* is widespread, primarily based on diverse strains of *Pseudomonas*, and that one of its likely functions is mucosal immunity.

Discovery of a missing step of the Taxol (anticancer drug) pathway using *S. cerevisiae* consortia

Leonardo Rios ORCID iD

University College London, London, United Kingdom

Abstract

The increasing demand for anticancer drugs, as well as the urgency of environmentally friendly production methods, makes evident the necessity to find alternative methods for Taxol synthesis, such as microbial cell factories. Nevertheless, the biosynthetic pathway is still not completely elucidated. This work contributes to the elucidation of this important pathway, highlighting the discovery of a long-sought missing taxane-10β-hydroxylase T1bOH.

Using cDNA-FLP assay data from *Taxus* cultures, exhaustive *in silico* studies revealed that a candidate enzyme could be the potential missing T1bOH gene in the Taxol pathway.

To validate the biological function of the candidate gene, we engineered and optimized an S*. cerevisiae* strain using CRISPR Cas9 (15 genome modifications) to produce T10bOH (10beta-hydroxytaxa-4(20),11-dien-5alpha-yl acetate) which is the substrate of the missing T1bOH gene. Nevertheless, it was found that the metabolic burden and redox balance of the engineered strain was not enabling the production of enough T10bOH. We, therefore, designed and engineered several *E. coli/S. cerevisiae* and *S. cerevisiae/S. cerevisiae* microbial consortia to divide the metabolic pathway. The best consortia consisting of 4 different specialized yeast strains finally allowed the detection, validation, and optimized production of up to 45 mg/L of the missing intermediate T1bOH.

In conclusion, this work highlights the powerful combination of *in silico* studies, metabolic engineering and microbial consortia for the discovery and optimization of elusive steps in a metabolic pathway. The successful synthesis of T1bOH will prove invaluable for the future elucidation of the biosynthesis of such an important drug.

Exploring the Skin Microbiome in Health and Disease

Invited talk: Microbiome mediated odour generation: It's the pits.

Barry Murphy ORCID iD

Unilever, Bebington, United Kingdom

Abstract

The generation of malodour on human skin is caused by the biotransformation of naturally secreted non-odorous precursor molecules into volatile odorants by members of the skin microbiome. Specifically in the axilla (underarm), malodour is mediated mainly by the bacterial metabolism of amino acid- and dipeptide-conjugated substrates originating from the apocrine gland secretions. The release of the subsequent thioalcohols e.g., 3-methyl-3-sulfanylhexan-1-ol and volatile fatty acids e.g., 3-hydroxy-3-methylhexanoic acid and 3-methyl-2-hexenoic acid, are part of the complex mixture that humans perceive as body odour. Numerous studies by Unilever and others have identified the bacterial composition of the axilla comprising the genera *Staphylococcus*, *Cutibacterium*, *Corynebacterium* as well as Gram-positive anaerobic cocci (GPAC) including *Anaerococcus* and *Peptoniphilus*. This presentation will summarise the recent advances in our understanding of the generation of axillary malodour focusing primarily on the generation of thioalcohol-based malodour predominately by the axillary commensal *Staphylococcus hominis*.

Invited talk: Modulating the host skin using the skin microbiome

Marc Güell ORCID iD

Universitat Pompeu Fabra, Barcelona, Spain. ICREA, Barcelona, Spain

Abstract

We will present our advancements in transforming Cutibacterium acnes into a synthetic biology platform tailored for skin applications. Initially, our focus was on leveraging this bacterium to achieve sustained alterations in the skin's microbiome, utilizing natural variants. The stable environment of its natural habitat, the sebaceous appendices, provides a robust foundation for persistent bioengineering. Our efforts led to notable successful colonization and interesting therapeutic effects.

In recent years, our work has expanded to include the development of robust tools for the precise genetic manipulation of C. acnes. This includes a variety of synthetic biology components, circuit designs, and methodologies for genetic modification. Notably, we have developed several biocontainment approaches for controlled application of genetically altered bacteria. We have engineered and characterized the effectiveness of various synthetic functions, such as sebum regulation, immune system interaction, and sensing.

Our vision is to introduce new functions to human skin by engineering these endogenous microbes.

Invited talk: The multi-faceted role of the microbiota in fortifying the barrier function of skin

Elizabeth Grice

University of Pennsylvania, Philadelphia, USA

Abstract

Skin is a protective barrier against the external environment and our first line of defense against pathogens, among other insults. Skin shapes our appearance but also safeguard internal organs and prevents water loss and dehydration. The skin microbiota is a key element that contributes directly and indirectly to the skin's main function as a barrier. Breach of the skin barrier and subsequent tissue repair also occur in the context of resident polymicrobial communities. Our lab investigates these microbial-mediated functions in skin barrier maintenance and repair, integrating analysis of clinical specimens with translational models of host-microbiota interactions and multi-omic approaches. In this talk, I will highlight mechanisms whereby the microbiota, and its products, directly contribute to skin barrier function, including 1) antagonism of pathogenic microbes and 2) interactions with keratinocytes that promote epithelial differentiation and repair. Targeting such mechanisms could prevent and treat skin barrier disorders and inhibit pathogenic colonization that exacerbates barrier dysfunction.

Invited talk: Bacterial-Fungal Interactions in the Skin Microbiome

Lindsay Kalan

University of Wisconsin-Madison, Madison, USA

Abstract

Bacteria have the genomic capacity to produce an array of small molecule metabolites. Within the human microbiome, the structure and function for the vast majority of these metabolites are unknown. Focusing on the skin, we have built a large library of diverse bacterial isolates that span the major phyla of the skin microbiome, including low abundant genera, for testing in phenotypic screens to measure metabolite mediated microbe-microbe interactions. We have found that bacterial members of the skin microbiome secrete metabolites that result in morphological changes in fungi. This includes human fungal pathogens such as *Candida albicans* and emerging multi-drug resistant pathogens such as *Candida auris*. We observe both selective and broad inhibition fungal growth and suppression of virulence factors such as formation of hyphae. We have also identified isolates that promote fungal biofilm formation under conditions where skin integrity is lost. Overall, our work suggests that fungi are sensing metabolites produced by skin-associated taxa that act to regulate their lifecycle in a context dependent manner. By characterizing these metabolites, we address a critical gap in knowledge regarding the role of metabolite-mediated interactions in the skin microbiome.

The bovine foot skin microbiota is associated with development of infectious digital dermatitis lesions

Veysel Bay ORCID iD[1], Amy Gillespie ORCID iD[2], Erika Ganda ORCID iD[3], Nicholas Evans ORCID iD[2], Stuart Carter ORCID iD[2], Luca Lenzi ORCID iD[2], Anita Lucaci[2], Sam Haldenby ORCID iD[2], Matthew Barden ORCID iD[2], Bethany Griffiths ORCID iD[2], Enrique Sanchez Molano ORCID iD[4], Rodrigo Bicalho ORCID iD[5], Georgios Banos ORCID iD[6], Alistair Darby ORCID iD[2], Georgios Oikonomou ORCID iD[2]

[1]Ege University, Bornova, Turkey. [2]University of Liverpool, Liverpool, United Kingdom. [3]Penn State University, Pennsylvania, USA. [4]The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, United Kingdom. [5]FERA Diagnostics and Biologicals, College Station, Texas, USA. [6]Scotland's Rural College, Edinburgh, United Kingdom

Abstract

Bovine digital dermatitis is an endemic infectious disease of cattle, causing lameness due to painful foot skin lesions.

In this longitudinal study, we used swabs to sample the foot skin from 259 Holstein Friesian dairy cattle 3-4 weeks prior to calving, and examined feet at one, four and 8-10 weeks after calving to record the development of infectious digital dermatitis lesions.

16S rRNA gene amplicon sequencing was carried out using the Illumina HiSeq 2500 platform, and taxonomic assignment of OTUs carried out using QIIME and the RDP classifier. Our analyses showed a tendency to decreased alpha diversity in samples from healthy feet that went on to manifest disease compared to those that remained healthy. Co-occurrence analyses showed increased competition in the bacterial communities of healthy feet that went on to become diseased. Response screening showed associations between the presence of *Succiniclasticum* spp., *Porphyromonas* spp., *Acholeplasma* spp., *Fastidiosipila* spp., *Peptoclostridium* spp. and *Prevotella* spp. and future development of BDD lesions. Shotgun metagenomic analysis of a small subset of samples (10) identified an increase in a functional pathway associated with polymer degradation, which may indicate increased connective tissue degradation occurring in healthy feet that went on to become diseased.

Overall these findings suggest changes in the bovine foot skin microbiota which result in disease are occurring in advance of the appearance of macroscopic lesions. This presents an opportunity to intervene using pre or pro-biotics to maintain a healthy skin microbiome as a disease prevention measure.

Learnings and key insights from a Microbiology Society workshop on microbiome safety

Aline Metris[1], Curtis Asante[2], Lindsay Hall[3,4]

[1]Unilever SEAC, Sharnbrook, United Kingdom. [2]Microbiology Society, London, United Kingdom. [3]Quadram, Norwich, United Kingdom. [4]University of Birmingham, Birmingham, United Kingdom

Abstract

With the advancement of New Generation Sequencing technologies, there has been an increase in research on the human microbiome and the implications of its composition on health and disease. For example, the composition of oral and skin microbiomes has been shown to have important roles in protecting human health through their resilience and stability. It has also been proven that the efficacy of some drugs is affected by human gut microbiota.

The current research landscape around the assessment of the safety of microbiome perturbations by chemicals and probiotics will be discussed at a workshop organised by the Microbiology Society on Wednesday 24 – Thursday 25 January 2024, in London. The event will bring together experts in skin, oral and gut microbiomes, bioinformatics, AI and *in vitro* models from both academia and the industry spanning food, cosmetics and therapeutics.

After expert views are sought on potential health implications mediated by product-induced human microbiome changes, ways to measure human microbiome perturbations and assess their safety will be discussed. Key insights from the workshop, including research needs in this rapidly evolving area, with emphasis on the skin microbiome, will be presented.

Study of skin immunity and host-microbiota communication: potential role of the Calcitonin Gene-Related Peptide

Emilie Hadjiev, Rached Ismail, Amine M. Boukerb, Chloé Catovic, Magalie Barreau, Sylvie Chevalier, Marc G.J. Feuilloley

UR4312 CBSA, University of Rouen Normandie, Evreux, France

Abstract

Skin is the largest neuroendocrine organ of the human body and numerous hormones and neurohormones diffuse into sweat and through the upper layers of the epidermis, constantly exposing the cutaneous microbiota to eukaryotic factors. Microbial Endocrinology investigates the ability of bacteria to respond to these neuropeptides.

In this context, it has been shown that the Calcitonin Gene-Related Peptide (CGRP) induces an increase in the virulence of a commensal cutaneous bacteria: *Staphylococcus epidermidis*.

Our current research is focusing on a wider range of bacteria in order to gain a better understanding of the impact of CGRP on various skin microbiota. A metagenomic study carried out on scalp samples and a further culture-based selection allowed the identification of 20 different strains. Among these, 4 were selected and tested for their virulence factors, notably cytotoxicity and inflammatory potential on the HaCaT human keratinocyte cell line.

The results obtained in our study indicate an impact of CGRP on the inflammatory potential of *Bacillus licheniformis* with an increase of 72% of IL8 production by keratinocytes. CGRP being involved in the modulation of the skin immune system, these results further encouraged us to look for a similar effect of CGRP in other Gram-positive and Gram-negative skin bacteria. The implication of CGRP on adhesion and biofilm formation has also been investigated. Preliminary results indicate other effects of CGRP. This suggests that the effect of CGRP could be species and even strain dependent, thus linked to epigenetic adaptation to the skin microenvironmental conditions.

An observational study using metagenomic approaches to understand the pathogenesis of microbial keratitis

Tobi Somerville ORCID iD[1,2], Ophthalmic Microbiology Collection Group[2], Malcolm Horsburgh ORCID iD[3], Alistair Darby ORCID iD[3], Stephen Kaye ORCID iD[2,1]

[1]Eye and Vision Sciences Department, University of Liverpool, Liverpool, United Kingdom. [2]St Paul's Eye Unit, Liverpool University Hospitals NHS Foundation Trust, Liverpool, United Kingdom. [3]Infection Biology and Microbiomes, University of Liverpool, Liverpool, United Kingdom

Abstract

Purpose

Microbial keratitis (MK) is an ophthalmological emergency that can lead to blindness. Isolation rates using standard culture techniques are poor, with microorganisms of unknown pathogenic significance commonly isolated. We performed a prospective observational study to improve understanding of the cornea microbiome in health and disease.

Methods

Samples were collected from the unaffected and MK-affected eyes of patients and healthy controls using minimally invasive corneal impression membrane sampling. Samples were processed using culture and two metagenomic approaches: 1) full-length 16S rRNA gene sequencing using nanopore sequencing technology and 2) shotgun metagenomic sequencing on the Illumina NovaSeq platform.

Results

150 MK and 20 healthy control patients were included. The affected eye isolation rate was 56.6% with coagulase-negative staphylococci (CoNS; 17%), *Staphylococcus aureus* (8%) and *Pseudomonas aeruginosa* (7%) the most frequently isolated bacteria. CoNS were the only microorganisms isolated from healthy controls. CoNS, *S. aureus*, alpha-haemolytic streptococci and diptheroids were isolated from unaffected eyes of MK patients. Microbiome analysis revealed lower microbial diversities for those culture positive cases which had known MK pathogens detected (*S. aureus*, *P. aeruginosa, Streptococcus pneumoniae, Moraxella* spp. and *Klebsiella* spp.). Virulence factor genes, including exotoxins, were detected from *P. aeruginosa* metagenome assembled genomes.

Conclusions

This is the first study to compare the corneal microbiome dysbiosis seen in the affected eye of MK patients with their unaffected eye and healthy controls using metagenomic sequencing and increases our understanding of MK pathogenesis. Future genomic sequencing could be used to identify virulence factors associated with poor disease outcomes in MK.

Investigating skin microbial interactions and metabolite production on artificial sebum

Danita Kasi Patel, Drupad K Trivedi

University of Manchester, Manchester, United Kingdom

Abstract

Sebum is a lipid-rich biofluid, secreted by sebaceous glands, which lubricates skin, and acts as a protective barrier against infection. Volatile organic compounds (VOCs) present in sebum have previously been identified as potential biomarkers for Parkinson's disease (PD). Additionally, *Malassezia* proliferation and seborrheic dermatitis are associated with a significantly increased risk of PD development. Recent work has also shown sebum composition changes in diseases such as PD, Alzheimer's, COVID-19 and Tuberculosis. We hypothesised that changing sebum composition requires skin commensal microbes to adapt metabolically.

We investigated the growth ability of three skin-specific bacteria, *Staphylococcus epidermis, hominis,* and *capatis,* and three sebum-specific bacteria, *Corynebacterium tuberculostearicum, simulans* and *Cutibacterium acnes* on artificial sebum in co-cultures for 24 hours on artificial sebum. Additionally, endogenous and extracellular metabolites were obtained during the logarithmic growth phase and analysed using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), with spectral matching performed to annotate metabolites, and subsequent pathway analysis. We then investigated the sebaceous microbiome of 30 healthy volunteers via matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). A dilution-to-extinction method demonstrated the micro-organisms necessary to maintain microbial growth on artificial sebum.

The data presented show *in vitro* adaptation to changing lipid feed and metabolic response of these bacteria. Microbial metabolic markers, *in vivo*, may potentially be useful substrates for disease diagnosis. Furthermore, the metabolic adaptation of skin commensal microbes may dictate relationship with other microbes, including pathogens. The changes in microbial communities on skin may modulate host immune response over time, warranting further studies.

Metagenomic insights into pig skin microbiota: a human-relevant skin microbiome model.

Michelle Rudden ORCID iD[1], Amber R. Stafford[1], Nina D.C. Rocha[1], Alexandria S. Kidd[1], Andrea Bell[2], Jeffrey Hart[2], Bob de Rooij[3], Matthew J. Hardman ORCID iD[1], Holly N. Wilkinson ORCID iD[1]

[1]University of Hull, Hull, United Kingdom. [2]Cica Biomedical Ltd, Knaresborough, United Kingdom. [3]Micreos Pharma, Bilthoven, Netherlands

Abstract

Staphylococcus aureus is a frequent opportunist of skin and a common biofilm-forming pathogen isolated from chronic wound infections. Understanding the complex interplay between *S. aureus* and host pathology is essential to develop selective therapies to manage chronic *S. aureus* infections. Here we employ a translationally relevant pig wound microbiome model to demonstrate the efficacy of a novel bacteriophage-derived endolysin (XZ.700) that selectively depletes *S. aureus*. Using long-read nanopore sequencing, we taxonomically defined the skin microbiome profile of two common pig wound models (Yorkshire Pig and Minipig). Notably, both species exhibited dominance by coagulase-negative *Staphylococcal* (CoNS) species, akin to humans. We show that *S. aureus* is naturally prevalent in both pig skin microbiomes (~10%) and can rapidly colonise the wound environment by out-competing commensal pig microbiota. XZ.700 treatment selectively inhibits *S. aureus*, restoring microbiome diversity and promoting wound healing. We whole genome sequenced (WGS) commensal microbiota and endogenous *S. aureus* strains from infected wound sites from both pig models. Comparative pan genomic analyses revealed significant strain specific genomic diversity and heterogeneity among commensal pig microbiota and *S. aureus* wound isolates. Species specific core genomic profiles were dominated by central metabolism genes whereas accessory genomes included functions associated with host colonisation and adaption, with *S. aureus* enriched in virulence and antibiotic resistance genes. This proof-of-concept study demonstrates pig skin as a new and important model of the human skin microbiome. It further validates the pig as a pre-clinical "model of choice" for translational wound studies, bridging *in vitro* studies to human clinical trials.

Lipidomics analysis of ovine footrot using liquid extraction surface analysis mass spectrometry: a pilot study

Rebecca Greatorex, Sidrah Rahman, Thomas White, Catrin Rutland, Rian Griffiths, Rachel Clifton

University of Nottingham, Nottingham, United Kingdom

Abstract

Ovine footrot is a polymicrobial infection of the interdigital skin of sheep. *Dichelobacter nodosus* is the causative agent, however *Fusobacterium necrophorum*, *Porphyromonas asaccharolytica* and *Mycoplasma fermentans* are also associated with disease. Changes in expression of genes involved in lipid metabolism have been reported in feet affected by footrot compared to healthy feet, however, the role of lipids in footrot pathogenesis has not been studied. Here we present a pilot study of direct biopsy sampling via liquid extraction surface analysis mass spectrometry (LESA-MS) analysis of lipid analytes from ovine foot tissues. Biopsy sections from three healthy feet and three feet with footrot were obtained post-mortem at an abattoir. These were cryo-sectioned and three replicate spectra obtained for each sample. Confirmed triacylglycerols (TGs) were more abundant in healthy tissue compared to footrot tissue whereas lipids identified via accurate mass as either phosphoserines or phosphothreonines were more abundant in footrot tissue. TGs are important for skin barrier function, therefore decreased abundance during footrot may reflect loss of integrity of the epidermis. Phosphoserines are a global immunosuppressive signal that can be exploited by intracellular bacteria to facilitate infection. This could represent a mechanism for the previously reported reduction in expression of pro-inflammatory cytokines by ovine cells infected with *M. fermentans* and requires further investigation. This work shows the potential for LESA-MS of tissue sections to provide useful information on pathogenesis of polymicrobial infections. Future work will focus on identifying the bacterial strains present in footrot tissue samples and additional analytes such as bacterial metabolites.

Revealing the diversity of corynebacteria in the human underarm

Reyme Herman[1], Barry Murphy[2], Gavin Thomas[1]

[1]University of York, York, United Kingdom. [2]Unilever, Port Sunlight, United Kingdom

Abstract

A large proportion of the human skin microbiome consists of cutibacteria, staphylococci and corynebacteria. While being generally nutrient limited, the different sites of the human skin can dictate the microbial species found. Here we are interested in the human underarm/axilla, an environment supplied with diverse types of metabolites derived from the three sweat glands: the eccrine, sebaceous and apocrine glands. We have reported the characterisation of various axilla associated staphylococci but skin corynebacteria is generally poorly studied with little known about its diversity in the axilla. In this study, we performed whole genome sequencing and subsequent bioinformatics analysis of over 150 isolates of axillary corynebacteria from one individual, complemented with isolates from three other individuals. We identified a range of species, including species previously not associated to the underarm and possible novel species. Bioinformatics analysis on their genomes also revealed diversity within each species, including further clarification of the distinction between species within the *Corynebacterium tuberculostearicum* species complex. Further analysis also revealed potential biosynthetic gene clusters which could pave the way to the identification of novel antimicrobials. Our study not only improves our knowledge of axillary corynebacteria but also greatly expands the number of corynebacterial genomes, complementing recent studies seeking to understand the diversity of skin corynebacteria.

Staphylococcal colonisation and interspecies competition in the skin microbiome

Joan Geoghegan, Tabitha Parker, Mary Turley, Sophie Dunn

University of Birmingham, Birmingham, United Kingdom

Abstract

During atopic dermatitis disease flares, the skin microbiome undergoes dysbiosis, with a reduction in microbial diversity resulting in an overrepresentation of *Staphylococcus aureus,* which in turn exacerbates the disease. Understanding the factors that influence bacterial colonisation of the stratum corneum is essential to inform effective methods to restore a healthy skin microbiome. Here we investigate bacterial adhesins that promote interaction with the stratum corneum during an atopic dermatitis flare. We demonstrate that *S. aureus* targets a number of corneocyte (terminally differentiated keratinocyte) and plasma proteins. We show that coagulase-negative staphylococci (CoNS) interfere with *S. aureus* adhesion to key ligands found within the stratum corneum and shed light on the mechanisms involved. In summary our data provide a deeper understanding of bacterial colonisation mechanisms and interspecies competition in the human skin microbiome.

Finding the Needle in the Haystack: Microbial Surveillance in Complex Samples

Invited talk: PATH-SAFE: a UK Government surveillance pilot for foodborne pathogens and AMR

Robin May ORCID iD

Food Standards Agency, London, United Kingdom. University of Birmingham, Birmingham, United Kingdom

Abstract

PATH-SAFE is a cross-government pilot to coordinate whole-genome surveillance for foodborne pathogens and antimicrobial-resistant strains across the food chain. Over the last two years, partners across the UK have been coordinating sampling and sequencing efforts in wastewater, on livestock, in milk and in the wider environment in order to start building a comprehensive picture of how pathogens move through the food chain. A large part of the programme has been building a 'user-friendly' database to allow easy upload and interrogation of surveillance datasets. Finally, PATH-SAFE has also been analysing multiple sequencing technologies for their suitability to 'in-field' use. Here we will present highlights from the programme thus far, including key findings to date and some of the advantages and challenges associated with largescale coordination of surveillance data.

Invited talk: Wastewater-based epidemiology: The challenge of finding needles in haystacks and the relevance of needles in a sea of needles

Andrew Singer ORCID iD

UK Centre for Ecology & Hydrology, Wallingford, United Kingdom

Abstract

Wastewater-based epidemiology is currently experiencing a renaissance. WBE's earliest incarnation was when John Snow source-apportioned cholera within a waste-contaminated well in 1854. It has evolved to the current near-real-time capability of pandemic coronavirus monitoring in 2020, and the London polio and worldwide monkeypox outbreaks in 2022+. Proposed legislation in Europe aims to monitor antimicrobial resistance within wastewater. What are the limits of WBE? How can WBE best be used to answer important public health questions? Are there ethical limits to what should be done? If ethical debates are not resolved, is the prospect of a global surveillance programme for epidemic and pandemic health threats in jeopardy? Important questions reside in the hallways of those who practice WBE, many of which will not be resolved but will continue to linger until we determine how we, as a society, are prepared to use this new capability. WBE represents a paradigm shift in population health surveillance, however, if the health community does not use the data effectively, it amounts to a monumental waste of time and money. The holistic integration of WBE into government and society will likely provide the best return on investment, but how to achieve this remains to be seen.

Invited talk: A robust eDNA toolkit for exploring oomycete pathogens of plants and animals

David Cooke ORCID iD[1], Eva Randall[1], Beatrix Keillor ORCID iD[1], Peter Cock ORCID iD[1], Leighton Pritchard ORCID iD[2], Debbie Frederickson-Matika ORCID iD[3], Sarah Green ORCID iD[3]

[1]The James Hutton Institute, Dundee, United Kingdom. [2]Strathclyde Institute of Pharmacy & Biomedical Sciences, Glasgow, United Kingdom. [3]Forest Research, Roslin, United Kingdom

Abstract

Oomycetes are an incredibly diverse group of water-borne heterotrophic organisms in the supergroup SAR (stramenopiles, alveolates and rhizarians). Many are parasitic and cause significant damage to economically and environmentally important organisms such as fish, crustacea, seaweeds, many crops and plants in natural ecosystems. Our understanding of the diversity, ecology and management of oomycete taxa is being advanced through new technologies. We have developed a method of environmental DNA (eDNA) metabarcoding based on in situ filtered water samples and have applied it to track plant pathogenic *Phytophthora* and downy mildew species. In parallel, we have exploited synthetic DNA control barcodes to provide an in-built and a robust quality control system to the analysis pipeline. In this paper we describe the application of the method and its expansion from an rDNA Internal Transcribed Spacer based assay to one based on an mtDNA barcode that extends the taxonomic range to diverse groups of 'dark oomycete taxa' that are unrepresented on international databases.

Targeted surveillance of Extended-Spectrum Beta-Lactamase producing Escherichia coli in care settings

Sarah Gallichan ORCID iD[1], Sally Forrest[1], Esther Picton-Barlow[1], Eva Heinz[1], Joseph Lewis[2], Nicholas Feasey[3], Fabrice Graf[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]University of St Andrews, St Andrews, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a global threat to modern medicine. Ineffective treatment of drug-resistant infections results in higher mortality, longer hospital stays and higher costs to the health system. Resistance to 3rd generation cephalosporins (3GC), that are often used empirically to treat severe bloodstream infections, is a leading cause of AMR-associated deaths. Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* (*E.* coli) are resistant to 3GC and have become widespread globally. Understanding transmission pathways of ESBL-producing *E. coli* is essential to implementing targeted prevention to interrupt transmission with the aim to reduce the number of infections. We established an optimised microbiological workflow for the targeted surveillance of ESBL-producing *E. coli* from stool samples and rectal swabs. We compare steps in the workflow from pre-enrichment: broth type, incubation times, and with or without antibiotic selection, and selective plating, to DNA extraction yielding high quality DNA for whole genome sequencing. Further, we applied our optimised workflow and sequenced stool samples from 7 patients to assess the within patient variation of ESBL-producing *E. coli* at the single nucleotide variant (SNV) level. We disclose an optimised method for ESBL-producing *E. coli* isolation from stool or rectal swab using a four-hour pre-enrichment in buffered peptone water without selection before plating on 1 µg/mL cefotaxime-supplemented MacConkey agar and extracting isolates using the Lucigen MasterPure DNA Purification kit. This cost-effective method is sufficient for the analysis of SNV variation between ESBL-producing *E. coli* isolated from patients with high enough resolution to inform genomic epidemiology and transmission modelling.

Applying and developing Nanopore Adaptive Sampling methods for surveillance of pneumococcal populations.

Samuel Horsfield ORCID iD[1,2], John Lees ORCID iD[1,2], Nicholas Croucher[2]

[1]EMBL-EBI, Cambridge, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

Streptococcus pneumoniae is a respiratory pathogen that can cause invasive systemic disease that disproportionately affects young children and the elderly. Drug-based treatment is becoming increasingly ineffective due to multi-drug resistance observed in *S. pneumoniae* globally. Pneumococcal Conjugate Vaccines (PCVs) are a crucial intervention in preventing invasive disease, with formulations targeting the most invasive 'serotypes' which contribute to the highest burden of severe disease.

Accurate and comprehensive surveillance is required to identify new invasive serotypes to include in vaccine formulations. Current surveillance methods are limited in their sensitivity and specificity, as well as resource costs. Nanopore Adaptive Sampling (NAS), is a real-time enrichment method, enabling rapid pathogen identification in metagenome samples. NAS has the potential to significantly reduce the resource requirements and improve accuracy of *S. pneumoniae* surveillance.

Despite its promise, the accuracy limitations of NAS in metagenome-based surveillance have not been explored previously. Here, we analyse NAS specificity, as well as sensitivity for novel serotype detection. We find that NAS cannot distinguish between *S. pneumoniae* and other streptococci when enriching for a whole genome, but is effective when targeting the capsular biosynthetic locus (CBL), the operon which defines the *S. pneumoniae* serotype. We also developed a novel pangenome graph-based algorithm to enrich for previously unobserved serotypes, which may be missed by the current NAS method which aligns to linear references. We show that graph-based NAS outperforms linear alignment when the reference database is missing a target CBL, making it well-suited for metagenome-based *S. pneumoniae* surveillance.

Scalable search of phylogenetic neighbours in SARS-CoV-2 databases using uvaia

Leonardo de Oliveira Martins ORCID iD[1], Alison Mather ORCID iD[1,2], Andrew Page ORCID iD[3]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Theiagen Genomics, Colorado, USA

Abstract

Manipulating SARS-CoV-2 (SC2) genomic data sets is challenging, especially when we need to select only a few sequences from a large database assumed to be representative of our sample. Many SC2 phylogenetic analyses rely on low numbers of ambiguous sites as a measure of quality, since ambiguous sites do not contribute to single nucleotide polymorphism (SNP) differences. We present a novel method, uvaia, for quickly extracting database sequences similar to query sequences of interest. By using measures of sequence similarity that also consider (IUPAC-coded) partially ambiguous sites, uvaia achieves higher granularity than SNP distance-based measures, without compromising their phylogenetic accuracy. By using a comprehensive set of all SC2 genomes sequenced at the Quadram Institute, we show that uvaia can detect differences even between sequences without SNPs. This difference is more pronounced between sequences with more ambiguous sites, which justifies current best practices in SC2 quality control. We furthermore show how uvaia can find sensible neighbours in a maximum likelihood context, which would otherwise be overlooked by SNP-based methods. Uvaia works with compressed files and it can use multiple cores, while using efficiently the computer memory by loading the database in batches. This way it is able to search against millions of genomes on a standard desktop, which may not even fit in memory or disk when uncompressed.

Rapid culture enrichment and host DNA depletion for same-day sequencing-based detection of bloodstream infections and antimicrobial resistance

Mohammad Saiful Islam Sajib ORCID iD[1], Kirstyn Brunker ORCID iD[1,2], Katarina Oravcova ORCID iD[1], Paul Everest ORCID iD[1], Catherine Wilson[3], Michael Murphy[3,4], Taya Forde ORCID iD[1]

[1]School of Biodiversity, One Health, and Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom. [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [3]Department of Microbiology, NHS Greater Glasgow and Clyde, Glasgow Royal Infirmary, Glasgow, United Kingdom. [4]School of Medicine, Dentistry & Nursing, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, United Kingdom

Abstract

Sepsis is a major public-health concern and existing diagnostics tools are suboptimal. Nanopore metagenomic-sequencing (mNGS) can facilitate rapid pathogen and antimicrobial resistance (AMR) detection, but identification is challenged by significant host versus bacterial DNA in bloodstream-infections (BSI). We developed a rapid mNGS-based chemical host DNA depletion (CHDD) workflow, validated with suspected BSI blood-culture samples and rapid culture-enriched spiked blood.

To assess CHDD efficiency, six protocols were benchmarked. The best performing protocol (M-15, developed in-house) was combined with rapid mNGS with/without adaptive sampling (AS) and tested on BACT/ALERT VIRTUO positive (n=14)/negative (n=3) clinical samples. To utilise M-15 prior to blood-culture flagging positive, a rapid-enrichment method was tested with 1-10 CFU of the top-15 bacterial species causing BSI spiked into BACTEC medium enriched with 10 mL sheep blood.

Six protocols removed 52.4-99.9% of host. Sequencing of simulated CHDD samples revealed ~10^4 CFU/mL of bacteria achieves ≥60% bacterial-DNA yield. CHDD BACT/ALERT positive clinical samples had ~98% bacterial-DNA and M-15 reduced 770x, AS 5.58x host versus no CHDD/AS controls. All 17 clinical samples matched blood-culture species results, and 38 AMR genes were detected. 8-hour BACTEC-enrichment yielded ≥10^4 CFU/mL for 12/15 species. Average bacterial yield following CHDD was 85.3% for these species versus 50.5% for the remaining 3/15.

M-15 mNGS, enables species and AMR detection under 8-hours of BACTEC positivity. The rapid protocol yields results <16 hours from sample collection, including 8-hours of culture-enrichment, and <8-hours for M-15 and sequencing. Further research is required to assess its effectiveness on sepsis patient outcomes.

Identification of over 5000 RNA Virus–Derived RdRp Sequences in almost 3000 Publicly Available Transcriptomic Data Sets

Ingrida Olendraite ORCID iD, Katherine Brown ORCID iD, Andrew Firth ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

RNA viruses are abundant and highly diverse and only a fraction of RNA virus species have been catalogued. To cost-effectively expand and characterise the RNA virome, we used77 family-level Hidden Markov Model profiles for viral RNA-dependent RNA polymerases to mine publicly available transcriptomic data sets. We identified 5867 contigs encoding RNA virus RdRps and analysed their diversity, phylogeny, and host associations. Among the 5867 RdRps there were 3228 unique sequences at the 90% similarity threshold. When compared with current Riboviria RdRps, 2961 sequences had identity scores below 90% (often taken as an Operational Taxonomic Unit threshold). Furthermore, 293 sequences had identities below 30%, representing the “dark matter” of Earth’s virome. All these new viruses were sequenced unintentionally as part of other RNA-seq projects, illustrating how existing scientific resources can be exploited for virus discovery.

In parallel, we also queried the NCBI nr/nt virus database, thus in total analysing over 15000 RNA virus RdRp sequences. In some cases the newly identified sequences more than doubled the members of known families (e.g. Iflaviridae, Narnaviridae, Chuviridae), while in others they formed new families or clades. We also described a novel plant virus which forms a clade within Mononegavirales and employs unique splicing within RdRp core motifs, multiple new Orthomyxoviridae-like clades/genera, and the deep evolution of the RdRp and its motif C. Our study expanded the known diversity of RNA viruses, and the 77 curated RdRp Profile Hidden Markov Models provide a useful resource for the virus discovery community.

Metatranscriptomics improves the laboratory diagnosis of infectious intestinal disease from human diarrhoeal samples

Edward Cunningham-Oakes ORCID iD[1,2], Yan Li[3], Blanca Perez-Sepulveda[3], Jay Hinton[3], Charlotte Nelson[4], K Marie McIntyre[5], Maya Wardeh[6,7,8], Sam Haldenby[4], Richard Gregory[4], Miren Iturriza-Gómara[3,9], Christiane Hertz-Fowler[4], Sarah O'Brien[5], Nigel Cunliffe[2,3], Alistair Darby[1,2,4]

[1]Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]NIHR Health Protection Research Unit in Gastrointestinal Infections, Liverpool, United Kingdom. [3]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [4]Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom. [5]School of Natural and Environmental Sciences, Newcastle University, Newcastle, United Kingdom. [6]Department of Computer Science, University of Liverpool, Liverpool, United Kingdom. [7]Department of Livestock and One Health, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [8]Department of Mathematics, University of Liverpool, Liverpool, United Kingdom. [9]Centre for Vaccine Innovation and Access, Program for Appropriate Technology in Health (PATH), Geneva, 1218, Switzerland, United Kingdom

Abstract

Background Conventional laboratory-based surveillance for gastrointestinal pathogens is time-consuming, potentially hindering timely outbreak detection. The INTEGRATE study explored the efficacy of metagenomic and metatranscriptomic sequencing for promptly diagnosing community-associated gastrointestinal infections.

Methods We performed an observational study using stool samples from 1,407 patients with acute gastroenteritis, recruited via general practitioners in the UK. 1,067 stool samples were processed using i) routine clinical methods, ii) a molecular multiplex real-time polymerase chain reaction (PCR) assay, and iii) DNA and RNA sequencing. The relationship between assigned taxonomy, routine clinical diagnostics, and PCR was determined with multivariable linear regression models.

Findings: Strong positive relationships were observed between metatranscriptomic reads and traditional diagnostics for five pathogens (*Campylobacter*, *Cryptosporidium*, *Salmonella*, *Rotavirus*, *Sapovirus*). Metagenomic sequencing displayed this relationship for two pathogens (*Campylobacter*, *Salmonella*). Additionally, metatranscriptomic reads showed strong positive relationships with PCR results for six pathogens (*Adenovirus*, *Campylobacter*, *Cryptosporidium*, *Norovirus*, *Rotavirus*, *Sapovirus*), while metagenomic data displayed this relationship for four pathogens (*Adenovirus*, *Campylobacter*, *Salmonella*, *Shigella*). A comprehensive transcriptomic profile of *Salmonella* Enteritidis was recovered from a later-confirmed *Salmonella* infection.

Interpretation Metatranscriptomics effectively identified diverse gastrointestinal pathogens, offering direct viral and bacterial gene expression profiling from stool samples. We propose using metatranscriptomics for the future surveillance of gastrointestinal pathogens.

‘One Health’ Genomic Surveillance of Avian and Human Influenza A Viruses Through Environmental Wastewater Monitoring

Aaron G. Canton[1], Andrew J. Lee[1], Marina Reyne[1], Cormac McSparron[2], James McKenna[3], Derek Fairley[3], Jennifer M. McKinley[2], Deirdre F. Gilpin[4], Ken Lemon[5], John McGrath[6], Connor G. G. Bamford[6]

[1]School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom. [2]School of Natural and Built Environment, Queen's University Belfast, Belfast, United Kingdom. [3]Regional Virus Laboratory, Belfast, United Kingdom. [4]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom. [5]Agri-Food and Biosciences Institute (AFBI), Belfast, United Kingdom. [6]School of Biological Sciences and Institute for Global Food Security, Queen's University Belfast, Belfast, United Kingdom

Abstract

Influenza A viruses (IAV) are significant pathogens of humans and other animals. Although endemic in humans and birds, novel IAV strains can emerge, jump species, and cause epidemics, like the latest variant of H5N1 in wild birds. Wastewater-based epidemiology (WBE) has very recently been shown to detect human IAV but whether it can detect avian-origin (or non-human) IAV, and if whole genome sequencing (WGS) can be used to discriminate circulating strains of IAV in wastewater remains unknown.

Using a pan-IAV RT-qPCR assay, six wastewater treatment works (WWTWs) across Northern Ireland (NI), were screened from August to December 2022 alongside respiratory syncytial virus (RSV) and SARS-CoV-2. A WGS approach using Oxford Nanopore technology was employed to sequence positive samples. Phylogenetic analysis of sequences relative to currently circulating human and avian IAVs was performed. We detected a dynamic IAV signal in wastewater from September 2022 onwards across NI. “Meta” whole genome sequences were generated displaying homology to both human and avian IAV strains. The relative proportion of human versus avian-origin IAV reads differed across time and sample site. A diversity in subtypes and lineages was detected (e.g. H1N1, H3N2, and several avian). Avian segment 5 and 8 related to those found in recent H5N1 clade 2.3.4.4b was identified.

WBE affords a means to monitor circulating human and avian IAV strains and provide crucial genetic information. As such WBE can provide rapid, cost-effective, year-round “one-health” IAV surveillance to help control epidemic and pandemic threats in conjunction with extant testing.

Building-level wastewater surveillance localizes influenza interseasonal variation and norovirus non-residential spread

Emma Germano, Tiffany Flores, River Kim, JJ Miranda

Barnard College, Columbia University, New York, USA

Abstract

Influenza and norovirus pose recurring and prevalent threats to public health. We aimed to leverage longitudinal building-level wastewater-based surveillance (WBS) over consecutive years to assess the feasibility of studying interseasonal variation and community spread. Our WBS program measured viral RNA in the sewage outflow of dormitories and non-residential buildings at an urban New York City college between 2020 and 2023. We were able to detect the arrival and estimate the case magnitude of seasonal influenza A. Wastewater test positivity strongly correlated with New York County clinical cases. Positive wastewater samples are also associated with campus clinical cases. The 2022 data stands in stark contrast to the 2021 results by revealing the more frequent and earlier presence of influenza A. It is further noteworthy that positive samples were not evenly distributed among buildings. Understanding the onset of seasonal influenza waves could increase the index of suspicion, encourage mitigation strategies, and guide vaccination recommendations. We were also able to detect the prevalent distribution of norovirus to a non-residential setting. In 2022, Both GI and GII noroviruses frequently appeared in the library at rates higher than in dormitories. We suspect that this presence may be the result of asymptomatic infections or behavioral pressure to work regardless of symptoms. Understanding the scope of norovirus distribution could promote heightened cleaning protocols in addition to awareness communications. Our results highlighting the ability of building-level WBS to localize influenza and norovirus incidence has the potential to guide public health intervention.

A taxonomy-free approach to classifying SARS-CoV-2 spike protein haplotypes from wastewater sequencing reveals circulating cryptic variants missed by clinical sequencing

John-Paul Wilkins, Francesco Rubino, Marina J. Reyne, Andrew Lee, Evan Troendle, David Simpson, Jennifer M. McKinley, Deirdre F. Gilpin, John McGrath, Christopher J. Creevey

Queen's University Belfast, Belfast, United Kingdom

Abstract

DNA sequencing of SARS-CoV-2 spike protein sequences from wastewater (WW) samples has been used to monitor the temporal and geographic spread of the virus from 2021 until recently. The resulting data is normally analysed by comparison to a database of known isolates (usually from clinical samples), but this runs the risk of misidentifying or discarding as noise unmatched, or 'cryptic', variants.

To address this, we developed a computational approach to identify cryptic sequences in WW samples from Northern Ireland, utilising reconstructed viral haplotypes for the spike protein. A sequence-only numeric classifier system was used to link WW spike sequences to clinical viral genomes in the same geographical region. Spike protein haplotypes exclusively found in WW samples across consecutive sampling timepoints would indicate the presence of cryptic strains not accounted for in the clinical sequencing.

The vast majority of spike protein haplotypes were found in both WW and clinical samples. However, a subset of re-occurring WW haplotypes did not match any in the clinical samples. These WW spike protein haplotypes showed clear, non-random patterns indicating timelines for the emergence and disappearance of cryptic variants circulating in Northern Ireland during the pandemic.

Our results show that WW viral genomic sequencing not only provides real-time data on the prevalence of known viral isolates but also contains a wealth of information on cryptic variants not captured by clinical sequencing. This supports the use and expansion of wastewater sequencing to monitor known circulating pathogens, and act as a sentinel for emerging and cryptic diseases.

Genetics and Genomics Forum

The hunger strikes back: forging a cunning aggressor, *Acinetobacter baumannii*

Woojun Park ORCID iD

Korea University, Seoul, Korea, Republic of

Abstract

The molecular processes governing the adaptive evolution of multidrug-resistant (MDR) pathogens in vitro and in vivo, especially in the absence of antibiotic pressure, are poorly understood. Genomic analysis of MDR A. baumannii cells cultured for 8,000 generations under starvation conditions (EAB1) or nutrient-rich conditions (EAB2) revealed significant genomic rearrangements, gene losses, and the addition of insertion sequence (IS) elements. Remarkably, thirty antibiotic-resistance genes retained their number and positions unchanged, showcasing exceptional stability throughout evolution. EAB1 exhibited diminished ability to form biofilms and adhere to lung epithelial cells. However, it displayed heightened invasiveness in mice, evading the immune system and spreading to host organs, ultimately resulting in host mortality. However, EAB2 exhibited a strong attachment to epithelial cells, leading to increased proinflammatory cytokine and chemokine synthesis. This study highlights how MDR A. baumannii adapted through genomic changes during starvation, resulting in altered biofilm formation and noncanonical invasive behavior.

Modelling gene flows into early eukaryotes

Tom Williams

University of Bristol, Bristol, United Kingdom

Abstract

The origin of eukaryotic cells - cells with a nucleus and mitochondria - was a key event in the history of life. Current hypotheses for eukaryotic origins emphasise the importance of symbiosis between Archaea and Bacteria, with the prevailing scenario invoking a symbiosis between a member of the Asgard archaea and an alphaproteobacterium that became the mitochondrion as a pivotal early event during eukaryogenesis. Beyond these two symbiotic partners, other genetic contributions have also been suggested, including - for example - the involvement of a Deltaproteobacteruin that might have provided the bacterial-type membrane of eukaryotic cells. However, testing these scenarios empirically has been challenging because it is difficult to disentangle signal from noise in the inference of ancient evolutionary relationships. Here, I will describe some new phylogenetic methods for testing hypotheses about the number of ”gene flows” (large-scale genetic contributions) into early eukaryotes. These methods provide an empirical framework for testing between the different scenarios, and I will present results that identify additional gene flows into eukaryotes, including from ancient Chlamydiae and other lineages, that have not traditionally been considered in origin-of-eukaryotes scenarios. Overall, analyses with these new methods suggest that the origins of eukaryotic genome chimerism are perhaps more complex and varied than has been considered previously.

The *Trypanosoma brucei* RNA/DNA hybrid interactome reveals a role for RAD51 in R-loop homeostasis and repair of VSG-localized DNA breaks during antigenic variation

Mark John Girasol[1,2], Jeziel Damasceno[2], Catarina Marques[2], Marija Krasilnikova[2], Jose Batista[2], Craig Lapsley[2], Ross Carruthers[3], Dario Beraldi[2], Pilarita Rivera[4], Emma Briggs[5], Richard McCulloch[2]

[1]College of Medicine, University of the Philippines Manila, Manila, Philippines. [2]Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom. [3]Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom. [4]College of Public Health, University of the Philippines Manila, Manil, Philippines. [5]School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom

Abstract

R-loops are three-stranded nucleic acid structures comprised of an RNA/DNA hybrid and a displaced single-stranded DNA, which usually arise when an elongating transcript reinvades the template DNA or in trans. They can serve as obstacles during replication and can cause DNA damage. Mapping R-loops in *Trypanosoma brucei* revealed widespread enrichment, including in subtelomeric Variant Surface Glycoprotein (VSG) expression sites, linking them to DNA damage and antigenic variation. However, the mechanisms that control many aspects of R-loop biology in the *T. brucei* genome remain unclear. Using RNA/DNA hybrid immunoprecipitation coupled with mass spectrometry, we identified four putative interactors that may be involved in VSG switching: the recombinases RAD51 and RAD51-3, a putative SNF2 chromatin remodeler (ATRX), and an ATP-dependent DEAD/H RNA helicase (DDX60). Loss of all proteins each led to nuclear genome damage and alterations in VSG expression dynamics. Loss of RAD51 resulted in a global decrease in R-loop abundance, while depletion of ATRX, DDX60, and RAD51-3 led to a global increase. Genome-wide mapping of R-loop distribution in RAD51 mutants using DRIP-seq indicated depletions in R-loops at genomic sites including VSG-associated 70-bp repeats. Using Breaks Labelling In Situ and Sequencing (BLISS) we found pronounced levels of DNA breaks that localize to the 3' end of the expressed VSG and become more abundant in RAD51 mutants. Our data reveal multiple unexplored activities that may influence R-loop function in the *T. brucei* genome and provide a mechanistic link between R-loops and the parasite's ability to evade host immunity through VSG switching.

Staphylococcus haemolyticus Diversity: Insights into Pathogenic Strains and Commensal Variation

Heather Felgate ORCID iD[1], Lisa Lamberte ORCID iD[2], Antia Acuna-Gonzalez ORCID iD[1], Janet Berrington ORCID iD[3], Jonathan Chapman ORCID iD[3], Leonardo de Oliveira Martins ORCID iD[1], Christopher J Stewart ORCID iD[3], Ad C. Fluit ORCID iD[4], Matt K. O'Shea[2], Jorunn Pauline Cavanagh ORCID iD[5], Lindsay Hall ORCID iD[1,2], Willem van Schaik ORCID iD[2], Mark A. Webber ORCID iD[1]

[1]Quadram Institute, Norwich, United Kingdom. [2]Birmingham University, Birmingham, United Kingdom. [3]Translational and Clinical Research Institute, Newcastle, United Kingdom. [4]University Medical Center Utrecht, Utrecht, Netherlands. [5]The Arctic University of Norway, Tromso, Norway

Abstract

Staphylococcus haemolyticus is a common skin commensal and important opportunistic pathogen capable of causing infections including bacteraemia. Previous studies have characterised isolates from patients but how these relate to the wider phylogeny of the species and commensal strains remains uncertain. To address this knowledge gap, we assembled and characterised a panel of 820 *S. haemolyticus* isolated from across Europe between 1975-2022, combined with 166 genomes in NCBI and created a phylogenetic tree based on the core gene alignment.

The phylogenetic tree shows significant variation across the species with a conserved core ~1800 genes. Closely related isolates were observed in multiple hospitals across countries highlighting likely transmission of successful clones. Genes associated with clinical isolates were involved in metal transport and isolates taken from blood had significantly more genes associated with iron homeostasis and high rates of carriage of *sodA.* Analysis of source metadata revealed the *crtOPNM* genes, involved in production of staphyloxantin, were significantly associated with isolates from neonatal blood. We also identified a significant reservoir of diverse plasmids and phages in these isolates.

In conclusion there are multiple variants of *S. haemolyticus* across Europe in circulation with some clones appearing to have developed increased pathogenicity in neonates compared to adults. Other variants appear more generalist and are associated with carriage on skin and in the gut, appearing to cause infection only occasionally in vulnerable patients. This better understanding of the epidemiology of *S. haemolyticus* provides a starting point to study future evolution of this important organism.

TraDIS-Xpress identifies mechanisms of action and synergies of a novel lipid IVA biosynthesis inhibitor

Emma Holden ORCID iD[1], Muhammad Yasir ORCID iD[1], Keith Turner ORCID iD[1], Mark Webber ORCID iD[1], Ian Charles[1,2], Ed Siegwart[3], Tony Raynham[3], Ajay Mistry[3], John George[3], Matthew Gilmour ORCID iD[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Oppilotech, London, United Kingdom

Abstract

The development of novel antimicrobials provides additional treatment options for infectious diseases, including antimicrobial resistant infections. There are many hurdles to antimicrobial development and identifying an antimicrobial's mechanism of action is a crucial step in progressing candidate molecules through the drug discovery pipeline. We used the genome wide screening method TraDIS-Xpress to identify genes in two model Gram-negative bacteria that affected sensitivity to three analogues of a novel antimicrobial compound (OPT-2U1). TraDIS-Xpress identified that all three analogues targeted the lipid IVA biosynthetic pathway in E. coli and Salmonella Typhimurium. Specifically, we determined that the antimicrobial target was likely to be LpxD, and validated this by finding a 5 log2-fold increase in the MIC of the OPT-2U1 analogues in E. coli when lpxD was overexpressed. LpxD is encoded by an essential gene in both species and therefore cannot be assayed via conventional transposon sequencing approaches. This showcases the novelty and efficacy of TraDIS-Xpress for the identification of drug targets. Synergies were identified between OPT-2U1 analogues combined with rifampicin or colistin, to varying strengths, in both E. coli and S. Typhimurium. Finally, genes involved in ATP synthesis and membrane signalling functions were also found to affect the synergy between colistin and OPT-2U1 analogues. This study has confirmed the predicted target pathway of OPT-2U1 and identified synergies which could be investigated for development of novel antimicrobial formulations. TraDIS-Xpress has proven a powerful tool to rapidly assay all genes (and notably, essential genes) within a bacterium for roles in dictating antimicrobial sensitivity.

Genomic relics of bacterial warfare in commensal *Escherichia coli* populations

Elizabeth Cummins ORCID iD[1], Rebecca Hall ORCID iD[2], Alan McNally ORCID iD[2], Samuel Sheppard ORCID iD[1]

[1]University of Oxford, Oxford, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

The pandemic multi-drug resistant (MDR) *Escherichia coli* clone of ST131 is a threat to public health globally. ST131 emerged in clinical settings in the UK during the early 2000s before expanding to become a dominant cause of *E. coli* bloodstream infections worldwide. MDR ST131 are capable of displacing commensal *E. coli* in the intestinal tract as observed in studies where travellers are rapidly colonised by MDR *E. coli* upon arrival to AMR endemic regions. However, the dominance of ST131 in the microbiome is transient and other *E. coli* return after a period of dysbiosis. The reasons for this are not understood. Specifically, while ST131 clearly has a major fitness advantage when colonising naïve hosts, it is not known how resident strains overcome displacement to rebalance the population. One explanation is that the alleles that confer beneficial colonisation phenotypes to ST131 are acquired by resident *E. coli,* over time allowing them to compete with the invading strains. To test our hypothesis, we quantified the prevalence of 234 ST131-specific core alleles in *E. coli* populations before (-1999) and after (2015-) the global ST131 epidemic. Of the ST131-core alleles, 37% (n=86) were completely absent in the pre-2000 data set whereas all ST131-core alleles were present in the post-2014 data set. Overall, 70% (n = 163) of the ST131-core alleles increased in prevalence in other *E. coli* lineages over this time. Quantifying the genomic scars of transient colonisation provides information about the chronology of pandemic clone emergence and the alleles allowing them to proliferate.

CarD: Coupling Gene Expression to DNA Supercoiling in Rhodobacter

David Forrest, David Grainger

Institute for Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

CarD is a major transcription factor involved in stabilising the melted DNA strands during transcription initiation. Whilst not found in γ-proteobacteria such as *E. coli,* CarD is essential in many bacteria such as the human pathogen *Mycobacterium tuberculosis* and the photosynthetic bacterium *Rhodobacter sphaeroides.*

Whilst investigating global transcription initiation in *R. sphaeroides*, we noticed a relationship between CarD, transcription, and DNA supercoiling. After perturbing negative supercoiling with the DNA gyrase inhibitor novobiocin, ChIP-seq revealed reduced binding of CarD and σ^{93} (the major σ factor) to chromosomal DNA. This was coupled with downregulation of CarD-dependent gene expression, suggesting CarD activity requires negative supercoiling. We next probed global DNA supercoiling patterns with Psora-seq, which uses the chemical psoralen to intercalate into negatively supercoiled DNA. Chromosomal regions with reduced CarD binding in novobiocin treated cells also had positive DNA supercoiling. *In vitro* transcription assays on DNA with increasing levels of negative supercoiling reaffirmed our findings. To analyse the CarD regulon, predicted and annotated functions were assigned from orthologous sequences. CarD predominantly regulated genes encoding proteins and RNAs functioning in metabolism and translation, including ribosomal and tRNA genes.

Our work shows CarD regulation of gene expression is dependent on the supercoiling state of the chromosomal DNA. Given that levels of DNA supercoiling fluctuate according to environmental conditions, coupling supercoiling to CarD offers another layer of gene regulation. As suggested by the predicted functions of the CarD regulon, this could allow *R. sphaeroides* to adapt its growth and cellular processes to the external environment.

A versatile protein Swiss army knife: *Mycobacterium abscessus* encodes diverse modular ESX substrates

Kieran Bowran ORCID iD[1], Patrick Moynihan ORCID iD[2], Tracy Palmer ORCID iD[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

Non-tuberculous mycobacteria including *Mycobacterium abscessus* are an emerging group of environmental pathogens capable of causing chronic infections in immunocompromised groups. *M. abscessus* prevalence has increased over the past decade within the UK cystic fibrosis population, together with extensive antibiotic resistance it is now a pathogen of notable concern. Like Bacilliota, mycobacteria encode type 7 secretion systems termed ESX systems; consisting of a multi-protein complex embedded in the inner cell membrane, involved in trafficking substrates. *M. abscessus* encode two ESX systems – ESX-3 and ESX-4. The function of ESX-3 is heavily implicated with iron scavenging of the environment and macrophages during infection; whereas the biological role of ESX-4 and its substrates remains elusive, with differing proposed roles amongst mycobacteria.

We undertook a large-scale bioinformatic analysis, identifying over 50 predicted ESX-secreted substrates within *M. abscessus*. These substrates possessed highly variable C-termini encompassing a diverse range of biochemical functions and cellular targets, each with an associated immunity protein akin to Bacilliota T7b substrates. *M. abscessus* strains were determined to heterogeneously encode between 10 – 12 ESX substrates at variable sites across the chromosome. Each of these variable chromosomal sites co-occurred with specific N-terminal alpha-helical stems of the WXG100 family. Furthermore, each alpha-helical stem was associated with specific small proteins known to facilitate secretion in T7b systems. Secretion of these substrates was confirmed to be solely attributed to ESX-4. The heterogeneous composition of these ESX substrates within *M. abscessus* and the distribution of ESX-4 loci across Actinomycetota indicates a competitive role for ESX-4 within the environment.

Microbial Life in Acid Mine Drainage: Detection of Novel Functions Through Metagenomics

Michael McDonald, Fiona Strathdee, Andrew Free

The University of Edinburgh, Edinburgh, United Kingdom

Abstract

Acid mine drainage (AMD) poses a worldwide threat to human and environmental health, particularly through freshwater contamination with mobile metallic pollutants. Central Scotland has been mined extensively and abandoned mine wastes have led to environmental degradation and elevated metal toxicity. Our work has focused on a previously mined site within the Central Belt, which is continually generating chemically hazardous and acidic effluent.

How the microbial community native to AMD leachate can persist and contribute to wider ecosystem functioning remains unclear. Current understanding of acidophiles' functional potential is limited despite their metabolic potential in aiding environmental recovery. Through metagenomic sequencing, reconstruction and annotation of metagenome assembled genomes (MAGs) using Nanopore Sequencing, we have observed functions previously not reported in members of low pH microbial communities. This metabolic potential includes strategies to counteract deleterious concentrations of heavy metals, mechanisms of acid-tolerance, and additional metabolic pathways which underpin wider biogeochemical cycling under extreme physiological constraints.

Our high-quality genome assemblies pertaining to taxa from within Pseudomonadota and Actinomycetota expand on the extremely limited literature on the metabolic repertoire of acidophiles. In particular, the reconstruction of a MAG from *Metallibacterium* provides novel insights into the metabolic roles of this poorly characterised organism and indicates its potential role in bioremediation. Our metagenomic findings provide a broad overview of the metabolic and biogeochemical potential of the community in this highly polluted site and can guide further work to explore routes to exploit the function of microorganisms which proliferate in this challenging and unique ecosystem.

Eco-evolutionary interactions of bacteria, phages, and antibiotics within cholera patients

Jesse Shapiro

McGill University, Montreal, Canada

Abstract

A century has passed since the discovery that virulent bacteriophages are associated with improved survival of cholera patients. Despite an increasingly detailed picture of the molecular mechanisms that govern phage-bacterial interactions, we lack an understanding of how these interactions impact disease severity. Here we report a year-long, nation-wide study of diarrheal disease patients in Bangladesh. Among the subset of cholera patients, we quantified *Vibrio cholerae* (prey) and its virulent phages (predators) using metagenomics and quantitative PCR, while accounting for antibiotic exposure using quantitative mass spectrometry. Virulent phage (ICP1) and antibiotics suppressed *V. cholerae* to varying degrees and were inversely associated with severe dehydration; aspects of these effects were dependent on resistance mechanisms. In the absence of anti-phage defenses, predation was 'effective' with a high predator to prey ratio that correlated with increased genetic diversity among the prey. In the presence of anti-phage defenses, predation was 'ineffective' with a low predator to prey ratio that correlated with increased genetic diversity among the predators. Our results link phage predation to disease severity, support phage-bacteria coevolution within patients, and suggest the ratio of phage to pathogen can serve as a biomarker for clinical, diagnostic, and epidemiologic applications.

Genome-scale metabolic modelling reveals *Klebsiella pneumoniae* metabolic traits associated with multidrug resistance.

Helena Cooper[1], Ben Vezina[1], Jane Hawkey[1], Kathryn Holt[1,2], Kelly Wyres[1]

[1]Monash University, Melbourne, Australia. [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

The *Klebsiella pneumoniae* Species Complex (KpSC) comprises seven closely-related *Klebsiella* taxa and causes nosocomial and multidrug resistant infections globally. Phenotypic assays have revealed metabolic diversity within the KpSC, which could influence the evolution of drug resistance through differential survival in niches that increase exposure to antibiotic resistance genes. However, this relationship remains largely neglected as metabolism experiments are time-consuming and cost-intensive.

Genome-scale metabolic models represent the metabolic network of an organism and have been used to investigate bacterial metabolism at scale. We have constructed a pangenome scale metabolic model representing 507 diverse KpSC isolates, which can generate strain-specific models and predict growth phenotypes with 95.4% median accuracy (n=124 distinct carbon sources in aerobic conditions). To identify metabolic traits associated with multidrug resistance, the KpSC pan model was utilised to build strain-specific models for 917 clinical isolates. Reaction, gene and predicted growth phenotype information were used in population-structure aware association analyses. Preliminary analyses identified traits previously associated with multidrug resistance (eg: mercury resistance), plus additional traits including restriction modification systems ($p < 3.70x10^{-4}$) and predicted growth using formamide ($p < 1.61x10^{-3}$). Initial genomic analyses indicate that the associated genes are located on plasmids, and ongoing work will determine whether antimicrobial resistance genes are co-localised on the same replicons.

This work demonstrates the utility of the KpSC pan metabolic model as a reference for population metabolism analyses that can identify associations between metabolic and clinically relevant traits. Such analyses highlight targets for novel drug development or surveillance of emerging drug resistant strains.

The evolution of a bacterial tRNA gene set by within-genome duplication events

Wing Yui Ngan ORCID iD[1], Florence Bansept ORCID iD[1,2], Arne Traulsen ORCID iD[1], Jenna Gallie ORCID iD[1]

[1]Max Planck Institute for Evolutionary Biology, Ploen, Germany. [2]Aix-Marseille Université, Marseille, France

Abstract

The transfer RNA (tRNA) content of cells affects the efficiency of protein synthesis. To study how organisms can adaptto novel translational demands, our laboratory uses two engineered strains of *Pseudomonas fluorescens* SBW25, each lacking one or more tRNA genes. Previously, we have shown that these slow-growing strains rapidly recover fitness in serial transfer evolution experiments by duplicating large chromosomal segments (up to 1 Mb), each containing a small (~100 bp) compensatory tRNA gene. However, while adaptive, these large duplications are mechanistically unstable and hence are unlikely to persist over longer evolutionary time scales. Here, we investigate the evolutionary fate of the duplications and the new tRNA gene copies that they contain. We extend the evolution experiment to 100 transfers (~700 generations) and characterize the evolving lines through isolate and population sequencing. We find that, within each evolving population, various duplication fragments rapidly arise and compete. Over time, progressively smaller – and hence, mechanistically more stable – duplication fragments arise and dominate in all lineages. The smallest of these is a duplication fragment of only 236 bp, encompassing the compensatory tRNA gene and promoter. Our results provide a detailed, real-time example of a bacterial tRNA gene set evolving in response to translational challenges.

The resistome of *Vibrio cholerae* and its variability between lineages

Avril Coghlan ORCID iD[1], Corin Yeats ORCID iD[2], Sina Beier ORCID iD[1], Florent Lassalle ORCID iD[1], Sam Dougan[1], Adrián Cazares ORCID iD[1], Anne Bishop ORCID iD[1], Alyce Taylor-Brown ORCID iD[1], Mathew Beale ORCID iD[1], David Aanensen ORCID iD[2], Matthew Dorman ORCID iD[1,3], Nicholas Thomson ORCID iD[1]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom. [2]Centre for Genomic Pathogen Surveillance, Oxford, United Kingdom. [3]University of Galway, Galway, Ireland

Abstract

Cholera, a disease characterised by acute watery diarrhoea, can kill within two days if left untreated. Since the 1960s there has been a worrying increase in resistance to many antimicrobials used to treat the most vulnerable patients. We set out to identify all antimicrobial resistance (AMR) genes and variants found in *Vibrio cholerae*, including to tetracyclines, quinolones and macrolides (the classes of antimicrobials recommended by the WHO for cholera treatment), and assess their effects on resistance phenotypes. To do this, we have collated an extensive data set of 6014 genomes from historical and recent *V. cholerae* isolates, along with manually curated metadata, including AMR phenotypes from laboratory tests. To study the distribution of these resistance determinants across the species tree, we created a database of *V. cholerae* genomes clustered by lineage using PopPUNK. Interestingly, we found that the repertoire of AMR factors in *V. cholerae* is highly lineage-specific, with a striking preponderance of resistance determinants in the current pandemic lineage 7PET. To leverage this resource for public health, we have released it as part of Pathogenwatch (https://pathogen.watch) as the Vibriowatch database. This platform provides easy-to-use bioinformatics tools to allow microbiologists and clinicians to use genomic data generated from isolates collected during a cholera outbreak, to answer key questions relevant to public health. These include whether the lineage of the outbreak of *V. cholerae* is 7PET; what is its predicted AMR profile; and how is the outbreak related to current and previous outbreaks in the region and around the world.

A synthetic genomics approach to elucidating gene sets that underly functional and effective symbiotic nitrogen fixation in rhizobia

Barney Geddes, Ahmad Al Amad, Garrett Levin

North Dakota State University, Fargo, USA

Abstract

Engineering cereals that form root nodules and fix nitrogen in association with rhizobia is a holy grail of biotechnology. Doing so will require an exhaustive understanding of the genetic programming that underlies how rhizobia form intracellular, nitrogen-fixing symbiosis with legume plants. To this end, we are taking a synthetic genomics approach to elucidate the base components of this programming. By using megaplasmid-cured rhizobial strains as chassis for the re-introduction of defined complements of genes and testing for recovery of symbiotic phenotype, we are elucidating the minimum subset of genes required to engage in symbiosis with legumes[1]. By applying this approach in the model rhizobium *Sinorhizobium meliloti*, we have identified some new gene sets required for optimal symbiosis with its *Medicago* hosts, and others that limit symbiotic effectiveness and lead to enhanced crop productivity once removed. Finally, we have explored adding heterologous gene sets to engineer "new-to-nature" symbiosis with alternate hosts. This has allowed us to predict genetic loci that, while not critical for symbiosis with native hosts, become important determinants of non-host nodule colonization and nitrogen fixation. Together these findings help set the stage for optimizing rhizobium-legume symbioses and developing new-to-nature symbiosis between rhizobia and engineered root-nodule forming cereals.

1. Geddes, B. A., Kearsley, J., Huang, J., Zamani, M., Muhammed, Z., Sather, L., Panchal, A., diCenzo, G., and Finan, T. M. 2021. Minimal gene set from *Sinorhizobium* (*Ensifer*) *meliloti* pSymA required for efficient symbiosis with *Medicago*. *Proceedings of the National Academy of Sciences.* **221** (2) e2018015118.

The emergence and diversification of a zoonotic pathogen from within the microbiota of intensively farmed pigs

Gemma Murray[1], Mukarram Hossain[2], Sebastian Bruchmann[3], Andrew Balmer[3], Josephine Herbert[4], Marta Matuszewska[3], Robert Mugabi[5], Ganwu Li[5], H2020 PIGSs Consortium[6], ZELS Myanmar Pig Partnership Consortium[3], Stephen Bentley[7], Ngo Thi Hoa[8], Maria Clavijo[5], Jerry Wells[6], Dan Tucker[3], Lucy Weinert[3]

[1]University College London, London, United Kingdom. [2]Cancer Research UK, Manchester, United Kingdom. [3]University of Cambridge, Cambridge, United Kingdom. [4]University of Portsmouth, Portsmouth, United Kingdom. [5]Iowa State University, Ames, Iowa, USA. [6]University of Wageningen, Wageningen, Netherlands. [7]Wellcome Sanger Institute, Cambridge, United Kingdom. [8]Oxford University Clinical Research Unit, Ho Chi Minh city, Vietnam

Abstract

There is concern that growth in livestock populations and changes in farming practices are driving the emergence of pathogens capable of causing disease in both livestock and humans. In this study we investigated how the livestock microbiota can act as a source of these emerging pathogens through examining *Streptococcus suis*, a ubiquitous component of the respiratory microbiota of pigs that is also a major cause of disease on pig farms and an important zoonotic pathogen. We analysed the genomes of 3,070 *S. suis* isolates taken from pigs, wild boar, and humans, from Europe, North America, Asia and Australia. Combining molecular dating, phylogeography, and comparative genomic analyses, we found that several pathogenic lineages of *S. suis* emerged in the 19th/20th centuries, during an early period of growth in pig farming. These lineages have repeatedly spread between countries and continents, mirroring trade in live pigs, and are distinguished by the presence of three genomic islands with putative roles in metabolism and cell adhesion, and an ongoing reduction in genome size. Through reconstructing the evolutionary histories of these islands we found that pathogenic lineages have consistently emerged from one subpopulation of *S. suis* and acquired genes from other pathogenic lineages. These results shed light on the capacity of the microbiota to rapidly evolve to exploit changes in their host population and suggest that the impact of changes in farming on the pathogenicity and zoonotic potential of *S. suis* is yet to be fully realised.

First insights into genomes from *Anopheles*-infecting *Wolbachia* strains.

Thomas Walker ORCID iD[1], Shannon Quek ORCID iD[2], Claire Jeffries[1], Louise Cerdeira ORCID iD[2], Louise Cerdeira[2], Janvier Bandibabone[3], Vishaal Dhokiya ORCID iD[1], Roland Bamou ORCID iD[4,5], Mojca Kristan[1], Louisa Messenger[1], Alexandra Gidley ORCID iD[1], Sean Tomlinson[2], Sean Tomlinson ORCID iD[2], Emily Hornett ORCID iD[2,6], Enyia Anderson[2], Cintia Cansado-Utrilla[2], Shivanand Hegde[2], Chimanuka Bantuzeko[3], Jennifer Stevenson[7,8], Neil Lobo[9], Simon Wagstaff[2], Christophe Nkondjio[4], Seth Irish[10], Eva Heinz ORCID iD[2], Grant Hughes ORCID iD[2]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom. [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [3]Centre de Recherche en Sciences Naturelles (CRSN/LWIRO), Sud-Kivu, Congo, the Democratic Republic of the. [4]Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon. [5]University of Dschang, Dschang, Cameroon. [6]University of Liverpool, Liverpool, United Kingdom. [7]Macha Research Trust, Macha, Zambia. [8]Johns Hopkins University, Baltimore, USA. [9]University of Notre Dame, Notre Dame, USA. [10]Centers for Disease Control and Prevention, Atlanta, USA

Abstract

Wolbachia are obligate bacterial endosymbionts that infect a diverse range of arthropod species. Infections in mosquitoes have been shown to inhibit the transmission of human pathogens, with the most prominent success being the 77% reduction of Dengue cases in Yogyakarta, Indonesia, following the introduction of *Wolbachia*-infected *Aedes aegypti* mosquitoes. Despite their large host range, high-density *Wolbachia* infections of *Anopheles* mosquitoes, including all major malaria vectors, have been difficult to conclusively prove, with identification frequently based only on sensitive nested PCR evidence, and no *Wolbachia* has yet been identified in the >2,500 genomes available via the *Anopheles gambiae* genome consortium. Recently, we have shown the presence of two novel strains of *Wolbachia* in high-density infections in the poorly studied species *Anopheles moucheti* (*w*AnM) and *An. demeilloni* (*w*AnD). Genomic analysis of the two *Wolbachia* shows a reduced genome for both strains when compared to their closest-related *Wolbachia* strains infecting *Drosophila simulans* (*w*No) and *D. mauritiana* (*w*Ma, *w*Mau), while still encoding for all major conserved biochemical pathways. Genome reduction in the *Anopheles*-associated *Wolbachia* are due to degenerated prophage genes and insertions that are normally frequent in *Wolbachia* of insects, and which are predicted to influence *Wolbachia* persistence in insect populations. Additionally, only *w*AnD was noted to contain uninterrupted cytoplasmic incompatibility factor genes, which are responsible for *Wolbachia*'s sexual parasitism phenotype. The identification of these two novel strains will further understanding of *Wolbachia* host range and opens up investigations for their use as malaria control tools.

Global genomic pathogen surveillance: a decade-long expedition in pneumococcal genomics and identification vaccine-escaping lineage

Stephanie Lo ORCID iD[1,2], Lesley McGee[3], Stephen Bentley[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]University of Bath, Bath, United Kingdom. [3]Centers for Disease Control and Prevention, Atlanta, USA

Abstract

Background

Streptococcus pneumoniae is one of the leading causes of bacterial infection in children under five. Pneumococcal Conjugate Vaccines (PCVs), targeting up to 20 of >100 known serotypes, has significantly reduced global mortality. However, the rise in invasive diseases caused by non-PCV serotypes negates the benefits of the vaccination program. We conducted the Global Pneumococcal Sequencing Project to assess PCV's impact on pneumococcal populations, aiming to inform future vaccine design.

Methods

We whole-genome sequenced 21,155 pneumococcal isolates with detailed epidemiological data from 59 countries, 1989-2018. We defined a novel genomic definition of pneumococcal lineage (or Global Pneumococcal Sequence Cluster, GPSC) using PopPUNK, and inferred serotype, and antimicrobial profile of 19 antibiotics from genome data.

Results

We clustered a global collection of pneumococcal genomes into 968 lineages, identifying ten major lineages with serotypes not in the PCV that globally expanded post-PCV introduction. Most of these lineages, prevalent worldwide, could express various serotypes. GPSC10, one such lineage, underwent frequent recombination, resulting in multidrug resistance and expression of 17 serotypes. Since the introduction of the first PCV, GPSC10 mediated vaccine evasion across multiple countries, and lately drove the increase of an invasive serotype 24F in Europe and Latin America. These findings led to the inclusion of serotype 24F in the forthcoming 25-valent PCV (IVT-25), but also expose the limitation of serotype-based vaccine design for lineages like GPSC10.

Conclusions

This project is a pioneering example of how pathogen genomic surveillance can be used to inform and improve bacterial vaccine design.

Imprecise host decontamination adversely affects microbial genomic and metagenomic analyses

Bede Constantinides ORCID iD, Derrick Crook ORCID iD

University of Oxford, Oxford, United Kingdom

Abstract

Microbial sequences generated from clinical samples are often contaminated with human host sequences containing personally identifiable information about the sample's donor. Unless suitably consented, these sequences must therefore be informatically discarded for ethical and legal reasons. However, in doing so, care must be taken to avoid inadvertently discarding microbial sequences, to the detriment of subsequent genomic and/or metagenomic analyses.

Using analysis of real and simulated sequence data, we show that popular host decontamination methods systematically remove sequences belonging to microbial genomes, giving rise to gaps in sequencing coverage, and adversely affecting downstream analyses such as variant calling. For seven clinically important human pathogens, more than 1 in 1000 (0.1%) bacterial reads were removed by a popular existing decontamination approach.

As well as wishing to highlight what we consider an overlooked problem in microbial genomics, we present a solution in the form of open source software for more accurate host decontamination. Under evaluation, our new approach removed 21-43 times fewer microbial sequences than existing methods, while typically removing more human sequences and taking less time. Optional use of a masked reference genome can further increase microbial sequence retention.

Infection Forum

Antibiotic resistance alters the ability of *Pseudomonas aeruginosa* to invade the respiratory microbiome

Selina Lindon[1], Sarah Shah[1], Danna Gifford[2], Maria A. Gomis Font[3], Divjot Kaur[1], Antonio Oliver[3], R. Craig MacLean[1], Rachel Wheatley[1,4]

[1]University of Oxford, Oxford, United Kingdom. [2]University of Manchester, Manchester, United Kingdom. [3]Instituto de Investigación Sanitaria Illes Balears (IdISBa), Palma de Mallorca, Spain. [4]Queen's University Belfast, Belfast, United Kingdom

Abstract

The emergence and spread of antibiotic resistance in bacterial pathogens is a global health threat. One important unanswered question is how antibiotic resistance influences the ability of a pathogen to invade the host-associated microbiome. Here we investigate how antibiotic resistance impacts the ability of the opportunistic bacterial pathogen *Pseudomonas aeruginos*a to invade the respiratory microbiome, by measuring the ability of *P. aeruginosa* spontaneous antibiotic resistant mutants to invade pre-established cultures of commensal respiratory microbes. We find that commensal respiratory microbes tend to inhibit the growth of *P. aeruginosa*, and antibiotic resistance is a double-edged sword that can either help or hinder the ability of *P. aeruginosa* to overcome this inhibition. The directionality of this help or hinderance depends on both *P. aeruginosa* genotype and respiratory microbe identity. Antibiotic resistance facilitates the invasion of *P. aeruginosa* into *Staphylococcus lugdunensis*, yet impairs invasion into *Rothia mucilaginosa* and *Staphylococcus epidermidis*. *Streptococcus* species provide the strongest inhibition to *P. aeruginosa* invasion, and this is maintained regardless of antibiotic resistance genotype. Our study demonstrates how antibiotic resistance can alter the ability of a bacterial pathogen to invade the respiratory microbiome and suggests that attempts to manipulate the microbiome should focus on promoting the growth of commensals that can provide robust inhibition of both wildtype and antibiotic resistant pathogen strains.

Involvement of oxidative stress in the infection process of *A. castellanii* by *L. pneumophila*

Alban Hay ORCID iD, Willy Aucher, Alexandre Crépin, Yann Héchard, Romain Villéger, Ascel Samba-Louaka

Université de Poitiers, Poitiers, France

Abstract

Oxidative stress is an imbalance between reactive oxygen species (ROS) and the antioxidant defenses in cells. The host's oxidative stress targets intracellular bacterial pathogens to limit their proliferation. *Legionella pneumophila* is an opportunistic pathogen that uses free-living amoebae such as *Acanthamoeba castellanii* as an environmental multiplication site. This amoeba shelters and provides nutrients for *L. pneumophila* efficient intracellular proliferation. Little is known regarding the modulation of the amoeba antioxidant defenses by pathogens. In this work, using a global proteomic approach, we demonstrated the ability of *L. pneumophila* to increase the number of proteins linked to antioxidant defenses in *A. castellanii* when infected with the wild-type (WT) strain rather than the homologous mutant Δ*dotA*. This mutant presents a defective type IV secretion system (T4SS) making it unable to multiply within the host. RT-qPCR analysis also revealed that at 6 h p.i., *L. pneumophila* WT increased the transcription of certain genes linked to antioxidant defense, unlike the Δ*dotA* mutant. Fluorescent labeling revealed a decrease in ROS quantity at 24h p.i. when amoebae were infected with *L. pneumophila* WT compared to *L. pneumophila* Δ*dotA.* These results seem to demonstrate the bacterium's ability to disturb the amoeba's antioxidant defenses to its advantage to reduce the quantity of ROS and proliferate. This study highlights how the pathogen could disrupt oxidative stress in *A. castellanii*.

A Copper Economy Underlies Synergy of *Candida albicans* and *Staphylococcus aureus* Dual-Species Biofilms.

Seána Duggan ORCID iD

MRC Centre for Medical Mycology at the University of Exeter, Exeter, United Kingdom

Abstract

When the fungus *Candida albicans* and the bacterium *Staphylococcus aureus* interact, they enhance each other's virulence and antimicrobial tolerance. As such, *C. albicans* and *S. aureus* co-infections result in worse disease, prolonged treatment, and poor patient outcome. We urgently need to investigate synergistic interactions in pathogens so we can understand and treat co-infections. Proteomics analysis on *C. albicans* and *S. aureus* dual-species biofilms demonstrated a reciprocal regulation of copper related proteins, whereby the fungus increases expression of copper machinery, and the bacterium decreases expression. Experiments tittering copper in growth media demonstrated that *C. albicans – S. aureus* dual-species biofilms are sensitive to changes in environmental copper, compared to their relative single species biofilms. Furthermore, copper availability governs biofilm composition as *C. albicans* dominates in copper deplete conditions while *S. aureus* dominates in copper replete conditions. Electron microscopy revealed that *C. albicans* hyphal structures are impeded by increased copper, indicating a copper mediated impact of fungal morphology in the dual-species biofilm. Experiments employing both fungal and bacterial copper machinery mutants demonstrated the copper dependent effects on dual-species biofilms is controlled by copper transport by the fungus. Finally, copper nanoparticles are shown to reduce the viability of *C. albicans* and *S. aureus* dual-species biofilms, thereby acting as a promising therapeutic for this co-infection. This work reveals a copper economy underlies synergy in *C. albicans* and *S. aureus* dual-species biofilms and demonstrates interfering with the copper status of the biofilm as a potential therapeutic avenue for addressing co-infections.

lasR is an ecological "keystone gene" in cystic fibrosis-associated polymicrobial airway infections

Eva Bernadett Benyei ORCID iD, Rahan Nazeer, Pok-Man Ho ORCID iD, Martin Welch ORCID iD

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Abstract

Pseudomonas aeruginosa (PA) is a frequent cause of airway infections in people with cystic fibrosis (CF). In the CF airways, often has to share the environment with a variety of co-habiting species. However, the influence of these co-habitants on PA biology (and *vice versa*) has proven exceptionally difficult to investigate, primarily due to the absence of a suitable experimental system. This impasse was recently broken with the development of a continuous-flow setup that enables very stable steady state co-cultures of three key CF pathogens (PA, *Staphylococcus aureus* and *Candida albicans*) to be maintained. We have been using this setup to investigate how so-called "pathoadaptive" PA genes influence inter-species interactions.

Perhaps the most intensely-studied pathoadaptive gene in PA is *lasR*. Mutants in *lasR* – a master regulator of quorum sensing in PA – arise with high frequency in CF, and are thought to be classic exemplars of "evolutionary cheats", affecting *intra*-species dynamics. In this work, we show that *lasR* is also a "keystone gene", whose loss-of-function also has a hitherto unsuspected impact on *inter*-species interactions. We show that in the absence of the wild-type, the polymicrobial community is destabilized by mutation of *lasR*, especially following antibiotic challenge. Transcriptomic analyses reinforce this notion; whereas the wild-type appears essentially agnostic to the presence of other species, the *lasR* mutant displays substantial differences in gene expression when grown in mono- *vs* polyculture. These data suggest that *lasR* is a "keystone gene" capable of influencing the ecology of the entire system.

A novel strategy to combat Group B Streptococcus neonatal disease by targeting interkingdom interactions with fungus Candida albicans

Joshua Elsey ORCID iD, Dr Angela Nobbs ORCID iD

University of Bristol, Bristol, United Kingdom

Abstract

Group B *streptococcus* (GBS) is a coloniser of the genitourinary tract and can be vertically transmitted from mother to neonate during birth. It carries pathogenic potential in neonates, causing neonatal sepsis and meningitis, killing approximately 150,000 annually. GBS can also associate with *Candida albicans*, with this inter-kingdom relationship boosting the GBS carriage rate in the genitourinary tract. Furthermore, the interaction is hypothesised to dampen the proinflammatory immune response to GBS, thereby promoting colonisation. This research investigated this relationship, performing co-association assays using 17 previously untested GBS clinical isolates and coaggregation assays to assess physical interaction. This highlighted 6 GBS isolates that experienced a significant increase in association with vaginal epithelial cells (VECs) when associated with *C. albicans,* and identified the Als3 receptor of *C. albicans* as crucial in this relationship, as *ΔAls3 C. albicans* mutants destroyed coaggregation. Cytokine sandwich ELISA's and proteomic profiling assessed whether dual-species infection dampened VECs immune response. The ELISA's found no significant difference in IL-8, CXCL1 or IL-1β production between VECs infected with monospecies GBS samples or dual-species GBS-*C. albicans* counterparts. However, proteomic profiling and bioinformatic analyses highlighted several immune pathways significantly downregulated during dual-species infection. It also showed strengthening of epithelial barrier integrity, suggesting the VECs prevent ascending infection, creating an environment favouring GBS colonisation. Overall, this research showed co-association between previously untested GBS clinical isolates and *C. albicans*, identifying Als3 mediated physical aggregation. It also suggested that inter-kingdom interactions have the potential to dampen aspects of the proinflammatory host cells' immune response.

Applying Droplet Fluidics to Mycobacterial Drug Discovery

Antonia Molloy[1], Alaa Kadhim[2], Xin Li[2], Jonathan Cox[1]

[1]Aston University, Birmingham, United Kingdom. [2]Sphere Fluidics, Cambridge, United Kingdom

Abstract

Droplet microfluidics have promising future applications in microbiology to miniaturise drug screening platforms at the single-cell level. There is an urgent need to design *in vitro* antibiotic susceptibility tests which reduce animal models and replicate the human infection environment. Here we have optimised picodroplet technology for *Mycobacterium abscessus* antibiotic susceptibility testing.

Picolitre water-in-oil droplets were generated using picodroplet technology with a pressure-pump and an image-based feedback system. A droplet stabilising surfactant (AM50-06) was synthesised based on existing know-how of Sphere Fluidics Limited and assessed for small molecule leakage out of droplets compared to commercial products. *M. abscessus* was encapsulated at the single-cell level in droplets and assessed for viability and proliferation by fluorescence microscopy.

Picodroplet technology was sufficient to generate monodisperse and stable droplets with mycobacterial culture media (coefficient of variation of <1%). The combination of imaging-based feedback and pressure driven pumping maintained a constant droplet volume. AM50-06 surfactant showed slower small molecule leakage (24 hours) compared to commercial surfactants (4 hours). *M. abscesuss* was encapsulated in droplets (1-10 cells per droplet), remained viable, and proliferation was successfully detected using open-source image-analysis software "CellProfiler™".

An assay platform was optimised of which *M. abscessus* can be encapsulated in monodisperse and stable droplets. Picodroplet technology allows each 'bioreactor' to be identical in volume. The synthesised surfactant shows promising results to reduce antibiotic leakage from each droplet and therefore individual microenvironments are maintained. Future work includes developing this platform for antibiotic discovery and rapid susceptibility testing as part of advancing efforts to tackle AMR.

Synergism between LPS and capsule in Extra-intestinal Pathogenic *Escherichia coli*: therapeutic implications

Naoise McGarry, Domhnall Roe, Stephen Smith

Trinity College, Dublin, Ireland

Abstract

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) is a major cause of urinary tract infections (UTI), bacteraemia, and sepsis. Often, ExPEC possess capsule polysaccharides and long-chain O antigen molecules which render them resistant to the bactericidal components of serum. CFT073 is a prototypic, urosepsis isolate of O6:K2:H1 serotype. This laboratory among others, has shown that strain CFT073 is serum-resistant, with virulence factors including capsule imparting resistance. In this study, it was found that rough and deep-rough lipopolysaccharide (LPS) mutants were more sensitive to serum and displayed reduced capsule compared to wild-type CFT073, suggesting that retention of capsule on the cell surface is dependent on a full LPS molecule. Interestingly, unretained capsule of LPS mutants was found in the culture supernatant. Subsequently, electrostatic interactions between capsule and LPS were shown to underpin the association of the K2-specific capsule antigen with the bacterial cell in CFT073 and other ExPEC isolates. This study also showed that targeting LPS charge or biosynthesis using sub-inhibitory polymyxins and a WaaG inhibitor resulted in increased serum sensitivity, increased antibiotic sensitivity, and reduced capsule association in CFT073. Interestingly, the dependency of capsule on LPS has been observed previously in several *Klebsiella pneumoniae* isolates, which indicates that the synergy between these polysaccharides is not just strain, serotype or species-specific but may be conserved across several pathogenic Gram-negative species. Therefore, using WaaG inhibitor derivatives or phage-derived capsule depolymerases to target LPS is a promising avenue for co-administration with antibiotics to reduce morbidity and mortality by reducing or eliminating surface capsule.

Evaluation of the role of IL-10 during *Campylobacter* and *Salmonella* infection in chickens

Ricardo Corona Torres ORCID iD[1], Dominique Meunier[1], David Hume[2], Rachel Hawken[3], Fiona Tomley[4], Lonneke Vervelde[1], Kellie Watson[1], Mike McGrew[1], Mark Stevens[1]

[1]The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom. [2]Mater Institute, University of Queensland, Brisbane, Australia. [3]Cobb Vantress, Colchester, USA. [4]Royal Veterinary College, London, United Kingdom

Abstract

Handling and consumption of poultry meat is an important risk factor for food-borne diseases in humans such as campylobacteriosis and non-typhoidal salmonellosis. *Campylobacter jejuni* and non-typhoidal *Salmonella*infections in poultry are not typically associated with clinical disease, albeit gastrointestinal inflammation has been reported in some lines. Interleukin (IL) -10 regulates intestinal inflammation and has been associated with the outcome of infections by *Eimeria* in chickens. In mice, loss of IL-10 is associated with spontaneous colitis and more severe inflammation in response to *Campylobacter* and *Salmonella*. We have used CRISPR/Cas9 genome editing to generate chickens with homozygous null or heterozygous mutations in exon 1 of the avian IL-10 gene. We challenged wild-type, heterozygous and homozygous null chickens separately with *C. jejuni* and *S.* Typhimurium to establish the role of IL-10 in the avian host. Bacterial colonisation of the caeca, spleen and liver was determined and we analysed gross pathology and histopathology in the gut. Our results indicate that chickens lacking IL-10 have elevated inflammatory responses at key sites of colonisation in the avian gut. Moreover, levels of bacterial colonisation of the caeca were lower in birds deficient in IL-10. Our findings add to understanding of how innate responses can control zoonotic pathogens in the avian reservoir.

In an ex vivo human spleen model, *Streptococcus pneumoniae* infection may stimulate macrophages production of GM-CSF

Neama Alnabati[1], Shiying Tang[1], Trisha Kanani[1], Francesco Flandi[2], Daniele Ghezzi[2], Tareq Al saoudi[1], Wen Chung[3], Ashley Dennison[3], Luisa Martinez-Pomares[4], Marco Oggioni[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Bologna, Bologna, Italy. [3]University Hospitals of Leicester, Leicester, United Kingdom. [4]University of Nottingham, Nottingham, United Kingdom

Abstract

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that is involved in the regulation of inflammatory immune responses and activation of various immune cells such as macrophages. The production of GM-CSF by macrophages is linked to the host's immune response to danger signals during infections which then promotes pathogen clearance. Although several studies in mice have shown the importance of GM-CSF, data on humans are still limited especially in *Streptococcus pneumoniae* (*S. pneumoniae*). *S. pneumoniae* is the leading cause of lower respiratory tract infections and is linked with a high morbidity and mortality rate. Therefore, to investigate how splenic macrophages respond to the infection, we utilised an *ex vivo* human spleen perfusion model with Clinical Trial number (NCT04620824). Spleens were collected, perfused and infected with a mix of *S. pneumoniae* serotypes for 6 hours and blood, and biopsies samples were collected for bacterial enumeration and immunofluorescence.

Here we present the first data of the immunofluorescence microscopy analysis of the production of GM-CSF by macrophages. Our data shows that there is a significant increase in early-time of GM-CSF production by CD163+ which is the large macrophage subtype in the human spleen. However, the production of GM-CSF by the perifollicular sheath CD169+ macrophages decreased in the late time, this was also in correlation with bacterial removal which also decreased.

To conclude, our data are the first indication of the role of GM-CSF during an *ex vivo* human spleen model which shows that it could have an important role in the inflammatory response and regulation of immune cells.

Identifying novel biomarkers of staphylococcal prosthetic joint infection

Leanne Sims[1], Emma Manners[2], Muhammad Yasir[1], Claire Hill[1], John Wain[1], Iain McNamara[3], Mark Webber[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]EMBL-EBI, Hinxton, United Kingdom. [3]Norfolk and Norwich University Hospital, Norwich, United Kingdom

Abstract

In the UK almost 200,000 primary knee and hip replacements are performed annually. Prosthetic joint infection (PJI) is a devastating complication affecting 1-2% of these. Most infections are attributed to *Staphylococcus aureus* or *Staphylococcus epidermidis*, though diagnosis of the latter is complicated by their presence in diagnostic samples as contaminants from the skin.

We have developed a model of PJI where strains of interest are grown in human synovial fluid obtained as diagnostic excess. We used transposon-directed insertion sequencing to identify genes in *Staphylococcus epidermidis* essential or detrimental for growth in human synovial fluid. We combined this dataset with an RNASeq experiment to discover genes upregulated upon exposure to synovial fluid in *S. epidermidis*.

There was good agreement between the datasets, which identified some key core pathways which were important for survival in synovial fluid. A specific gene cluster showed potential as a biomarker for PJI - upregulated in synovial fluid samples and protected during transposon mutagenesis experiments. A defined single gene mutant was obtained and demonstrated compromised growth in synovial fluid in comparison to the wild type.

This work shows an in vitro model of PJI can be used to explore the molecular mechanisms that allow organisms to grow in synovial fluid and identify potential biomarkers for improved diagnostics.

Antibiotic persister reservoirs of *Salmonella* are determined by bacterial secretion machinery *in vivo*

Joshua Newson[1], Sarah McHugh[2], Flavia Gürtler[1], Erik Bakkeren[3], Wolf-Dietrich Hardt[1]

[1]ETH Zurich, Zurich, Switzerland. [2]University of Lausanne, Lausanne, Switzerland. [3]University of Oxford, Oxford, United Kingdom

Abstract

The effectiveness of antibiotics is threatened by antibiotic persistence, in which a subpopulation of susceptible bacteria endure lethal antibiotic pressure. Little is known about how persister cells form within an infected host *in vivo.* Intracellular pathogens such as *Salmonella* achieve infection using protein secretion machinery termed type-three secretion systems (T3SS) to translocate bacterial effector proteins into host cells. Here, we aimed to understand how antibiotic persistence arises *in vivo,* and how the major virulence factors of *Salmonella* contribute to antibiotic treatment failure.

In mouse models of infection, we observed antibiotic persistent populations that survived treatment with a range of clinically relevant antibiotics. Extended exposure failed to reduce the size of the persister population, and re-isolated persisters demonstrated similar virulence and antibiotic persistence in re-infection experiments. We found that regrowth of persister cells after antibiotic treatment is dependent on the T3SS-2, which facilitates intracellular survival and replication. This regrowth led to migration of *Salmonella* from the spleen to the gut, suggesting persister formation enables transmission to new hosts. This robust colonisation of the gut by recovered persisters could be attributed to relatively few founding bacteria based on genetic tagging and qPCR analysis. Using a range of mutants each deficient for multiple T3SS-2 effectors, we characterised how distinct functional cohorts of effectors contribute to bacterial recovery after antibiotic treatment.

Together, these data describe how virulence factors of *Salmonella* contribute to the formation of an antibiotic-persistent reservoir that cannot be eliminated by antibiotic treatment, with broader implications for the therapeutic control of intracellular bacterial pathogens.

Developing an arterial microfluidic model of vascular disease to investigate the crosstalk between *Porphyromonas gingivalis* and vascular endothelial cells.

Chloe Smith[1], Prachi Stafford[1], Daniel Kelly[1], Christine Le Maitre[2,1]

[1]Sheffield Hallam University, Sheffield, United Kingdom. [2]University of Sheffield, Sheffield, United Kingdom

Abstract

Background: Atherosclerosis can be exacerbated by periodontitis, a chronic oral inflammatory disease associated with the bacteria *Porphyromonas gingivalis.* *P gingivalis* constitutes 47% of bacterial DNA found in atherosclerotic plaques of periodontitis patients and has been proposed to contribute to endothelial dysfunction and lesion development following its entry into the circulation through ulcerated periodontal pockets, although this is poorly understood. **Aims**: Develop an *in-vitro* vascular microfluidic model to investigate *P. gingivalis*-vascular endothelial cell (VECs) interplay under physiological environments.

Methods: Using the ibidi® microfluidic system, optimal adherence of HUVECs to the µ-Slide$^{0.4}$, shear stress and flow duration required for cell realignment was determined. The influence of dynamic flow (25 dyne/cm^2 for 48h) v/s static controls on proliferation (Ki-67), and expression and localisation of ZO-1 and VE-cadherin was investigated pre-and post-infection with *P. gingivalis* (MOI:1:100 for 24h). Infectivity rates were quantified via ImageJ and Mann-Whitney U test used to assess significance ($P \leq 0.05$).

Results: Proliferation of HUVECs was decreased under flow v/s static conditions. ZO-1 and VE-cadherin expression were unaffected under flow, whilst *P. gingivalis* decreased VE-cadherin expression under static conditions. Percentage of cells infected with *P. gingivalis* was decreased under flow (39%) 75% CI (23.94, 54.06) versus static (61%) 75% CI (42.91, 78.76) (p =0.1000).

Conclusions: *P. gingivalis* infects VECs under high shear stress although to a lower level than static conditions, and decreased adhesion molecule expression. Future work will explore branched flow at 30˚ and 45˚ to simulate blood vessels bifurcation, enhancing understanding of periodontal pathogens' role in atherosclerosis and informing patient treatment.

SARS-CoV-2 infection enhancement by Amphotericin B and Nystatin

Dung Nguyen[1,2], Stephen Laidlaw[1,2], Xiaofeng Dong[3], Miraz Rahman[4], Clive Page[4], Matthew Wand[5], Mark Sutton[5], Julia Tree[5], Rachel Milligan[6], Max Erdmann[6], David Matthews[6], Andrew Davidson[6], Julian Hiscox[3], Miles Carroll[1,2]

[1]Centre for Human Genetics, University of Oxford, Oxford, United Kingdom. [2]Pandemic Sciences Institute, University of Oxford, Oxford, United Kingdom. [3]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [4]Institute of Pharmaceutical Science, King's College London, London, United Kingdom. [5]UK Health Security Agency, Salisbury, United Kingdom. [6]School of Cellular and Molecular Medicine, Faculty of Life Sciences, University Walk, University of Bristol, Bristol, United Kingdom

Abstract

Severe coronavirus disease 2019 (COVID-19) patients who require hospitalisation are at high risk of invasive pulmonary mucormycosis. Amphotericin B (AmB), which is the first line therapy for invasive pulmonary mucormycosis in low- and middle-income countries, has been shown to promote or inhibit replication of a spectrum of viruses. In this study, we investigated the impact of AmB, Nystatin, Natamycin, Fluconazole and Caspofungin on SARS-CoV-2 infection *in vitro*. Results show AmB and Nystatin actually increase SARS-CoV-2 infection in Vero E6, Calu-3 and Huh7 cells. At optimal concentrations, AmB and Nystatin increase SARS-CoV-2 replication by up to 100- and 10-fold in Vero E6 and Calu-3, respectively. The other tested antifungals had no impact on SARS-CoV-2. Drug kinetic studies indicate that AmB enhances SARS-CoV-2 infection by promoting virus cell entry. Additionally, knockdown of genes encoding for interferon-induced transmembrane (IFITM) proteins 1, 2, and 3 suggests AmB enhances SARS-CoV-2 cell entry by overcoming the antiviral effect of IFITM3 protein. This study further elucidates the role of IFITM3 in viral entry and highlights the potential dangers of treating COVID-19 patients, with invasive pulmonary mucormycosis, using AmB.

Exploring the potential of the mitochondria of Cryptococcus neoformans as a drug target.

Dmytro Prasolov, Campbell Gourlay, Elizabeth Edrich

University of Kent, Canterbury, United Kingdom

Abstract

Cryptococcus neoformans is an opportunistic fungal pathogen that predominantly affects immunocompromised individuals, causing as many as 181,000 deaths annually. One of the hallmark features of this fungus is its ability to persist within the host in a dormant state for decades. It has been observed that as many as 70% of children in densely populated areas in the USA are exposed to this yeast. The identification of multidrug-resistant strains as early as 1999, coupled with the recent inclusion of *C. neoformans* in the fungal priority pathogen list by the World Health Organization, underscores the urgent need for new drug development. One potential target for the development of novel drugs is the mitochondria, which play a central role in vital life processes such as energy production, ergosterol biosynthesis, and iron homeostasis. Unlike human cells, *C. neoformans* possesses an alternative oxidase system encoded by the *AOX1* gene. In many fungal pathogens AOX allows them to maintain mitochondrial function despite oxidative stress. Nitric oxide is of particular interest as it is a compound naturally produced by macrophages to kill pathogens. Here we present evidence of our investigation of Aox1 function in *C. neoformans*. Our data suggest that Aox1 is important for production of virulence factors such as the capsule as well as for maintaining essential processes such as aerobic respiration during the inhibition of the oxidative phosphorylation.

Bayesian Phylodynamic Approaches Reveal Ancestral Host Association of Group B Streptococcus Clonal Group 103/314: Insights from Human and Cattle Populations

Antonia Hilbig ORCID iD[1], Chiara Crestani ORCID iD[2], Roman Biek ORCID iD[1], Samantha Lycett ORCID iD[3], Tiziana Lembo ORCID iD[1], Ruth Zadoks ORCID iD[4], Swaine Chen ORCID iD[5], Laura Oliveira ORCID iD[6], Tatiana Pinto ORCID iD[6], Taya Forde ORCID iD[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Institut Pasteur, Paris, France. [3]University of Edinburgh, Edinburgh, United Kingdom. [4]University of Sydney, Sydney, Australia. [5]Genome Institute of Singapore, Singapore, Singapore. [6]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract

Group B Streptococcus (GBS) showcases varied host associations among clonal complexes, including host generalists and specialists. Notably, clonal group (CG) 103/314, isolated globally from humans and cattle, causes dairy cow mastitis worldwide and is an emerging human pathogen in China. Our focus is to explore how CG103/314's host associations have evolved and to identify the genetic factors driving host shifts, given the favourable conditions in human-dairy cow interactions.

We analysed over 240 whole genomes spanning three decades and 18 countries across five continents. Using these sequences, we constructed a time-scaled phylogeny from the core genome. Host and host associated gene loci (lac.2 operon, scpB-lmb, and locus3) were modelled as discrete traits. Additionally, we explored a structured coalescent approach, assuming transmission within a host species is more frequent than between host species, distinguishing between spill-over and sustained transmission. Finally, we evaluated antimicrobial resistance and virulence factor profiles to understand their impact on the success of lineages.

Multiple host jumps of CG103/314 occurred from human to bovine populations since the 1940s. The lac.2 operon is closely linked to bovine association, but remains present in many human lineages. ScpB-lmb is mostly absent, indicating low virulence, but acquired by a few lineages recently. Locus 3 genes, commonly associated with fish infections in other CGs, were found in most lineages, despite CG103/314 never being linked to fish disease.

Our study showcases GBS-CG103/314 as versatile host generalist, revealing its capacity for adaptation and providing a valuable example for pathogen dynamics in a one health framework.

Introduction of targeted molecular ciprofloxacin resistance testing on positive nucleic acid amplification testing (NAAT) *Neisseria gonorrhoea* samples in NHS Grampian

Ijeoma Okoliegbe ORCID iD[1], Chithra Naveen[2], Nicola Ewen[1], Gary Stuart[1], Noha El Sakka[1]

[1]NHS Grampian, Aberdeen, United Kingdom. [2]University of Aberdeen, Aberdeen, United Kingdom

Abstract

Gonorrhoea, a prevalent global and UK sexually transmitted infection, has seen an alarming rise in cases, with Scotland experiencing a 50% surge since 2019. This has led to unprecedented demands for management within specialist services. In line with guidelines, gonorrhoea can be treated with ciprofloxacin if susceptibility is known. However, in the diagnostic pathway, samples are first tested for *Neisseria gonorrhea* (NG) using nucleic acid amplification testing (NAAT). The Sexual Health clinic then recalls some patients for culture and reference laboratory susceptibility testing. This study aimed to understand the local five-year (2018-2023) gonococcal epidemiology. In addition, we explored the introduction of reflex molecular ciprofloxacin resistance assay on positive NG NAAT samples to help inform ciprofloxacin susceptibilities and aid clinical decision-making.

Retrospective analysis show a total of 151,063 samples were tested for NG in NHS Grampian with 1.47% (n=2216) positivity. Of these, 62.27% (n=94,069) of NG NAAT samples were females while 37.26% (n=56,285) were males. Sub-group analyses showed 0.85% (n=796/94,069) positivity in female samples and 2.52% (n=1418/56,285) positivity in male ($p<0.0001$). A four-fold (0.82% vs 3.25%) yearly increase in NG positivity was observed. This was driven by the 16-25 age group, where NG positivity increased from 1.21% to 5.48% in the last 2yrs.

Introduction of ciprofloxacin molecular assay showed 100% concordance with reference laboratory results, with a two-thirds reduction in turnaround time.

The introduction of molecular ciprofloxacin assay will aid prompt treatment decisions and ensure efficient use of clinic time with the removal of patient recalls and double sampling.

A OneHealth approach to prediction and prevention of foodborne Shiga-toxin producing E. coli (STEC) O157 outbreaks

Neil Cunningham ORCID iD

United Kingdom Health Security Agency, London, United Kingdom. Lewisham and Greenwich NHS Trust, London, United Kingdom

Abstract

Background

Shiga-toxin producing *E. coli* (STEC) O157 is a foodborne pathogen associated with gastrointestinal illness and severe disease (34% hospitalisation rate, 5-15% develop haemolytic uraemic syndrome (HUS)). In 2022, the largest UK outbreak of STEC O157 was identified since the implementation of routine Whole Genome Sequencing (WGS). A national-level investigation was undertaken to investigate the cause.

Methods

WGS was used to identify outbreak cases of STEC O157. Food exposure information and food chain investigations were used to identify the likely source. Evidence generation included gathering information on crop rotation combined with analysis of meteorological and land use data.

Results

259 (STEC) O157 t5.5294 cases were confirmed across the UK. Descriptive epidemiological analyses supported salad produce as the vehicle. Contextual analysis of WGS data, international communications, and case onset dates relative to supply chains supported a UK grown, nationally distributed food item with a short shelf life, with salad leaf production by a single grower the likely cause. Weather and land use data revealed an isolated and unusual heavy precipitation event at the location of the grower in an otherwise drought-like situation, corroborated by land-use data indicating land used to grow salad for human consumption.

Conclusion

Due to the nature and potential severity of STEC infection, early detection and investigation is important. The ability to respond rapidly to emerging outbreaks is limited by delays in seeking treatment, and short product shelf-lives. The use of One Health data is a potential tool for evidence generation and risk mitigation strategies in future outbreaks.

Emerging rhabdoviruses and human infection in Uganda.

James Shepherd ORCID iD[1], Shirin Ashraf[1], Jesus Salazar-Gonzalez[2], Maria Salazar[2], Robert Downing[3], Hanna Jerome[1], Joseph Mpanga[3], Chris Davis[1], Lily Tong[1], Sreenu Vattipally[1], Linda Atiku[3], Nicola Logan[1], Ezekiel Kajik[3], Yafesi Mukobi[3], Cyrus Mungujakisa[3], Michael Olowo[3], Emmanuel Tibo[3], Fred Wunna[3], Hollie Jackson Ireland[1], Iyanuoluwani Owolabi[1], Ana da Silva Filipe[1], Josephine Bwogi[3], Brian Willett[1], Julius Lutwama[3], Daniel Streicker[1], Pontiano Kaleebu[3], Emma Thomson[1]

[1]University of Glasgow-MRC Centre for Virus Research, Glasgow, United Kingdom. [2]MRC/UVRI & LSHTM Uganda Research Unit, Entebbe, Uganda. [3]Uganda Virus Research Institute, Entebbe, Uganda

Abstract

Le Dantec virus (LDV), the type species of the genus *Ledantevirus* within the *Rhabdoviridae* has been previously associated with human disease in Africa, but has gone undetected since the 1970's. We detected LDV in a human case of acute febrile illness in Uganda by metagenomic sequencing and confirmed a convalescent serological response to the viral glycoprotein using ELISA, pseudovirus-based neutralisation and immunocytochemistry. To investigate the extent of LDV infection in the human population of Uganda we screened sera from a national cohort of 997 individuals by ELISA, showing that exposure is geographically heterogeneous, with 76% of individuals testing by positive by ELISA in Western Uganda, but lower sero-prevalence in other areas. A pseudovirus-based neutralisation assay against a panel of related viruses demonstrated that a proportion of sero-positivity in Uganda could be explained by cross-reactivity to other viruses within *Ledantevirus*, indicating exposure to both LDV and related ledanteviruses in the Ugandan population. To investigate further we performed environmental sampling of peridomestic animal species in Uganda, revealing the presence of a novel ledantevirus in the widespread rodent species *Mastomys erythroleucus*, suggesting rodents may act as vector species for African ledanteviuses. *Ledantevirus* infection is common in Uganda but is geographically heterogeneous and may be driven by both LDV and other members of the genus. Further studies of patients presenting to healthcare with fever are required to determine the contribution of bothe LDV and other ledanteviruses to acute febrile illness in Uganda

Microbes as Sentinels and Solutions in a Changing World

Invited talk: Microbial manufacturing of synthetic fuels: from academic pursuit to industrialisation.

Nigel Scrutton ORCID iD

C3 Biotechnologies Ltd, Manchester, United Kingdom. University of Manchester, Manchester, United Kingdom

Abstract

The scaled microbial production of commodity chemicals and fuels is challenging but remains a primary goal of the bioeconomy as we transition to renewable resources as feedstocks. To address this challenge, we have engineered biology to produce biocompatible chemicals that are precursors of synthetic fuels. Chemical conversion then gives the target synthetic fuel in a coupled process to generate surrogates of existing fuels. A prominent example is kerosene which is used as an aviation and household fuel. In this presentation, I will discuss the journey from academic science through to the industrialisation at pilot plant scale of microbial synthetic kerosene production, focussing on challenges, limitations and solutions. Synthetic fuels are advantageous compared to petrochemical counterparts and can be used as drop-in replacements. A first-generation pilot plant built by C3 Biotechnologies Ltd for microbial synthetic kerosenes will be discussed alongside testing of the produced fuel. Plant optimisation should lead to future cost-effective production and enable distributed manufacture of a range of kerosene-like fuels. As production is transitioned to use renewable feedstocks, microbial bioproduction can offer strategic and cost advantages. For example, localised production would reduce burdensome costs of fuel distribution from petrochemical refineries to point of use, especially for use in remote and more challenging regions of the world.

Invited talk: Soil microbiomes: gatekeepers of ecosystem carbon cycling

Ashish Malik[1], William Pallier[1], Lisa Cole[1], Steven Allison[2]

[1]University of Aberdeen, Aberdeen, United Kingdom. [2]University of California, Irvine, USA

Abstract

Soil microbiomes act as gatekeepers of the global soil-atmosphere carbon exchange, either by decomposing plant-fixed organic matter so it is respired back into the atmosphere or by stabilisation of their dead biomass (necromass) in the soil mineral matrix. Understanding how soil microbial processes operate over spatial and temporal scales to influence the terrestrial carbon budget remains a big challenge. In this talk, I will present findings from my research group on how we address this challenge by combining multi-omics, stable isotopes, geochemistry, and modelling tools. First, I will introduce a novel theoretical framework that is being successfully employed to translate the immense taxonomic and functional diversity of microbiomes into ecologically meaningful information (as traits) that can be modelled and upscaled. Then, I will present empirical work on the ecophysiological mechanisms of microbiome-mediated decomposition and stabilisation of soil carbon along land use and climate change gradients. I will synthesise findings from our UK soil studies to assess the microbial role in determining the levels of soil carbon changes in response to agricultural land use intensification and peatland restoration. In a Mediterranean system, we found that drought stress impacts plant litter decomposition by microorganisms and investigated the key microbial traits at different scales from populations to communities. Our results demonstrate variable mechanisms by which soil microbiomes and other interacting factors influence carbon accumulation or loss in different terrestrial ecosystems. This knowledge can inform manipulation of microbial processes to sequester carbon in soil to aid climate-resilient sustainable agriculture and ecosystem restoration.

Invited talk: Hitting the sweet spot: using fungi to make better for people & planet food ingredients

Jeremy Jentys

The Supplant Company, Cambridge, United Kingdom

Abstract

The Supplant Company takes abundant and renewable agricultural side-streams and uses fungal enzymes to turn them into healthier and more sustainable replacements for the world's most pervasive food ingredients.

Its flagship offering, Supplant™ Sugars from Fiber, can functionally and economically replace sugar in food. As well as making a positive environmental impact, it has less than half the calories of sugar, a lower glycaemic response compared to glucose, and is prebiotic.

Sugar reduction is one of the biggest long-term macrotrends in the food and drink industry. Sugar free beverages, such as Coke Zero, have long been available but Cake Zero is yet to hit the mainstream. The Supplant Company is working to change this and will share the journey of its innovative food ingredient from Cambridge lab to customers' lunchboxes.

supplant.com

Invited talk: The role of microorganisms in remediating metal contamination; a case study using citric acid to recover metals from mine waste and enhance environmental restoration.

Laura Newsome

University of Exeter, Environment & Sustainability Institute, Penryn, United Kingdom. Sellafield Ltd., Whitehaven, United Kingdom

Abstract

Mine waste is a serious global issue, polluting the environment with toxic metals and metalloids, causing problems for humans and ecology. However, some metals in mine tailings are of economic value, and therefore recovering metals from mine waste not only improves the environment but could be a source of revenue.

Microorganisms colonise and inhabit mine wastes, and they influence the environmental fate of metals through their metabolic activity, biogeochemical cycling, and detoxification mechanisms. Bioremediation strategies for metals may involve stimulating microbial activity to immobilise metals, or to solubilise metals which can then be recovered for reuse or disposal via appropriate engineered routes.

The Philippine Remediation of Mine Tailings project is focussed on finding new ways to manage and monitor metal mine wastes, clean up metal pollution, and to encourage soil development to allow the land to be reused. Tailings from a Cu-Au mine were obtained and the testing of a range of environmentally-benign and novel solvents led to citric acid being selected for further study.

Laboratory experiments were undertaken to assess how mine waste microorganisms interacted with citric acid. Low concentrations of citric acid effectively leached Cu and were fully biodegraded resulting in neutralised pH. High concentrations leached slightly more Cu but inhibited microbial citrate degradation and selected for fungi. Citrate was less effective at leaching metals but was rapidly biodegraded forming an abundance of biomass. Therefore, it is recommended that as well as leaching metals, remediation strategies should consider how microbial activity can contribute to achieving long-term environmental restoration.

Invited talk: Microbe-based solutions and challenges in reducing methane emissions from ruminants, animal wastes, rice paddies, and landfills

Mary Ann Bruns ORCID iD

Penn State University, University Park, PA, USA

Abstract

Microbes as solutions for the climate crisis are the focus of a five-year scientific portfolio announced in 2021 by the U.S. American Academy of Microbiology. As a Task Force member for the "Climate Change and Microbes" portfolio, I will present key findings from AAM's June 2023 colloquium, "The Roles of Microbes in Mediating Methane Emissions." Because of methane's greater warming potency and shorter lifespan than CO2, lowering atmospheric methane today is the most effective way to decelerate near-term temperature rise and "buy more time" before global temperatures reach tipping point. To that end, microbial approaches to reduce methane from four major sources will be discussed: ruminants, animal wastes, rice paddies, and landfills. For ruminants, rumen-modifying feed additives can reduce methanogenesis by altering microbial metabolic pathways, while direct chemical inhibitors and vaccines specifically target methanogens. Approaches to reduce methane emissions from animal wastes include composting or diversion to anaerobic digesters. In rice paddies, methane can be reduced by managing water to enhance oxic conditions and methanotrophy or by applying alternative electron acceptors to paddy soils to outcompete methanogenesis. Sustained sulfur cycling in paddy soils could be achieved by inoculating with filamentous "cable bacteria" that grow to lengths that span oxic and anoxic soil zones and cause depletion of substrates for methanogenesis. Landfill methane emissions can be reduced by capturing landfill gas to use for heat, electricity, or production of polyhydroxybutyrate plastic by methanotrophs. Overall, microbial knowledge can be deployed to a much greater extent to mitigate methane from these major sources.

Invited talk: Decoding the secret language of soils: chemical signalling in the rhizosphere and its effects on plant health.

Dunc Cameron ORCID iD

University of Manchester, Manchester, United Kingdom

Abstract

“Despite the staggering technological achievements of agriculture, we owe our

entire existence on this planet to a six-inch layer of soil and the fact that it rains”. The soil is the foundation of most terrestrial life on the Earth yet soil as a resource and its staggering complexity is often ignored, undervalued and underappreciated, non more so than the amazing array of organisms that make soil their home. The soil is a repository of information as well as an information super highway where signals are exchange between the soil organisms including plants, animals and microbes. These, usually chemical, signals have been hard to detect and to decode but advances in analytical methodologies, both biochemical and statistical, now allow us to eaves drop on these ‘conversations’ as never before. Here I have I explore some recent examples from my team illustrating how understanding the nature of plant-microorganism interactions, and the mechanisms that regulate them, provides an unparalleled opportunity to functionalise microbiomes for plant health in sustainable agricultural ecosystems.

Invited talk: Pathogenic Vibrios: the microbial barometer of climate change

Craig Baker-Austin

Cefas, Weymouth, United Kingdom

Abstract

Globally, the diverse bacterial genus *Vibrio* is the most important group of bacterial pathogens found in marine and coastal waters. These bacteria can cause an array of human infections via direct exposure to seawater or through the consumption of seafoods grown and cultivated in coastal and estuarine settings. Because these bacteria grow in warm, low salinity water and their abundance tracks ambient environmental conditions linked to temperature they have been deemed a microbial "barometer" of climate change. Critically, we may be on the cusp of an alarming global increase in diseases associated with these bacteria. A worldwide increase in seafood consumption, the globalization of seafood trade, the more frequent use of coastal waters for recreational activities and, finally, climate change, all contribute to greatly increased human health risks associated with these bacteria. Coupled to an increasingly susceptible population to more serious infections, we are likely to see a marked increase in reported cases including fatalities in the near future (2050). This presentation uses contemporary case studies to illustrate how the risk profiles of pathogenic vibrios have transformed in the last two decades - in particular in response to changing climatological and meteorological drivers such as marine coastal warming and extreme weather events such as heatwaves and storms.

Bacterial community responses to environmental change

Kaitlin Schaal ORCID iD[1], Manlio De Domenico ORCID iD[2], Shai Pilosof ORCID iD[3], James Hall ORCID iD[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Università degli Studi di Padova, Padua, Italy. [3]Ben Gurion University of the Negev, Beer-Sheva, Israel

Abstract

Anthropogenic environmental changes can have a profound impact on the soil microbial communities that are crucial for ecosystem functioning and food security, but existing work in this area has been limited by experimental systems that poorly capture the spatial structures intrinsic to soil or are difficult to scale up, and lack of crosstalk between modellers and experimentalists. Across study systems, interactions within microbial communities have been found to be generally negative or neutral, although specific types of environments may promote positive interactions. As environments change, interactions may evolve, depending on their mechanisms, shedding light on microbial competition and cooperation over time. Using a combination of soil microbial communities grown in simple soil microcosms and multi-layer network modelling and agent-based modelling approaches, we examine how interactions among the member species are impacted by environmental changes, and how these interactions evolve. We focus on low-level antibiotic presence, mercury presence, and increased nutrient levels as environmental changes, all of which are relevant by-products of human activity near soils. We explore the effect of environmental change on the balance of positive and negative interactions over time as species evolve, and the impact of plasmids and HGT on community- and individual-level responses to environmental perturbations. This work will allow us to synthesize across biological levels – plasmid to species to communities – as we seek to better understand the effect of human-induced environmental changes on microbial communities, especially their robustness and overall ecological function.

Impacts and Implications of Airborne Microorganisms in a Warming Atmosphere

Stephan Schuster ORCID iD

SCELSE, Singapore, Singapore

Abstract

Amid escalating concerns regarding climate change and air pollution, the intricate interplay between climate dynamics and air microbiomes remains inadequately understood. Our research team is dedicated to an in-depth exploration of bioaerosol dynamics through metagenomic analysis. This will establish linkages between resultant environmental microbiomes and a spectrum of physico-chemical factors. We will further evaluate potential implications of climate driven bioaerosol dynamics on human health. Consequently, our research initiative entails a comprehensive analysis of bioaerosol dynamics across distinct climate regimes, encompassing alpine, temperate, and tropical environments. Using high-volumetric air sampling technologies, we have conducted environmental time series that offer high temporal and taxonomic resolution. In an interdisciplinary approach that integrates expertise from aerobiology, medicine, atmospheric physics, and climate modelling, we aim at assessing the impact of raising global temperatures on atmospheric bioaerosols and the global dispersal of airborne microorganisms. Our bioaerosol detection methodologies can be applied to both, historical and contemporary air samples, enabling to examine the bioaerosol dynamics preceding the current and most acute climate crisis. By integrating biological, chemical, and physical measurements collected from pristine alpine and metropolitan areas from temperate and tropical settings, we investigate the potential interconnections between climate-driven alterations in airborne microbial dynamics and their consequential effects on human and ecosystem health.

The role of tree diversity in ecosystem functionality: investigating pathogen resilience through microbial diversity

Lisa Lamberte[1], Ruth Mitchell[2], Seumas Bates[3], Norman Dandy[3], Chris Nichols[4], Robert Jackson[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]The James Hutton Institute, Aberdeen, United Kingdom. [3]Bangor University, Bangor, United Kingdom. [4]The Woodland Trust, Grantham, United Kingdom

Abstract

Increased plant diversity significantly influences ecosystem functionality on a macro scale. However, its impact on microbial-scale ecosystem functionality remains unclear. Recent research underscores the critical role of leaf microbiota in bolstering plant health by their contributions to defence against foliar pathogens. Addressing the impact of increased tree diversity on leaf microbiomes and their role in resisting pathogen infection is crucial for advancing nature-based tree pathogen management strategies.

In the context of the interdisciplinary, multi-institutional project DiversiTree, we investigate the potential of diversified woodlands in countering pathogen-induced decline, focusing on Scots Pine (*Pinus sylvestris*) and Sitka Spruce (*Picea sitchensis*), two of the UK's prominent conifer species.

We characterized microbial communities in pine needles from trees in monoculture and diverse culture plots using amplicon sequencing and culturing techniques. Notably, trees in diverse culture plots exhibited core microbial communities, yet intriguing differences hint at potential microbe-tree associations.

Furthermore, *Bacillus velezensis*, identified in needles from trees in diverse culture plots, displayed promising biocontrol activity against the fungal pathogen *Dothistroma*, the agent causing Dothistroma needle blight disease. We evaluated biocontrol activities of bacteria consortia from monoculture and diverse culture plot-associated trees, including *Bacillus velezensis*, both *in vitro* and *in planta*. These findings provide a foundation for understanding microbial community dynamics and their implications for woodland health and disease resistance. Exploring how tree diversity shapes leaf microbiomes and influences their interactions with pathogens could inform strategies for fostering resilient woodlands.

Microbial bioinoculants obtained from pristine grassland can build soil organic carbon in cropland soil

Lisa Cole ORCID iD[1], Tim Goodall ORCID iD[2], Cécile Gubry-Rangin ORCID iD[1], Emily MacDonald[1], Nicole Cochrane[1], Ashish Malik ORCID iD[1]

[1]University of Aberdeen, Aberdeen, United Kingdom. [2]UK Centre for Ecology & Hydrology, Oxford, United Kingdom

Abstract

Agricultural intensification can cause soil degradation, as a result many arable soils have lost soil organic carbon (SOC) compromising terrestrial C stores and contributing to greenhouse gas emissions. Therefore, restoring this belowground SOC offers an opportunity to help mitigate climate change. The soil microbiome is responsible for transforming plant materials that enter soil into stable forms of SOC, and the microbiome of pristine grassland soils especially supports this process due to its high carbon use efficiency. This suggests that grassland microbiomes could be recruited to help restore SOC in degraded soils. To examine this theory, we established a mesocosm study where we reciprocally transferred microbiomes derived from historically undisturbed grassland soil and neighbouring cropland soil into their sterile counterparts, collected at two UK sites. We examined microbial biomass and community assemblage of the introduced microbiome in soils after 50 and 229 days. We also monitored soil C partitioning as respiration throughout the study and as SOC at the end of the study. We observed that soil conditions selected for distinct bacterial communities irrespective of the initial introduced microbiome, suggesting that communities were filtered by their environment. This raises questions around the persistence and therefore efficacy of microbial inoculations for soil recovery and highlights the need for careful land management to promote beneficial microbiomes for ecosystem services. Despite this, we observed that a grassland-derived microbiome was able to increase SOC in cropland soil after 7 months, albeit at one site, supporting the notion that soil microbial inoculants can benefit environmental restoration.

Microbes in our Waterways: Surveillance, Significance and Solutions

Invited talk: On the trail of cryptic SARS-CoV-2 wastewater lineages.

Thomas Peacock ORCID iD

The Pirbright Institute, Woking, United Kingdom. Imperial College London, London, United Kingdom

Abstract

Veterinary coronaviruses are well known to show either (or both) respiratory or gastrointestinal (GI) tropism while human coronaviruses are almost exclusively respiratory infections. SARS-CoV-2 RNA has been found to be shed at high levels in human stool, allowing the efficient tracking of community infections by wastewater sampling. This high level of shedding has been linked to active SARS-CoV-2 infections in the GI tract. SARS-CoV-2 infections in immunosuppressed individuals can give rise to infections that can last for months or years, with viruses showing distinct patterns of virus evolution during that time. Chronic infections are thought to be the source of long branch length 'variants of concern' such as Alpha, Beta or Omicron.

One oddity of wastewater sampling is that on occasion highly mutated SARS-CoV-2 lineages will appear for extended periods of time, in highly localised areas. These lineages are never found in the community from nasal/oropharyngeal surveillance, even during periods when surveillance has been extremely high. Several hypotheses have tried to explain the source of these lineages – gut-tropic chronic infections or infections of sewer resident rodents such as rats or mice are two potential explanations.

In this talk I will outline new evidence that highly mutated cryptic wastewater lineages most likely represent gut-tropic long term chronic human infections using a combination of epidemiology, genomic surveillance, and virological techniques.

Invited talk: Wastewater based epidemiology: informing public health strategy for pathogen and AMR surveillance

John McGrath[1], Andrew Lee[1], Marina Reyne[1], Hannah Saadi[1], Jennifer McKinley[1], Connor Bamford[1], Simon Cameron[1], Derek Fairley[1,2], Chris Creevey[1], Deirdre Gilpin[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Belfast Health and Social Care Trust, Belfast, United Kingdom

Abstract

Wastewater based epidemiology (WBE) uses biological and non-biological indicators present in sewage to provide information on the overall health of a community. As such it can provide timely public health intelligence about the trends of community pathogen prevalence, alongside the detection of known and the emergence of new variants of concern or interest. Where clinical testing is predominantly focused on secondary care, and thus potentially biased towards more clinically vulnerable patient cohorts and pathogens associated with more severe outcomes, WBE offers relatively unbiased sampling of pathogen burden across the whole population.

As in other jurisdictions worldwide, Northern Ireland (NI) developed a WBE programme for the detection and monitoring of SARS-CoV-2. This presentation will describe the NI WBE programme and the research involved in establishing methodologies for pathogen and AMR detection in wastewater, both at a sewage treatment works and at the individual building level. By developing a range of qPCR assays, alongside whole genome sequencing coupled with bioinformatic and population modelling pipelines, we have shown that WBE is not only effective for SARS-CoV-2 surveillance but also influenza A (both human and avian), respiratory syncytial virus, enterovirus D68, norovirus and adenovirus monitoring. Moreover through the UK's PATHSAFE programme we have used WBE (and air microbiome sampling) to determine both the pathogen load and the type and level of antimicrobial resistance (AMR) genes (and AMR bacteria) within a care home.

Invited talk: Wastewater-based surveillance of pathogens and antimicrobial resistance (AMR) in Wales

Reshma Silvester ORCID iD[1], William B. Perry[2], Gordon Webster[2], Laura Rushton[2], Amy Baldwin[2], Daniel A. Pass[3], Thom Clough[1], Kata Farkas[1], Peter Kille[2], Andrew J. Weightman[2], Peter Robins[1], Davey L. Jones[1]

[1]Bangor University, Bangor, United Kingdom. [2]Cardiff University, Cardiff, United Kingdom. [3]Compass Bioinformatics, Cardiff, United Kingdom

Abstract

Wastewater-based surveillance serves as an effective tool for tracking the dissemination of pathogens and antimicrobial resistance (AMR) within communities, aiding in the monitoring and prediction of public health risks. We used metagenomic approaches and bioinformatics to investigate the abundance of pathogens and antibiotic resistance genes (ARGs) in wastewater from hospital sewage networks, wastewater treatment plants (WWTPs), and receiving coastal waters and shellfish, in Wales. Our findings reveal that hospital effluents harbour a wide range of pathogens and exhibit elevated levels of AMR and antibiotics when compared to WWTPs. None of the treatment facilities succeeded in fully eliminating these pollutants from the wastewater, raising concerns about the continuous introduction of ARGs and pathogens through treated effluents into aquatic environments, such as rivers and coastal waters. The data on pathogen prevalence and persistence, alongside hydrological, weather, and wastewater discharge data, were used in mathematical models to estimate the extent of coastal areas impacted by wastewater contamination and to assess the associated human health risk. Future work should focus on identifying more effective treatment methods in the WWTPs and to explore the implementation of hospital wastewater pretreatment measures before release into municipal sewer systems, to minimize risk to human health.

Invited talk: From poo to policy: microbiological insights from the Welsh National Wastewater Monitoring Programme

William Perry ORCID iD[1], Arthur Morris[1], Alvaro Garcia Delgado[2], Rachel Williams[2], Andrew Mack[1], Amy Baldwin[1], Kata Farkas[2], Thomas Connor[3], Gareth Cross[4], Davey Jones[2,5], Owen Jones[1], Isabelle Durance[1], Peter Kille[1], Andrew Weightman[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]Bangor University, Bangor, United Kingdom. [3]Public Health Wales, Cardiff, United Kingdom. [4]Welsh Government, Cardiff, United Kingdom. [5]Murdoch University, Murdoch, Australia

Abstract

The COVID-19 pandemic brought the value of wastewater-based epidemiology (WBE) into sharp focus, providing a method of monitoring microbiological markers from large portions of the population. The Welsh National Wastewater Monitoring Programme started in September 2020 with 19 wastewater treatment works (WwTW) across Wales, using RT-qPCR to monitor levels of SARS-CoV-2. With time, the programme grew, and at its peak was monitoring 47 WwTW 5 days a week, as well as hospitals and other sources of wastewater. Additional pathogen and public health markers, including influenza, enterovirus, respiratory syncytial virus (RSV) and norovirus, and antimicrobial resistance (AMR) markers. These data streams proved to be a vital source of information for Welsh Government, and the timely insights gained were used to inform public health policies. A metagenomic approach to identify SARS-CoV-2 variants in wastewater was developed and implemented towards the end of 2021, just before the Omicron variants became dominant. It has been particularly valuable in monitoring changes in lineage abundances across Wales. A cross-correlation analysis between clinical and wastewater data have shown strong correlations, with little to no lag time. Additionally, utilising wastewater detected variants in conjunction with Bayesian hierarchical modelling, it is possible to detect early shifts in variant evolution, providing a potential early warning system for novel variants. Wastewater monitoring has proven to be a robust, cost-effective, tool for monitoring microbiological markers for health. Maintaining WBE infrastructure will be vital for future pandemic preparedness, and preparedness for other threats to public and broader One Health.

A rapid on-site Loop-mediated isothermal amplification (LAMP) technology for the detection of Shiga toxin-producing *Escherichia coli* (STEC) in water

Zina Alfahl ORCID iD[1,2], Sean Biggins[2,3], Owen Higgins[2,3], Alexandra Chueiri[2,3], Terry J. Smith[2,3], Catherine Burgess[4], Dearbháile Morris[1,2], Jean O'Dwyer[5,6], Paul D. Hynds[6,7], Martin Cormican[1,2], Liam P. Burke[1,2], Louise O'Connor[2,3]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Galway, Ireland. [2]Centre for One Health, Ryan Institute, University of Galway, Galway, Ireland. [3]Molecular Diagnostics Research Group, College of Science & Engineering, University of Galway, Galway, Ireland. [4]Teagasc Food Research Centre, Ashtown, Dublin, Ireland. [5]School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland. [6]Irish Centre for Research in Applied Geosciences (iCRAG), University College Dublin, Dublin, Ireland. [7]Environmental Sustainability and Health Institute, Technological University Dublin, Dublin, Ireland

Abstract

Background: Shiga toxin-producing *Escherichia coli* (STEC) is an important water-borne pathogen that causes hemolytic-uremic syndrome. All STEC serogroups harbour genes that can produce at least one Shiga toxin (*stx1* and/or *stx2*), which constitute the main virulence factors of STEC. The aim of this study was to develop a Loop-mediated isothermal amplification (LAMP) test which enables rapid real-time STEC detection using a portable on-site technology.

Methods: Water samples (n=33) were collected from rivers (n=16), groundwater wells (n=16) and agricultural drain (n=1) from the Corrib catchment in Galway. Water samples (100 ml) were passed through a 0.22 µm filter and buffer was added to elute captured cells. An aliquot of the eluate was tested directly using LAMP assays targeting *stx1*, *stx2* and *E. coli phoA* genes. The average time from sample collection to final results is 40 minutes. Previously published real-time PCR assays targeting *stx1* and *stx2* genes were used to confirm the results

Results: The limit of detection of the LAMP assays was <10 CFU/reaction. Overall, *stx1* and *stx2* were detected in 20/33 (61%) and 11/33 (33%) samples; respectively, while *E. coli* (*phoA*) was found in 30/33 (91%) samples. Using PCR tests for confirmation, all samples tested positive for *stx1* and *stx2* genes.

Conclusion: We describe a simple, transferable and efficient diagnostic technology which can be applied to carry out simple on-site analysis of various water sources. This method offers the potential to test drinking water on-site allowing swift decisions to be made in relation to usage by local authorities.

Toxic cyanobacteria journey from water to agricultural soil: Their colonization in soil and impacts on crop productivity, soil microbiome, and cyanotoxin risks

Jiyoung Lee ORCID iD, Yuehan Ai, Nicholas Basta

Ohio State University, Columbus, USA

Abstract

Properly managing the enormous amount of drinking water treatment residuals (WTRs) generated daily is crucial, with land application being a common solution. However, concerns arise when harmful cyanobacterial blooms (HCBs) affect the source water, prompting questions about the impact of HCB-affected WTR on soil and crops. Thus, this study aimed to: 1) characterize WTRs, focusing on cyanotoxins, nutrients, and microbiome; 2) track the dynamic of microbiome, microcystin (MC), and viable toxic cyanobacteria in WTR-amended soil; and 3) assess the WTR application impact on soybean productivity and the food safety risk. Two WTRs, their source water from two lakes in Ohio, USA, with varying HCB intensities, were applied to greenhouse soil (5% rate by dry weight) for soybean cultivation, Ohio's most common and essential crop. Both WTRs contained MC, saxitoxin, and β-Methylamino-L-alanine (BMAA). Nanopore sequencing revealed different core cyanobacterial genera, but both showed MC-producing *Planktothrix* and *Microcystis*. To investigate the dynamic of total and viable MC-producing cyanobacteria in WTR-amended soil, MC-synthesis genes were quantified in the extracted soil DNA and RNA, respectively. MC-producing *Planktothrix* survived better in soil than MC-producing *Microcystis*. Colonized MC-producers continuously released the toxin, elevating MC concentrations in the WTR-amended group at harvest. While soybean MC concentrations posed no health risk based on the MC in the edible portion of soybean, WTR addition impaired soybean productivity. Therefore, WTR application rates need to be systematically adjusted to minimize the long-term negative impacts of toxic cyanobacteria and other WTR biochemical attributes on food security and environmental health.

Wide distribution of plasmid mediated quinolone resistance gene, *qnrS*, among *Salmonella* spp. isolated from canal water in Thailand

Jirachaya Toyting[1], Neunghatai Supha[2], Yuwanda Thongpanich[2], Jeewan Thapa[1], Chie Nakajima[1,3,4], Yasuhiko Suzuki[1,3,4], Fuangfa Utrarachkij[2]

[1]Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Japan. [2]Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok, Thailand. [3]Global Institution for Collaborative Research and Education, Hokkaido University, Sapporo, Japan. [4]Division of Research Support, Hokkaido University Institute for Vaccine Research & Development, Sapporo, Japan

Abstract

Antimicrobial resistance (AMR) is a growing public health concern. The aquatic environment, such as a canal, is regarded as an AMR gene dissemination pathway. Canals in Thailand serve people as consumption resources for agriculture and daily use, where the presence of AMR genes carrying bacteria is a crucial threat. This study aimed to examine the prevalence of Plasmid-Mediated Quinolone Resistance (PMQR) determinants in *Salmonella* isolated from canal water in Thailand. In total, 333 water samples were collected between 2016 and 2020 from six canals in Thailand. *Salmonella* spp. was isolated by bacterial culture, and PMQR genes were identified by the specific polymerase chain reactions. The results revealed that 92.2% of water samples were positive for *Salmonella* spp., in which serogroup B and serogroup C were the majority serogroups. A considerable proportion (35.6%) of *Salmonella* isolates carried PMQR genes, predominantly *qnrS* (97.2%, n = 140/144). Furthermore, *qnrB*, *qnrD, oqxAB*, and *aac(6')-Ib-cr* were also detected. Among PMQR-positive isolates, six isolates carried more than one PMQR gene. The prevalence of PMQR-positive *Salmonella* isolates in this study was higher than in all previous studies conducted in Thailand. To our knowledge, this is the first report of *qnrD* in *Salmonella* spp. isolated from environmental water in Thailand. In conclusion, the high prevalence of *Salmonella* in water and PMQR-positive isolates may raise public health concerns. This study highlights the importance of including environmental samples in the surveillance program, which is essential for AMR problem-solving in the One Health approach and for planning sustainable intervention strategies.

Nature-based solution to eliminate cyanotoxins in water using biologically enhanced biochar

Linda Lawton[1], Jane Moore[1], Anjali Jayakumar[2], Sylvia Soldatou[3], Ondřej Mašek[4], Christine Edwards[1]

[1]Robert Gordon University, Aberdeen, United Kingdom. [2]University of Newcastle, Newcastle, United Kingdom. [3]University of Aberdeen, Aberdeen, United Kingdom. [4]University of Edinburgh, Edinburgh, United Kingdom

Abstract

Climate change and intensive eutrophication are increasing the occurrence and intensity of toxic cyanobacterial blooms in drinking water supplies. Conventional water treatment struggles to eliminate cyanobacteria and their toxins resulting in the need for expensive tertiary treatments. We designed a nature-based solution for treating drinking water in rural communities of Sri Lanka, using biochar produced from agricultural waste (coconut shell, rubber wood and cinnamon wood). This provides a low-cost porous support for the immobilization of microbial communities, forming biologically enhanced biochar (BEB). Highly toxic microcystin-LR (MC-LR) was used to influence the microbial colonization of the biochar by natural lake water microbiome. The BEBs were challenged with a range of microcystins, cyanobacterial extracts and live cells over 11 months, always resulting in rapid elimination of toxins and even reduction in cyanobacterial cell numbers. 16S metagenomic analysis demonstrated the microbial communities were diverse and included genera such as *Sphingopyxis* sp. which is frequently reported to degrade microcystins. This proof-of-concept study demonstrates a sustainable, scalable and safe nature-based water treatment solution.

Antibiotic resistant *Citrobacter* spp. in wastewater and surface water

Jarmila Lausova ORCID iD[1,2], Iva Sukkar[2,3], Lenka Davidova Gerzova[2], Monika Dolejska[1,2,3,4]

[1]Department of Biology and Wildlife Diseases, University of Veterinary Sciences Brno, Brno, Czech Republic. [2]Central European Institute of Technology, University of Veterinary Sciences Brno, Brno, Czech Republic. [3]Biomedical Center, Charles University, Pilsen, Czech Republic. [4]Department of Clinical Microbiology and Immunology, The University Hospital Brno, Brno, Czech Republic

Abstract

Citrobacter spp. represents an important reservoir of clinically relevant antibiotic resistance genes (ARGSs). Although members of this genus are commensals of humans and animals or commonly found in water or soil, some of them can cause serious diseases such as urinary or respiratory tract infections, peritonitis or meningitis. These can spread from hospitals via wastewater into the environment. The aim of these study was to characterize antibiotic resistant *Citrobacter* spp. in wastewater, treated water and receiving river using whole genome sequencing (WGS).

From a collection of 154 *Citrobacter* spp. obtained during wastewater screening in three cities in Czechia, a total of 78 strains were selected for WGS based on PCR screening of beta-lactamase genes. The strains originated from hospital sewage (n=28), inflow (n=23) and outflow (n=15) of the municipal wastewater treatment plan (mWWTP) and river upstream (n=5) and downstream (n=7) the WWTP. A total of 54 different sequence types (ST) were detected among the collection, of which 17 were novel ST and five belonged to the most common ST19. A total of 104 variants of ARGs encoding resistance to 11 antibiotic classes were detected with average of five ARGs per strain. Sixteen strains originating from hospital sewage and outflow of the mWWTP carried carbapenemase genes $bla_{\text{GES-5}}$ (n=10), $bla_{\text{NDM-4}}$ (n=2), $bla_{\text{VIM-1}}$ (n=2), $bla_{\text{VIM-4}}$ (n=1) and $bla_{\text{OXA-48}}$ (n=1). Three strains from river downstream the mWWTP carried *mcr-10* gene.

The presence of carbapenem-resistant *Citrobacter* spp. in mWWTP effluent poses a risk to the environment and emphasises the importance of environmental surveillance.

Microbial Physiology, Metabolism and Molecular Biology Forum

Multidrug resistance plasmids commonly reprogramme expression of metabolic genes in *Escherichia coli*

Rebecca J Hall ORCID iD[1], Ann E Snaith[1], Matthew J N Thomas[2], Michael A Brockhurst[2], Alan McNally[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Manchester, Manchester, United Kingdom

Abstract

Multidrug resistant *Escherichia coli* is a leading cause of global mortality. Transfer of plasmids carrying genes encoding beta-lactamases, carbapenamases, and colistin resistance genes between lineages is driving the rising rates of hard to treat nosocomial and community infections. Multidrug resistance (MDR) plasmid acquisition commonly causes transcriptional disruption, and whilst a number of studies have shown strain-specific fitness and transcriptional effects of an MDR plasmid across diverse bacterial lineages, fewer studies have compared impacts of different MDR plasmids in a common bacterial host. As such, our ability to predict which MDR plasmids are the most likely to be maintained and spread in bacterial populations is limited. Here, we introduced eight diverse MDR plasmids encoding resistances against a range of clinically important antibiotics into *E. coli* K-12 MG1655 and measured their fitness costs and transcriptional impacts. The scale of the transcriptional responses varied substantially between plasmids, ranging from >650 to <20 chromosomal genes being differentially expressed. However, neither the scale of regulatory disruption nor the plasmid size correlated with the magnitude of the plasmid fitness cost, which also varied between plasmids. The identities of differentially expressed genes varied among plasmids, although expression of certain metabolic genes and functions were convergently affected by multiple plasmids, including the downregulation of genes involved in L-methionine transport and metabolism. Our data show the complexity of interaction between host genetic background and plasmid genetic background in determining the impact of MDR plasmid acquisition on *E. coli*.

ParB, do you really need CTP to segregate DNA?

Kirill Sukhoverkov ORCID iD, Tung Le ORCID iD, Govind Chandra ORCID iD

John Innes Centre, Norwich, United Kingdom

Abstract

Low-copy plasmids require sophisticated segregation machinery for reliable inheritance. The most common mechanism for bacterial DNA partition system is a tripartite ParABS system, which consists of a centromere *parS* DNA site and DNA-binding proteins ParA and ParB which are capable of hydrolyzing ATP and CTP nucleotides, respectively. To move replicated plasmids apart, the canonical ParB-type proteins first load on *parS* site and then upon binding to CTP form a clamp that can slide away thus vacating *parS* site to spread to adjacent DNA. The ParB-DNA complexes then bind to ParA, and by repetitive cycles of ParA-ParB binding-unbinding move the DNA cargo to opposite cell poles. Here, we first describe a CTP-independent ParABS system which is involved in the maintenance of a conjugative *S. coelicolor* A3(2) plasmid SCP2. We demonstrate, that a ParB-like protein SCP2.04c, which we renamed as ParT, loads onto DNA via *parS* site, spreads in the flanking region, despite the lack of CTP binding domain and any NTPase activity. Using *in vitro* reconstitution of ParT-*parS* system we show that *parS* site alone catalyses ParT transition from loading to a sliding state. We found that such CTP-independent spreading mechanism is widely spread among bacterial species and may play a role in many more biological processes besides DNA partition. Overall, our study demonstrates that parS mediated CTP independent clamping is an alternative mechanism for protein spreading on DNA and such proteins form a distinct subclass of ParB-like enzymes.

Contribution of Catalase to Antibiotic Tolerance in *Staphylococcus aureus*

Skylar Gay ORCID iD[1], Nikki Wagner[2], Michelle Angeles-Solano[2], Sarah Rowe[2], Brian Conlon[2]

[1]University of Virginia, Charlottesville, VA, USA. [2]University of North Carolina, Chapel Hill, NC, USA

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for over 11,000 deaths annually due to severe infections. MRSA exhibits antibiotic tolerance when treated with clinically utilized antibiotics posing a large challenge clinically. Antibiotic tolerance is the ability of bacteria to persist in the presence of bactericidal antibiotics in the absence of genetically encoded resistance and is often accompanied by low metabolic activity and decreased ATP. Exposure to Reactive Oxygen Species (ROS) induces higher levels of antibiotic tolerance. The ability to combat ROS results in higher metabolic activity leading to more antibiotic-mediated killing. One way MRSA combats ROS is with catalase which detoxifies H2O2 to H2O and O2. In a mutant lacking catalase, we expected to see higher antibiotic tolerance due to the inability to combat ROS. We tested the JE2::ΔkatA in macrophage infections where phagocytosed S. aureus was exposed to oxidative stress. Additionally, this mutant was tested in biofilm with the addition of clinically relevant antibiotics and antioxidants. The JE2::ΔkatA mutant exhibits a significant phenotype of low antibiotic tolerance, contrary to the initial hypothesis. Further investigation of this phenotype revealed a significantly higher amount of ATP within the mutant versus the WT. This suggests a relationship between catalase and antibiotic tolerance. Additionally, this raises the question of ATP consumption during stress response as catalase appears to be a major consumer of ATP under ROS conditions.

Rolling in the DtpT: Deciphering the role of a neglected peptide permease in *Staphylococcus aureus* growth and infection

Imran Khan ORCID iD[1], Alice Cannon[2], Sigurbjorn Markusson[3], Sandy Macdonald[1], Holly Wilkinson[2], Christopher Mulligan[4], Simon Newstead[3], Joanne Parker[3], Marjan Van Der Woude[1], Gavin Thomas[1]

[1]University of York, York, United Kingdom. [2]Hull York Medical School, Hull, United Kingdom. [3]University of Oxford, Oxford, United Kingdom. [4]University of Kent, Canterbury, United Kingdom

Abstract

During an infection, growth of the bacterial pathogen *Staphylococcus aureus* is heavily reliant on the acquisition of host-derived peptides and amino acids as nutrient sources. Peptide utilisation in this organism is known to be facilitated by at least two transport systems: the ATP-binding cassette system Opp3 and the proton-dependant oligopeptide transporter DtpT. While Opp3 has previously been implicated in collagen utilisation in the context of skin infections, the physiological role of DtpT is comparatively understudied. Furthermore, the substrate specificities of both transporters remain poorly characterised despite their fundamental importance to cellular metabolism.

Here, we apply Phenotype Microarrays (BIOLOG) to compare utilisation of 282 small peptides by wild-type *S. aureus* JE2 against strains with disruptions in the known peptide transporters. From this, we observe that DtpT is required for the utilisation of around a third of the tested peptides. Moreover, these data demonstrate an apparent preference for glycine, glutamate and aspartate-containing peptides among the putative DtpT substrates, which may point toward the distinct role of this system. In order to further characterise the specificity of the transporter, we utilise a cell-free transport assay in which the intact DtpT protein is reconstituted into proteoliposomes. Finally, we describe ongoing experiments aiming to utilise an *ex vivo* human skin infection model to assess the importance of the *S. aureus* peptide permease systems during skin colonisation. Overall, our findings represent the most comprehensive investigation of peptide utilisation by *S. aureus* to date, and provide the first clues toward the distinct physiological function of DtpT.

RNA Hairpin-Mediated Transcription Termination at High Temperature in *Thermus aquaticus*

Nicholas P Bailey ORCID iD, Amber Riaz-Bradley, Yulia Yuzenkova

Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

RNA hairpin-mediated intrinsic termination is one of the two major transcriptional termination mechanisms in bacteria and serves an important role in insulating regulation between operons. Thermophilic bacteria have biotechnological applications in high-temperatures processes, which could be assisted by improving understanding of transcription in these organisms. RNA structures are generally unstable at high temperatures, and so the efficiency and importance of hairpin-mediated termination is unclear for thermophilic organisms. We sought to address this question by mapping RNA 3' termini across the genome of the extreme thermophile *Therumus aquaticus* (*Taq*) by TermSeq. Results indicated numerous termini within genes, suggesting frequent intragenic pausing or termination. RNA hairpins were computationally predicted upstream of the majority of *Taq* termini. Thermal stability of *Taq* hairpins was predicted to be substantially greater than those identified from the mesophile *Escherichia coli* based on previously published data. However, dinucleotide shuffled versions of the *Taq* terminators showed only a mild reduction in predicted thermal stability, indicating a general propensity of the GC-rich genome of *Taq* to form spurious hairpins. A native *Taq* terminator was able to trigger intrinsic termination for *Taq* RNA polymerase *in vitro*, albeit with low efficiency (~35%). We present two competing hypotheses; A) in *Taq*, intrinsic termination efficiency is low *in vivo*, and intragenic termination triggered by spurious hairpins helps to insulate from read-through transcription B) in *Taq*, intrinsic termination is higher *in vivo* than was observed *in vitro*, potentially aided by an as-yet unidentified termination factor.

Characterisation of novel bacteriophages in a hyper-adapted strain of *Enterocloster clostridioformis* associated with gut microbiome dysbiosis.

Suzie Humphrey ORCID iD[1], Angeliki Marouli[1,2], Katja Thuemmler ORCID iD[1], Dónal Wall ORCID iD[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom

Abstract

Enterocloster clostridioformis (previously known as *Clostridium clostridioforme*) is a commensal bacterium that is present at low levels in the human intestine but becomes abundant in the gut of those with metabolic disorders, such as type 2 diabetes and obesity, where the microbiome balance is disturbed (dysbiosis). The mechanisms underpinning the development of dysbiosis are unknown. We have isolated a strain of *E. clostridioformis* (LM41) that reproduces the outgrowth phenotype associated with human dysbiosis, but does so to an unusually high extent, comprising up to 60% of the total gut microbiota in *Fmr1*-KO mice. Genome sequencing of strain LM41 revealed an atypically high proportion of mobile genetic elements, with a particularly high abundance of prophages. Bioinformatic analysis predicted that of the 15 prophages present, 7 were intact, 3 were defective, and 5 could not be classified. We observed diversity in the lysogeny mechanisms utilised by the prophages and identified carriage of a variety of genes with roles in mediating restriction modification and genetic diversity, which may affect the ability of LM41 to take up and retain exogenous DNA. Experimental analyses indicate that LM41 prophages respond differently to classical inducing agents and exhibit bactericidal effects against other strains of *E. clostridioformis*. This work begins to shed light on the contribution of prophages to the lifestyle of poorly characterised members of the microbiome with roles in dysbiosis.

Bacteriocin outer membrane binding and translocation in Klebsiella pneumoniae

Angela Sun, Nicholas Housden, Melissa Webby, Colin Kleanthous

University of Oxford, Oxford, United Kingdom

Abstract

Bacteriocins are diffusible protein toxins deployed by bacteria in competition with their neighbours. Because of their potent action and high target specificity, bacteriocins have attracted interest as potential alternative therapeutics for antibiotic-resistant infections. For bacteriocins to exert killing activities, they must deliver their single, C-terminal cytotoxic domain into cells. In Gram-negative bacteria, this requires transport across the outer membrane (OM), which is driven by the proton motive force across the inner membrane (IM). Here, we report a novel OM transport mechanism of a nuclease bacteriocin klebicin G (KlebG) which was found to be active against the nosocomial pathogen *Klebsiella pneumoniae*. We identified the trimeric porin OmpK35 as both the receptor and OM translocator for KlebG and demonstrated that import is dependent on the energised Tol system. We have also solved the cryo-EM structure for the OmpK35-KlebG complex which shows tridentate binding of the bacteriocin to the porin surface: two β-hairpin motifs associate with two subunits in such a way as to permit insertion of a transport-activating (Tol-binding) epitope through the one unoccupied subunit to the periplasm. Further biophysical dissection of this interaction shows that KlebG binds with nanomolar affinity to OmpK35 and requires both its β-hairpins for high-affinity binding and toxicity. Porin-recognition by β-hairpin motifs is likely to be a common surface recognition feature in Gram-negative bacteria since a similar mode of binding has recently been described for bacterial conjugation systems in the *Enterobacteriaceae*.

The biofilm matrix of *Pseudomonas aeruginosa* is a distinct extracellular compartment which selectively traps key secreted proteins

Rahan Nazeer, Martin Welch

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Abstract

Pseudomonas aeruginosa (PA) is both a 'professional secretor' and a prolific biofilm former. Consequently, many secreted factors need to pass through the biofilm matrix. This raises the question of whether any of these secreted factors are selectively trapped in the matrix, forming a distinct biofilm "matrixome".

To address this, we developed a method to separate biofilm cells from the matrix and from the culture supernatant, yielding clearly-differentiated "exo-compartments" for proteomic analysis. This revealed that the matrix does indeed selectively trap key secreted factors, while allowing others to pass through into the "true secretome". One of the most abundant matrix-enriched proteins was an uncharacterised protein, PA2668. Encoded immediately adjacent to PA2668 is an uncharacterised Type II Secretion (T2S) system, designated as the *hpl* cluster. Given that the substrates of many T2S systems are also encoded adjacent to their cognate secretion machinery, it seems likely that PA2668 may be exported through the Hpl system. dN/dS analyses indicate that the *hpl* cluster and PA2668 are highly conserved across clinical isolates suggesting functional importance. To investigate this further, we have purified PA2668 – which bears no similarity to any previously characterised protein - and have begun a detailed functional characterisation. Single chain Fv antibodies are being used to assess the expression and localization of PA2668, and both PA2668 and the *hpl* cluster have been cleanly-deleted for proteomic definition of the Hpl secretome and phenotypic analyses. *LacZ* fusions and DNA pulldowns are being used to assess how expression of the Hpl machinery and PA2668 are regulated.

Single molecule studies of fungal virulence factor candidalysin reveal self-assembly interactions and multimodal kinetics

Katherine Schaefer[1], Elizabeth Conley[1], Charles Russell[2], Robert Pyron[2], Francisco Barrera[2], Gavin King[1]

[1]University of Missouri, Columbia, MO, USA. [2]University of Tennessee, Knoxville, TN, USA

Abstract

Candidalysin (CL) is a fungal virulence factor secreted by Candida albicans which is crucial for damaging mammalian cells and immune activation during infection. We have previously shown the toxicity mechanism relies on spontaneous assembly of CL into polymers prior to membrane binding and pore formation. A critical component of pore structure may be the loops formed by closure of linear CL polymers. Using multiple biophysical techniques, we performed studies to determine CL assembly rates and probed the interactions that guide CL oligomerization and polymerization. Understanding the mechanism required single-molecule visualization of CL complexes. We utilized atomic force microscopy (AFM), a technique capable of direct visualization of CL assemblies in physiologically relevant temperatures and buffer solution. Differentiation of CL particle types observed in AFM, such as linear polymers, loops, and larger complexes with secondary polymerization sites, led to the determination of multiple assembly rates. Additionally, CL interactions were probed under varying concentrations and ionic strength. The AFM results were supported by mass photometry and other biophysical techniques. Taken together, these data provide insight into the novel mechanism of this fungal peptide toxin and pave the way for therapeutic development.

Spatial organization of *Pseudomonas aeruginosa* aggregates mediates tolerance to antibiotic therapies

Alexa Gannon, Sophie Darch ORCID iD

University of South Florida, Tampa, USA

Abstract

In the context of disease, bacterial aggregates have been isolated from both chronic *and* acute infections and can be formed by bacteria, archaea, and fungi. As an opportunistic pathogen, *Pseudomonas aeruginosa* (*Pa*), causes disease in those whose immune systems or barrier functions are compromised. This includes those with chronic and acute wounds, medical devices, and chronic infection in the lungs of people with the genetic disease cystic fibrosis (CF). Once chronic *Pa* colonization is established, a large proportion of the infecting bacteria grow within airway sputum as aggregates (~10-1000 cells). Previous studies of *Pa* cells in large, well-mixed flask cultures, (macro-scale biofilm structures) have contributed significantly to our understanding of *Pa* growth, communication systems, and mechanisms *Pa* utilizes to become tolerant to many antibiotics. However, growth in this context does not closely recapitulate that of actual infection – growth as aggregates. This discrepancy signifies a significant gap in translating our knowledge of biofilm biology into meaningful insights for human health. Using a synthetic cystic fibrosis (CF) sputum media (SCFM2), we have identified a subset of genes critical for aggregate formation. Disruption of these genes results in a spectrum of spatial phenotypes that result in micron-scale changes in organization of *Pa* cells within aggregates. We show for the first time a direct relationship between changes in spatial organization and tolerance to antibiotic therapies. These data significantly contribute to our understanding of aggregate physiology during infection, with the potential develop new therapeutic strategies against *Pa* aggregates and other aggregate forming multi-drug resistant pathogens.

Optimisation of lipopeptide titres in *Bacillus* using active learning and high-throughput mass spectrometry

Ricardo Valencia Albornoz ORCID iD[1], Diego Oyarzún ORCID iD[2], Karl Burgess ORCID iD[1]

[1]School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom. [2]School of Informatics, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Integration of machine learning and high throughput measurements are essential to drive the next generation of the design-build-test-learn (DBTL) cycle in synthetic biology. Here, we report the use of active learning in combination with metabolomics for optimising production of surfactin, a complex lipopeptide resulting from a non-ribosomal assembly pathway, in *Bacillus subtilis*. We designed a media optimisation algorithm that iteratively learns the yield landscape and steers the media composition toward maximal production. The algorithm led to a 160% yield increase after three DBTL runs as compared to an M9 baseline. Metabolomics data helped to elucidate the underpinning biochemistry for yield improvement and revealed Pareto-like trade-offs in production of other lipopeptides from related pathways. We found positive associations between organic acids and surfactin, suggesting a key role of central carbon metabolism, as well as system-wide anisotropies in how metabolism reacts to shifts in carbon and nitrogen levels. Our framework offers a novel data-driven approach to improve yield of biological products with complex synthesis pathways that are not amenable to traditional yield optimisation strategies.

Complexation of CcmB with CcmACD safeguards heme translocation for cytochrome c maturation

Haichun Gao ORCID iD

Zhejiang University, Hangzhou, China

Abstract

Cytochromes *c*, essential proteins in respiration and photosynthesis, require a post-translational maturation process for the covalent attachment of heme. In organisms hosting System I maturation machinery, CcmB is the only one out of 8-9 Ccm components that remains functionally undefined. In this study, we identify and substantiate CcmB as heme efflux pump. We then show that complexation with CcmACD ensures heme translocated by CcmB to be used for the maturation only. Importantly, structural analysis and atomistic molecular dynamics simulation reveals that CcmB absorbs heme from the membrane to a heme pocket formed in the dimer interface of the transmembrane helix-bundles. Our data, by providing detailed insight into the conformational landscape of CcmB during heme entry, fill in the missing link in our understanding of the heme translocation for cytochrome *c* maturation.

The ClpX protease is essential for inactivating the CI master repressor and completing prophage induction in *Staphylococcus aureus*

Mohammed Thabet ORCID iD[1,2], José Penadés ORCID iD[3], Andreas Haag ORCID iD[4]

[1]School of Infection & Immunity, University of Glasgow, Glasgow, United Kingdom. [2]Department of Biology, Faculty of Science, Al-Baha University, Al-Baha city, Saudi Arabia. [3]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom. [4]School of Medicine, University of St Andrews, St Andrews, United Kingdom

Abstract

Bacteriophages (phages) are the most abundant biological entities on Earth, exerting a significant influence on the dissemination of bacterial virulence, pathogenicity, and antimicrobial resistance. Temperate phages integrate into the bacterial chromosome in a dormant state through intricate regulatory mechanisms. These mechanisms repress lytic genes while facilitating the expression of lysogenic genes such as integrase and the CI master repressor. Upon bacterial SOS response activation, the CI repressor undergoes auto-cleavage, producing two fragments with the N-terminal domain (NTD) retaining significant DNA-binding ability. The process of relieving CI NTD repression, essential for prophage induction, remains unknown. Here we show a specific interaction between the ClpX protease and CI NTD repressor fragment of phages Φ11 and 80α in *Staphylococcus aureus*. This interaction is necessary and sufficient for prophage activation after SOS-mediated CI auto-cleavage, defining the final stage in the prophage induction cascade. Our findings unveil unexpected roles of bacterial protease ClpX in phage biology.

Reference:

Thabet, M. A., J. R. Penadés and A. F. Haag (2023). "The ClpX protease is essential for inactivating the CI master repressor and completing prophage induction in *Staphylococcus aureus*." Nat Commun **14**(1): 6599.

Behavioral split in motility endurance under carbon starvation reveals an ecological dichotomy among motile copiotrophs

Johannes Keegstra ORCID iD[1], Zach Landry[1], Sophie Zweifel[1], Francesco Carrara[1], Clara Martínez-Pérez[1], Estelle Clerc[1], Ben Roller[2], Martin Ackermann[3], Roman Stocker[1]

[1]ETH, Zurich, Switzerland. [2]University of Vienna, Vienna, Austria. [3]EAWAG, Duebendorf, Switzerland

Abstract

Many copiotrophic bacteria experience a feast-famine lifestyle in heterogeneous environments, where they experience starvation in between infrequent encounters with rich nutrient hotspots. Motility increases the encounters with such hotspots by 100-1000 fold, but is also energy demanding to a starving cell, giving rise to a risk-reward trade-off. Here, we used videomicroscopy and particle tracking to quantify the behavioral response of 26 strains from 18 different species of marine bacteria over two days of carbon starvation. Our results reveal a dichotomy in the motility behavior in response to carbon starvation: there are species that cease motility within hours (`limostatic'), whereas others retain motility for at least two days (`limokinetic'). The biomass during starvation remained constant for species that ceased motility but decreased approximately 10 % per day for the species that remained motile, of which several species accumulated energy storage compounds (PHB and polyphosphate) before starvation. This shows strains need to convert biomass into energy to fuel motility, but doing so extends their search behavior and thus increases the chance of future biomass gain. Using machine-learning classifiers we identified a genetic component associated with this dichotomy, sufficiently robust to predict the response of an additional set of marine strains with >80% accuracy. Overall, these results expand our understanding of foraging strategies in bacteria, revealing a dichotomy among copiotrophs: risk-prone foragers that retain motility during starvation, and risk-averse foragers that rapidly give up motility during starvation.

Dissecting the *Escherichia coli*'s cell chaining phenotype

Adam Gicgier[1,2], Magdalena Lechowska[1], Joanna Szczepaniak[1], Colin Kleanthous[1]

[1]University of Oxford, Oxford, United Kingdom. [2]John Innes Centre, Norwich, United Kingdom

Abstract

The outer membrane (OM) constitutes an integral component of the Gram-negative bacterial cell envelope, functioning as a robust barrier that impedes the influx of large molecules and plays a pivotal role in multidrug resistance. The Tol-Pal system is composed of three inner membrane proteins (TolQ, TolR, TolA), a periplasmic protein (TolB) and an OM lipoprotein (Pal), all of which are known to be recruited to the septum during cell division to stabilise the OM constriction. Mutations within this system give rise to a pleiotropic *tol* phenotype, characterized by a loss of antibiotic resistance, blebbing, and cell chaining. Unexpectedly, it was shown that instead of failing to invaginate the OM, Δ*tol-pal* cells chain due to the peptidoglycan (PG) septation impairment. However, the direct link between TolA and PG remodelling is yet to be disentangled. *cpoB* is a part of the *tol-pal* operon however its absence does not compromise OM stability. It is thought to work with TolA to modulate the transpeptidase activity of PBP1B at the septum. Here, we demonstrate that the loss of *cpoB* in *tolA* cells significantly diminishes cell chaining, providing compelling evidence for CpoB's involvement in the *tol* phenotype. Using both electron and fluorescence microscopy, we dissect the chaining phenotype, categorizing the effects based on failure in either OM invagination or PG synthesis regulation. We propose a model in which the PG splitting defect arises from the hyperactivity of CpoB at the septum, inducing an imbalance between the glycotransferase and transpeptidase activities of PBP1B.

Dissection of the epigenetically controlled gene network in aflatoxigenic fungi to improve agricultural productivity and food safety

Pranay Agarwal ORCID iD, Ozlem Bayram, Kevin Kavanagh, David Fitzpatrick

Maynooth University, Maynooth, Ireland

Abstract

Aspergillus spp. contribute significantly to annual food losses through the production of mycotoxins like aflatoxin B1 (AFB1) and Sterigmatocystin (ST). These mycotoxins, part of a broader group of secondary metabolites (SMs) produced by Aspergillus spp., are regulated by molecular mechanisms driven by post-translational modifications.

CclA, a H3K4 methyltransferase, is a key component of the novel MERCK complex, consisting of five subunits, which plays a regulatory role in SM and development. Deletion mutants of this complex display defects in various aspects of Aspergillus spp., such as sexual fruit body formation, sporulation, hyphal growth, and secondary metabolite production. This project aims to uncover the MERCK complex's role in the regulation of SM biosynthesis.

The study analyzes proteomic and transcriptomic changes in *cclA* mutants of *Aspergillus nidulans* and *Aspergillus flavus* at the primary metabolism (PM) stage (20h growth) and the secondary metabolism (SM) stage (48h growth) compared to the wild type (WT). CclA significantly downregulates over 1300 genes at both stages of growth in both species.

In *A. nidulans*, CclA upregulates several genes involved in phosphate metabolism and cell-wall organization, while downregulating genes associated with ST biosynthesis at PM stage. At the SM stage, it upregulates genes involved in ST production and downregulates genes involved in Terpenoid and Austinol production.

In *A. flavus*, CclA upregulates AT production and several genes involved in amino acids' metabolism, while downregulating genes associated with transmembrane transport at PM stage. At SM stage, it upregulates genes involved in phosphate metabolism and downregulates genes in transmembrane transport.

SpoVG as a Global RNA Regulator of *Bacillus subtilis* Toxin Production

Andreas Lawaetz[1], Swati Mahapatra[2], Niki Christopoulou[3], Pedro Rei[3], Sander Granneman ORCID iD[3], Emma Denham ORCID iD[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Warwick, Coventry, United Kingdom. [3]University of Edinburgh, Edinburgh, United Kingdom

Abstract

On the battleground of biology, microorganisms adopt a multitude of strategies to ensure the survival of their genes. *Bacillus subtilis* is a widely studied Gram-positive model organism that undergoes a range of differentiation states in response to stress, including genetic competence, biofilm formation, and sporulation. Much is understood about the transcriptional regulation involved in these processes, but little is known about the regulation beyond transcription, largely due to the absence of known global RNA-binding proteins. In this study, we employed Cross-Linking and Analysis of cDNAs (CRAC), RNA-seq and proteomics to study the RNA-binding protein SpoVG in *B. subtilis*. Our investigation revealed that SpoVG binds RNA with a preferential binding to genes encoding antimicrobials, multidrug exporters, motility components, and prophage elements. Using motif analysis, we found a differential binding affinity of SpoVG towards coding and non-coding transcripts. RNA-seq and proteomics analysis of a *ΔspoVG* strain revealed enriched categories of antibacterial compounds, toxins, anti-toxins, and prophages, correlating well with its RNA-binding profile. Phenotypic analyses revealed altered patterns of biofilm formation, abnormal migration and swarming along with perturbed production of several antimicrobial compounds. Using reporter fusion assays along with northern and western blots we are able to verify our omics data and evidence suggests that SpoVG exerts its gene regulatory function by increasing transcript stability. Our study provides new insights to the roles of RNA-binding proteins in *B. subtilis* and demonstrates a novel function for SpoVG as a global regulator of toxin production through protein-RNA interactions.

Microbiota-Immune System and Vaccine Interplay (collaboration with British Society for Parasitology and Protistology UK)

Invited talk: Identification of human monoclonal antibodies against Shigella (ShimAbs) for vaccine acceleration

Elena Boero[1], Giacomo Vezzani[1], Maria Michelina Raso[1], Giampiero Batani[2], Emanuele Roscioli[2], Assia Duatti[1], Roberta Di Benedetto[1], Francesco Nannini[2], Matteo Ridelfi[2], Eleonora Marini[2], Simona Tavarini[3], Chiara Sammicheli[3], Carlo Giannelli[1], Gianmarco Gasperini[1], Makrina Totsika[1], Francesco Berlanda Scorza[1], Mariagrazia Pizza[4], Miren Iturriza[1], Omar Rossi[1], Claudia Sala[2], Francesca Micoli[1], Rino Rappuoli[5]

[1]GVGH, Siena, Italy. [2]Monoclonal Antibody Discovery Laboratory, Fondazione Toscana Life Sciences, Siena, Italy. [3]GSK, Siena, Italy. [4]Imperial College, London, United Kingdom. [5]Fondazione Biotecnopolo di Siena, Siena, Italy

Abstract

Shigella is a primary contributor to global diarrheal infections, disproportionately affecting vulnerable populations in areas with inadequate sanitation. Multi- and extensively-drug-resistant strains have been associated with recent outbreaks, exacerbating the problem. The complex pathogenesis of *Shigella* and the diversity of the O-antigens (OAg) in more than 50 serotypes make vaccine development challenging.

Here a Reverse Vaccinology 2.0 approach has been used to discover human monoclonal antibodies (ShimAbs) that could help identify shared epitopes among various serotypes, reveal so far neglected protein antigens, and create tools to accelerate vaccine discovery and development.

A high-throughput pipeline was established to isolate ShimAbs from individuals vaccinated with altSonflex1-2-3 (NCT05073003), a vaccine based on 4 different OAgs. Functional antibodies were selected through high-throughput innovative bactericidal and adhesion/invasion inhibition assays. Potent OAg-specific ShimAbs with cross-bactericidal properties against different *Shigella* were identified, confirming preclinical data showing the ability of altSonflex1-2-3 to induce antibodies functional also against a panel of *Shigella* strains carrying OAgs not included in the vaccine. ShimAbs preventing intestinal invasion and targeting previously disregarded proteins were pinpointed. Overall, these findings will facilitate the exploration of new shared sugar epitopes and the identification of novel target antigens, thus supporting the design and development of simplified vaccines with broad coverage. Finally, ShimAbs will be used to dissect the molecular anatomy of protection from shigellosis and facilitate the identification of immune correlates of protection against *Shigella*. The approach proposed can be easily extended to other pathogens and shows the interplay between mAbs and vaccine technologies to fight infectious diseases.

Funding: Wellcome Trust, GSK

Invited talk: Digestive tract microbiota and metabolome are associated with humoral response to COVID-19 vaccination in immunosuppressed individuals

James Alexander ORCID iD

Imperial College, London, United Kingdom

Abstract

Individuals with digestive tract disorders such as inflammatory bowel disease (IBD) or liver cirrhosis may be immunocompromised as a consequence of their disease or its treatment, and exhibit attenuated humoral immune responses to COVID-19 vaccination. The microbiota and its functional metabolic output, which are perturbed in IBD and liver cirrhosis, play an important role in shaping host immune responses. Our work explores whether the microbiota and metabolome could explain variation in anti-SARS-CoV-2 vaccination responses in immunosuppressed patients. Using faecal, saliva and serum samples prospectively collected from patients with liver disease and IBD in two large UK-wide studies of patients undergoing vaccination against SARS-CoV-2, we performed 16S rRNA gene amplicon sequencing, nuclear magnetic resonance (NMR) spectroscopy and ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS). We found associations between antibody responses to vaccination with both gut and oral microbiota and metabolome. Notably, in IBD patients, gut microbiota beta diversity was lower in poorer responders to vaccination, whilst trimethylamine, isobutyrate and omega-muricholic acid were associated with better response, and succinate, phenylalanine, taurolithocholate and taurodeoxycholate were associated with poorer response. In salivary microbiota, alpha diversity increased after vaccination and oral pathobionts were associated with a better antibody response. Our data suggest that there is an association between the microbiota and variable serological response to vaccination against SARS-CoV-2 in immunocompromised patients. Microbial metabolites including trimethylamine may be important in mitigating immunosuppression-induced attenuation of the immune response.

Invited talk: R21/Matrix-M™ vaccine: a turning point in the control and elimination of malaria?

Mehreen S. Datoo, Adrian V.S. Hill

Jenner Institute, University of Oxford, Oxford, United Kingdom

Abstract

In the last few years, significant progress has been made in malaria vaccine development with the first WHO recommendation of a malaria vaccine, RTS,S/AS01. However, initial supply of this vaccine is limited and will not meet the needs of the target population. Compounding this, global progress against malaria has stalled, as well as increases recorded in malaria mortality.

The R21/Matrix-M™ malaria vaccine received WHO policy recommendation and prequalification at the end of 2023. It is a circumsporozoite (CS) protein-based vaccine, and is the first malaria vaccine to date to reach the WHO-specified goal of ≥75% efficacy in African children over two years, with efficacy maintained over four years. This efficacy was first reported in the phase II trial in Burkina Faso and the larger, ongoing phase III trial is demonstrating similar results to date.

R21/Matrix-M™ has demonstrated a good safety profile, induced high antibody responses to the central repeat of the CS protein (NANP) and provided high-level efficacy in UK and African adult and infant populations.

Key data on safety, immunogenicity and efficacy from the phase I-III trials will be presented that supported the regulatory approvals and licensure in several African countries as well as WHO endorsement.

The Serum Institute of India Pvt. Ltd have committed to manufacturing up to 200 million doses of R21/Matrix-M™ annually. High-level efficacy of this vaccine, combined with the commitment to large-scale supply, at low cost ($3.9/dose), should have a significant impact now, and in the long term, on the lives of those in malaria endemic areas.

Authors listed are presenting on behalf of the R21/Matrix-M Phase III Trial Group*:

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(23)02511-4/fulltext

Mehreen S. Datoo DPhil[1], Alassane Dicko PhD[2], Halidou Tinto PhD[3*], Jean-Bosco Ouédraogo MD[4] Mainga Hamaluba DM[5,6], Ally Olotu DPhil[7^], Emma Beaumont MSc[8], Fernando Ramos Lopez MSc[1], Hamtandi Magloire Natama PhD[3], Sophie Weston MSc[1], Mwajuma Chemba MSc[7], Yves Daniel Compaore MD[4], Djibrilla Issiaka PhD[2], Diallo Salou PhD[3], Sharon Omenda MBChB[5], Alison Lawrie PhD[1], Philip Bejon PhD[5], Harish Rao PhD[9], Daniel Chandramohan PhD[8], Rachel Roberts MSc[1], Sandesh Bharati MD[8], Lisa Stockdale PhD[10], Brian M. Greenwood FRS[8], Katie J. Ewer PhD[10], John Bradley PhD[8], Cyrus S. Poonawalla DSc[9], Prasad S. Kulkarni MD[9], Umesh Shaligram PhD[9], Adrian V. S. Hill FRS[1, 10+] and the R21/Matrix-M™ Phase III Trial Group*.

The gut microbiota in XCR1+ knockout chickens, with and without infectious bronchitis virus vaccination

Laura Glendinning ORCID iD[1], Mick Watson[2,1], Adam Balic[3,1]

[1]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [2]DSM, Heerlen, Netherlands. [3]The University of Melbourne, Melbourne, Australia

Abstract

The chicken immune system and microbiota play vital roles in maintaining gut homeostasis and protecting against pathogens. It is well known that the immune system plays a crucial role in shaping and maintaining the chicken gut microbiota, and that the gut microbiota itself contributes to immune system maturation. XCR1+ conventional dendritic cells (cDCs) are located in the gut-draining lymph nodes and play a major role in gut homeostasis. These cDCs sample antigens in the gut luminal contents and limit the inflammatory response to gut commensal microbes by generating appropriate regulatory and effector T-cell responses. XCR1 is a chemokine receptor exclusively expressed by cDCs in chickens and a subset of cDCs in mammals. Mice lacking XCR1 show decreased intraepithelial and lamina propria T-cells and an increased susceptibility to gut inflammation. It is likely that these cells play similar roles in sustaining gut homeostasis in chickens, and we hypothesised that chickens lacking XCR1 were likely to contain a dysbiotic caecal microbiota.

In this study we compare the caecal microbiota of chickens that were either heterozygous or homozygous XCR1 knockouts, that had or had not been vaccinated for infectious bronchitis virus (IBV). We used short-read (Illumina) and long-read (PacBio HiFi) metagenomic sequencing to reconstruct 670 high-quality, strain-level metagenome assembled genomes. We found no significant differences between alpha-diversity, beta-diversity or the abundance of specific microbial taxa between genotypes. However, IBV vaccination was found to correlate with significant differences in the richness and beta-diversity of the microbiota, and to the abundance of forty bacterial genera.

Uncovering Novel Mechanisms of Commensal *Lactiplantibacillus plantarum* to induce Type-I Interferon cytokines and inform Microbiome Immunomodulatory Therapies

Selvin Solis ORCID iD[1], Elaina Maldonado ORCID iD[1], Jorge Gutierrez-Merino ORCID iD[1], Carlos Maluquer de Motes ORCID iD[1], Subhankar Mukhopadhyay ORCID iD[2]

[1]University of Surrey, Guildford, United Kingdom. [2]King's College London, London, United Kingdom

Abstract

The gastrointestinal tract is home to a diverse range of microbes that interact with host cells, conferring a homeostatic and healthy microenvironment. However, we are still unsure which of these many commensal microbes are the main drivers that restore or protect health from disease. Much less is the information concerning the molecules these key commensals possess to interact with the immune system. Our recent investigations have shown that certain species and strains of lactobacilli specifically *Lactiplantibacillus plantarum* with high self-aggregative phenotype significantly activate type I interferon (IFN-I) cytokine production in macrophages. Such cytokines are essential against microbial infections and auto-immune disorders. We have proved that cGAS predominantly drives this IFN-I activation upon sensing bacterial DNA, which ultimately activates the cytosolic sensor STING. Furthermore, transcriptomics revealed expression of certain cell wall proteins in high IFN-I inducers including bacterial adhesins and aggregation-promoting factors, both influencing its potential to interact with macrophages for subsequent internalization via non-opsonic scavenger receptors. This was further confirmed with commensals lacking cell wall proteins for loss of function and conversely gain of function tests with a heterologous host transformed with adhesins. Thus, such an interplay between surface proteins is crucial for the activation and magnitude of these IFN-I cytokines potentially leading to different degrees of microbe-host interactions. Understanding this molecular crosstalk between the microbiome and mammalian cells will inform how specific molecules of commensals stimulate host responses at mucosal sites that, in unhealthy individuals, are exacerbated or inhibited thus, paving the way for major therapeutic and vaccine discoveries.

Navigating the Future of Antimicrobial Resistance: Innovative Strategies in Diagnostics and Surveillance

Transposable elements can be used to track the transmission of blaCTX-M genes

Gabriel Trueba ORCID iD[1], Liseth Salinas ORCID iD[1], Joseph Eisenberg ORCID iD[2], Jay Graham ORCID iD[3]

[1]Instituto de Microbiologia, Universidad San Francisco de Quito, Quito, Ecuador. [2]Department of Epidemiology, Ann Arbor, USA. [3]University of California, Berkeley School of Public Health, Berkeley, USA

Abstract

Bacterial transposable elements (Tns), like other mobile genetic elements, can pick up pieces of DNA that often contain genes coding for adaptive functions, such as antimicrobial resistance. Tns, containing antimicrobial resistance genes, move among different plasmids and from plasmids to chromosomes, making it difficult to track the transmission of ARGs: among human bacteria, from food-animal bacteria to human bacteria, or from human bacteria to food-animal bacteria. To better understand transmission, we studied 20 E. coli isolates harboring blaCTX-M allelic variants from an Ecuadorian semirural community. Sixteen of the blaCTX-M genes were in plasmids and 4 were in chromosomes. In all plasmids and chromosomes, the blaCTX-M genes (and flanking sequences) were bracketed by two IS26 transposable elements and showed evidence of recent movement among plasmids and from plasmids to chromosomes; some of these transpositions may have occurred in the same community. Consistent with this finding, we observed different blaCTX-M allelic variants and different flanking sequences in 2 Ecuadorian locations. In summary, we suggest that the use of DNA sequences from the blaCTX-M gene and its flanking regions may be useful to track the transmission of these genes in the community.

Bats are potential carriers and dispersers of antimicrobial-resistant bacteria within the One Health context

Natália C. Gaeta ORCID iD, João Eduardo Cavalcante Brito, Juliana Maria Nunes Batista, Beatriz Gagete Veríssimo Mello, Tiago Roberto Brito, Daniel Ubriaco Oliveira Gonçalves de Carvalho, Ricardo Augusto Dias ORCID iD, Marcos Bryan Heinemann ORCID iD

University of São Paulo, São Paulo, Brazil

Abstract

Bats play a crucial role in the ecology and epidemiology of various diseases, warranting a deeper understanding of their potential as vectors for antimicrobial-resistant bacteria. A study conducted between 2021-2022 captured 248 bats across urban-rural locations during the four seasons of the year, in the largest metropolitan region of Brazil. The culture and isolation of Gram-negative bacteria in fecal swabs revealed higher bacterial diversity in males. Enterobacteria, predominantly *E. coli*, *E. cloacae*, *S. marcescens, C. freundii*, and *E. hormaechei*, were isolated, with notable drug resistance, particularly to amoxicillin + clavulanic acid and cefoxitin. Most resistant isolates were found in adults, males, residential areas, and during the summer. Out of 63 clinically important resistance genes, six were examined in fecal swabs, using PCR. The genes $bla_{CTX\text{-}M}$, *sul1, tet(A), aadA*, and *oqxA* were identified. *Mcr-1* and *vanA* were not found. Thirty-one samples had > 1 gene, while four samples showed both $bla_{CTX\text{-}M}$/*sul1*, and one each had $bla_{CTX\text{-}M}$/*tetA* and $bla_{CTX\text{-}M}$/*aadA*. Additionally, eight samples had *tet(A)/sul1*, two had *tet(A)/oqxA*, and one had *tet(A)/addA*. Lastly, one sample carried all six genes. Notably, bats carried multiple resistant bacteria and resistance genes, suggesting their potential role in disseminating antimicrobial resistance in Brazil's largest metropolitan region. The study emphasizes the significance of male and adult bats in carrying antimicrobial-resistant bacteria, particularly during the reproductive season (summer). This study highlights the importance of continued monitoring and research in understanding their role in the complex dynamics of antimicrobial resistance dissemination within the One Health context.

Phenotypic antibiotic susceptibility testing for last-resort antibiotics based on bacterial nanomotions provides results in two hours

Marta Pla Verge, Gino Cathomen, Grzegorz Jozwiak, Laura Munch, Danuta Cichocka, Alexander Sturm ORCID iD

Resistell, Basel, Switzerland

Abstract

With the increasing prevalence of multi-drug resistant bacteria, last-resort antibiotics like ceftazidime-avibactam and cefiderocol have become crucial. Due to the lack of rapid diagnostic tools and their absence in most automated antibiotic susceptibility tests (AST), empirical initiation of therapy is common for critically ill patients. We propose a rapid phenotypic AST using the Resistell Phenotech, measuring bacterial nanomotions – or vibrations - within two hours. The growth-independent AST was developed and tested on clinical E. coli and K. pneumoniae isolates, recording nanomotions for ceftazidime-avibactam and cefiderocol. Machine learning algorithms achieved predictive models with 93% accuracy for spiked positive blood cultures, providing results within two hours. This rapid and accurate nanomotion AST challenges the conventional delayed gold-standard tests in hospitals, potentially transforming the assessment of antibiotic susceptibility and informing treatment decisions faster, thereby curbing the spread of antimicrobial resistance for critical drugs.

VIDIIA Hunter: an artificial intelligence-assisted rapid diagnostic platform for infectious diseases and antimicrobial resistance

Aurore C. Poirier ORCID iD[1], Ruben D. Riaño Moreno[2], Leona Takaindisa[1], Jessie Carpenter[2], Jai W. Mehat ORCID iD[1], Abi Haddon[3], Mohammed A. Rohaim ORCID iD[4], Craig Williams[5], Peter Burkhart[5], Chris Conlon[6], Matthew Wilson[6], Matthew McClumpha[6], Anna Stedman ORCID iD[1], Guido Cordoni ORCID iD[1], Manoharanehru Branavan[7], Mukunthan Tharmakulasingam ORCID iD[1], Nouman S. Chaudhry ORCID iD[1], Nicolas Locker ORCID iD[1], Anil Fernando ORCID iD[1], Wamadeva Balachandran[7], Mark Bullen[6], Nadine Collins[3], David Rimer[2], Daniel L. Horton ORCID iD[1], Muhammad Munir ORCID iD[4], Roberto M. La Ragione ORCID iD[1]

[1]University of Surrey, Guildford, United Kingdom. [2]Vidiia Ltd, Guildford, United Kingdom. [3]Royal Surrey County Hospital, Guildford, United Kingdom. [4]Lancaster University, Lancaster, United Kingdom. [5]The Royal Lancaster Infirmary, Lancaster, United Kingdom. [6]GB Electronics (UK) Ltd, Worthing, United Kingdom. [7]Brunel University London, Uxbridge, United Kingdom

Abstract

Accurate and rapid diagnostic tests are key to halting the spread of infectious diseases and facilitate appropriate selection of treatments. For most infectious diseases, current gold standard tests rely on PCR and are highly accurate, but time-consuming and require expensive and specialised lab-based equipment. Therefore, there is an urgent need for tests that can be performed with minimal equipment and training.

In collaboration with Brunel University, Lancaster University, the NHS, GB Electronics Ltd and Vidiia Ltd, we developed a rapid and inexpensive diagnostic platform that utilises loop-mediated isothermal amplification (LAMP) and a portable smart diagnostic device. Automated image acquisition, and an Artificial Intelligence model embedded in the VIDIIA Hunter 6 device remove subjectivity associated with results interpretation.

A LAMP assay designed for the detection of COVID-19 was validated with the VIDIIA Hunter, using 400 clinical samples. When compared to RT-qPCR, the test was shown to be highly specific (100%) and sensitive (98–100% depending on viral load), with a limit of detection of 1.4 copies of RNA/µL. Using this data, our CE-IVD and MHRA approved platform has been approved for medical use in the UK, under the UKHSA Medical Devices Regulations 2022.

In collaboration with the University Hospitals of Leicester NHS Trust, LAMP assays for the detection of the five main carbapenemase gene families reported in the UK are currently being validated on the VIDIIA Hunter using human clinical samples harbouring carbapenemase-producing Enterobacterales. This system could provide a time and cost-effective diagnostic platform for infectious diseases, in resource-limited settings.

Molecular epidemiology of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* spp complex from Aberdeen, Scotland

Kareem Mahdy[1], Hisham Altayb[2,3], Noha El Sakka[1], Bruno Silvester Lopes ORCID iD[4,5]

[1]Aberdeen Royal Infirmary, NHS Grampian, Aberdeen, United Kingdom. [2]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. [3]Center of Artificial Intelligence in Precision Medicines, King Abdulaziz University, Jeddah, Saudi Arabia. [4]School of Health and Life Sciences, Teesside University, Middlesbrough, United Kingdom. [5]National Horizons Centre, Teesside University, Darlington, United Kingdom

Abstract

Extended-spectrum β-lactamases (ESBLs) are found in *Klebsiella* spp, an opportunistic pathogen associated with severe cases of hospitalisation. We whole genome sequenced twenty-five ESBL-producing blood culture isolates from NHS Grampian during 2019-2022.

DNA extraction and genome sequencing were performed by MicrobesNG v20230314. NovaSeq 6000 Illumina sequencing platform was used and *de novo* assemblies of Nanopore and Illumina reads were assembled using the Unicycler pipeline v.0.4.0. Genomes were annotated using RAST and typed by multi-locus sequence typing. Antibiotic resistance genes were detected using ResFinder, Kleborate. Plasmids were assembled using plasmidSPAdes and queried against BLASTN.

Twenty-two isolates were identified as *Klebsiella pneumoniae* whereas three belonged to *K. penumoniae* spp complex. All isolates were resistant to ceftazidime, cefotaxime, and cefuroxime. The $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes were present in 71% (n=17), 42% (n=10) and 96% (n=24) isolates respectively. The $bla_{\text{CTX-M-15}}$ was observed in 64% (n=16) isolates. Seven isolates had $bla_{\text{CTX-M}}$ on plasmids ranging from 1.6 kb - 96 kb, with two also possessing a chromosomal copy. The $bla_{\text{SHV-12}}$, $bla_{\text{SHV-7}}$, $bla_{\text{CMY-2}}$, and $bla_{\text{DHA-1}}$ ESBLs occurred in six isolates. The *mcr-9.1* was present on 2.5 kb and 203 kb plasmids in two isolates. Multi-locus sequence typing identified ST-13 (n=3), ST-200 (n=2) and 20 unique strains.

The coexistence of *mcr-9.1* along with ESBLs in *Klebsiella* spp is alarming and threatens the efficacy of critically important antimicrobials. *K. pneumoniae* ST13 observed in our study has been identified in 33 different countries highlighting its global dissemination. Whole genome analyses should provide better insights into the molecular dynamics and evolutionary trajectory of *K. penumoniae* spp complex.

CRISPR-Maze Runner: A targeted approach to combat superbugs and tackle antimicrobial resistance

Ayesha Nethmini[1], Gayan Gunatilake ORCID iD[1], Lalinka Herath ORCID iD[2,3], Kajan Muneeswaran[3], Vishvani Sudarshan[1], Akeel Shahan[2]

[1]International Institute of Health Sciences, Colombo, Sri Lanka. [2]Wayamba University of Sri Lanka, Makandura, Sri Lanka. [3]University of Colombo, Colombo, Sri Lanka

Abstract

This study introduces an innovative CRISPR-based genetic circuit designed for the targeted degradation of β-lactamases in superbugs, addressing their critical role in antimicrobial resistance (AMR). The circuit strategically integrates an AmpR promoter and TetR repressor gene, employing an AND gate genetic logic. In the absence of AMR microbes, the repressor remains active, preventing the expression of guide RNA and eGFP. However, in the presence of beta-lactamase-producing superbugs, the repressor is deactivated, allowing the expression of guide RNA and eGFP marker. The Cas9 protein encapsulated in nanoparticles is delivered directly to the gastrointestinal tract for precise targeting. The Novel CRISPR-Maize Runner exploits an AND gate genetic logic, ensuring specificity in depleting β-lactamases via CRISPR-mediated knockouts. This strategy acts as an effective maze, restricting escape routes for the superbugs. Potential real-world applications of this CRISPR-based strategy include its use in healthcare settings for the targeted treatment of antibiotic-resistant infections, livestock management to mitigate AMR in agriculture, and environmental control to curb the dissemination of antibiotic resistance genes. The CRISPR-Maize Runner emerges as a versatile tool in the ongoing battle against superbugs and holds promise for innovative solutions in diverse sectors, combating the global challenge of antimicrobial resistance.

What's lurking in the environment? Novel beta-lactamases found in Pseudomonas isolates

Alexander Kingdon ORCID iD, Richard Goodman, Ralfh Pulmones, Adam Roberts ORCID iD

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Many mechanisms of antimicrobial resistance existed in the environment long before their occurrence in clinically relevant pathogens, hence, studying the resistance profiles of environmental microbes could identify novel resistance genes prior to their transfer into pathogenic species. To this end, we screened a set of 884 environmental isolates for the presence of extended spectrum beta-lactamases and specifically meropenem resistance. Overall, >50% grew on beta-lactam-supplemented agar and ~24% were phenotypically meropenem resistant. We selected a sub-set of 41 isolates for 16S identification and then sent six non-aeruginosa Pseudomonas isolates for whole-genome sequencing. All six strains had annotated AmpC-type beta-lactamase genes, however, only three were detected in the ARG-ANNOT database and no matches were found when searching using Resfinder or CARD. The six encoded protein sequences were between 47-57% identical to the closest known AmpC-type beta-lactamase, PRC-1. Hence, the identified strains could represent a novel environmental reservoir of resistance within *Pseudomonas* populations.

A longitudinal study of extended-spectrum beta-lactamase producing Enterobacterales from freshwater sites of contrasting land-uses in New Zealand

Rose Collis[1], Amanda Gardner[1], Lynn Rogers[1], Patrick Biggs[2], Meg Devane[3], Brent Gilpin[3], Margaret Leonard[3], Adrian Cookson[1]

[1]AgResearch, Hopkirk Research Institute, Palmerston North, New Zealand. [2]Molecular Epidemiology and Public Health Laboratory, School of Veterinary Science, Massey University, Palmerston North, New Zealand. [3]Institute of Environmental Science and Research, Christchurch, New Zealand

Abstract

Antimicrobial resistance is a global public health concern. Infections caused by antimicrobial resistant bacteria are difficult to treat, increasing the risk of serious disease. Rivers are directly impacted by contaminants from diverse land-use activities, therefore the objective of this work was to take a One Health approach to understand the impact of local land-use on the detection of waterborne antimicrobial resistant bacteria.

A longitudinal study where 298 freshwater samples from 41 separate New Zealand (NZ) sites were collected, occurred between October 2022 and July 2023. Sites where the local land-use was urban, dairy, avian, sheep and beef, low impact, and mixed (sheep, beef, and dairy) were included. Extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E) including *E. coli*, *Citrobacter* spp. and *Klebsiella* spp. were isolated from 28 water samples where urban (25), dairy (1), avian (1), and sheep and beef (1) were the identified land-use. Faecal source tracking data indicated that human was the dominant faecal source for 23 of 28 ESBL-E positive samples.

Assembly of closed genomes indicated the presence of *E. coli* ST-38, ST-68 and ST-131, sequence types commonly associated with human urinary tract infections, suggesting contamination with urban wastewater. Two *E. coli* (ST-410 and ST-617), harboured the carbapenemase-encoding gene bla_{NDM-5}, and two other *E. coli* (ST-131 and ST-8131), carried plasmids containing genes encoding the enteroaggregative *E. coli* adherence property. This is the first study to assess the impact of land-use on ESBL-E in waterways at a national scale in NZ and has highlighted urban as the most common land-use associated with EBSL-E.

Prokaryotic Stress Responses – their diversity and regulation

Invited talk: Understanding the role of membranes in microbial responses to stress

Stefano Pagliara ORCID iD[1], Urszula Lapinska[1], Ka Kiu Lee[1], Mark Blaskovich[2], Krasimira Tsaneva-Atanasova[1]

[1]university of exeter, exeter, United Kingdom. [2]university of queensland, brisbane, Australia

Abstract

Phenotypic variations between individual microbial cells play a key role in the resistance of microbial pathogens to stress, including treatment with antibiotics. Nevertheless, little is known about phenotypic variations in antibiotic accumulation. We hypothesise that, besides variations in intracellular processes,[1-3] phenotypic diversification in microbial response to stress is driven by fundamental cell-to-cell differences in drug transport rates. To test this hypothesis, we employed microfluidics-based single-cell microscopy and libraries of fluorescent antibiotic probes.[4] This approach allowed us to rapidly identify phenotypic variants that avoid antibiotic accumulation within populations of *Escherichia coli*, *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, and *Staphylococcus aureus*.[5] Crucially, we found that fast growing phenotypic variants avoid macrolide accumulation and survive treatment without genetic mutations.[5] The opposite is true for the antimicrobial peptide tachyplesin, in which case slow growth allows for low intracellular accumulation and survival. We used this new knowledge to eradicate variants that displayed low antibiotic accumulation through the chemical manipulation of their outer membrane inspiring new avenues to overcome current antibiotic treatment failures.[4-7]

[1] Goode et al. mBio 12, 00909 (2021).

[2] Bamford et al. BMC Biology 15, 121 (2017).

[3] Goode et al. ACS Infectious Diseases 7, 1848 (2021).

[4] Stone et al. RSC Chemical Biology, 1, 395 (2020).

[5] Łapińska et al., *eLife*, 11:e74062 (2022).

[6] https://doi.org/10.1101/2023.09.21.558807.

[7] Conners et al. Nature Communications, 12 6316 (2021).

Invited talk: How the infection environment influences P. aeruginosa pathogenesis

Kendra Rumbaugh

Texas Tech University Health Sciences Center, Lubbock, USA

Abstract

Pseudomonas aeruginosa is one of the most commonly isolated pathogens found in wounds, but its pathogenesis varies greatly depending on the type of wound it inhabits. The same strain of *P. aeruginosa* can be the cause of both a biofilm-associated chronic wound infection and a rapidly spreading septic infection. We and other groups have demonstrated that environmental conditions, host responses and the presence of other microbes are all factors that influence the pathogenesis and infection trajectory of *P. aeruginosa.* Importantly, local conditions within the wound can also affect the efficacy of treatments. Genetic tools can now be used to reveal the conditions *P. aeruginosa* faces *in vivo*, determine how it responds to them, and help predict what therapeutic strategies will be most effective in a given type of infection.

Invited talk: How environmental factors affect rates of switching to antibiotic-tolerant states in Mycobacteria

Vijay Srinivasan[1], Marco Mauri[1], Miriam Clincy[2], Martin Evans[3], Nguyen Thuy Thuong Thuong[4], Rosalind Allen[1]

[1]Friedrich Schiller University, Jena, Germany. [2]Fachhochschule Esslingen, Esslingen, Germany. [3]University of Edinburgh, Edinburgh, United Kingdom. [4]Oxford University Clinican Research Unit, Ho Chi Minh City, Vietnam

Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, kills over 1 million people per year, and successful treatment requires very long courses of antibiotic treatment. One of the reasons that TB is difficult to treat is that *Mycobacterium tuberculosis,* like other Mycobacteria, can enter antibiotic-tolerant "dormant" physiological states. Working with *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* and the clinically relevant antibiotic rifampicin we investigate some of the factors affecting the rates of switching into and out of dormant states. We also develop mathematical models to rationalise our data and predict the outcome of different treatment strategies.

Invited talk: Inner conflicts of Salmonella persisters during infection

Sophie Helaine, Molly Sargen

Harvard Medical School, Boston, USA

Abstract

Bacterial persistence, characterized by chronic and relapsing infections, is a major threat to human health as these infections cause considerable morbidity and frequently require multiple courses of antibiotics. Such long-lasting infections are caused by a variety of bacterial pathogens including *Mycobacterium tuberculosis*, *Salmonella*, *Pseudomonas, Staphylococcus aureus* and pathogenic *Escherichia coli*. During infection, *Salmonella* specifically respond to engulfment by host macrophages by forming high proportions of antibiotic persisters. These persisters escape the combined action of the antibiotic and host immune killing mechanisms for prolonged periods of time by adopting a non-growing state. The molecular mechanisms that govern persister survival are not all understood, but these antibiotic persisters have been consistently proposed to overcome antibiotics thanks to a relative inactivity. We will discuss the immense activity that is taking place in these growth arrested cells during their interaction with macrophages.

Invited talk: Fessing up: what mycobacteria reveal under stress

Digby Warner ORCID iD

University of Cape Town, Cape Town, South Africa

Abstract

Understanding *Mycobacterium tuberculosis* physiology and metabolism is critical to the development of novel interventions – including new antimycobacterial drugs – for tuberculosis (TB), a leading cause of mortality owing to a single infectious agent and a major contributor to antimicrobial resistant deaths globally. Here, I will provide two examples of our work utilizing molecular tools and microscopy to investigate gene function in mycobacteria under applied stress. First, by combining inducible CRISPR-interference, fluorescence-based transcriptional reporters, and quantitative, image-based analyses, we are developing a morphological profiling ("phenoprinting") platform to infer hypothetical gene function and to deduce drug mechanism-of-action. As a second example, I will describe ongoing efforts towards the identification of so-called "anti-evolution" compounds that might limit the capacity for resistance acquisition by targeting the mycobacterial mutasome, a mutagenic DNA repair system that has been implicated in DNA damage-induced drug resistance and host adaptation. In addition to highlighting the utility of image-based analyses in elucidating gene function in *M. tuberculosis*, I hope to amplify the call for continued development of novel tools to understand the biology – and potential vulnerabilities – of an infectious agent that is exquisitely adapted to its obligate human host.

The evolution of bacterial resistance to Type 6 Secretion System toxins

William Smith ORCID iD[1], Marek Basler ORCID iD[2], Michael Brockhurst ORCID iD[1]

[1]University of Manchester, Manchester, United Kingdom. [2]University of Basel, Basel, Switzerland

Abstract

The bacterial Type 6 Secretion System (T6SS) is a toxin-injecting nanoweapon, and a prolific mediator of competition in plant- and animal-associated microbial communities. Envelope stress response pathways can confer innate resistance to T6SS toxin effectors, but we have yet to understand the factors controlling when, and how, this resistance emerges *de novo*. This is a major gap in our understanding of microbial ecology, since resistance to anti-competitor weapons is predicted to drastically alter community composition and invasion susceptibility. To address this knowledge gap, we combine directed evolution *in silico* and *in vitro* to examine how susceptible bacteria evolve when confronted with different competitor T6SS toxins. In both our models and experiments, we find that different toxins generate contrasting evolutionary outcomes: milder toxins select for consistent resistance, whereas stronger toxins or toxin combinations limit resistance evolution by creating genetic bottlenecks, driving resistant lineages extinct before they can reach high frequency. Using whole-genome sequencing, we also show that bacteria can gain resistance to toxins via modifications to envelope stress response, lipopolysaccharide and cell wall biogenesis pathways, and that these modifications frequently confer cross-resistance or collateral sensitivity to other T6SS toxins. By illuminating evolutionary responses to intermicrobial competition, our research guides the manipulation of plant- and human-associated microbial communities, informing future therapeutic or biocontrol strategies.

Elucidating how bacterial pathogens adapt to immune cell oxidative stress using single-molecule microscopy

Fiona Sargison ORCID iD[1], Claire Qu[2,1], Clara Kummerer[1], Amy Moores ORCID iD[1], Valentine Lagage[1], Stephan Uphoff ORCID iD[1]

[1]Department of Biochemistry, Oxford, United Kingdom. [2]Emory University, Atlanta, USA

Abstract

Increasing antibiotic resistance is putting greater pressure on our immune system to fight bacterial infections. Pathogenic bacteria can survive attack by immune cells, such as macrophages, but it remains unclear how. To address this question, I have developed a super-resolution microscopy method to image bacteria after macrophage infection. This novel approach allows the real-time visualisation of the movement and function of individual protein molecules within living cells. I discovered that proteins which sense, and repair, DNA damage become active in intracellularly encapsulated bacteria. This provides a direct and novel insight into how bacteria withstand the damage that is caused by immune cells, and thereby helps us identify weaknesses in bacterial defences that could be explored as targets for novel therapeutics. Furthermore, I detected errors in DNA repair, which can result in mutations that accelerate pathogen evolution. Building upon these proof-of-concept results promises novel insights into bacterial evolution and host-pathogen interactions.

Global RNA interactome of nitrogen starved Escherichia coli uncovers a conserved post-transcriptional regulatory axis required for optimal growth recovery

Josh McQuail[1], Gianluca Matera[2], Tom Gräfenhan[2], Thorsten Bischler[2], Per Haberkant[3], Frank Stein[3], Jörg Vogel[2], Ramesh Wigneshweraraj[1]

[1]Imperial College London, London, United Kingdom. [2]HIRI, University of Würzburg, Würzburg, Germany. [3]EMBL Heidelberg, Heidelberg, Germany

Abstract

The RNA binding protein Hfq has a central role in the post-transcription control of gene expression in many bacteria. Numerous studies have mapped the transcriptome-wide Hfq-mediated RNA-RNA interactions in growing bacteria or bacteria that have entered short-term growth-arrest. To what extent post-transcriptional regulation underpins gene expression in growth-arrested bacteria remains unknown. Here, we used nitrogen (N) starvation as a model to study the Hfq-mediated RNA interactome as *Escherichia coli* enter, experience, and exit long-term growth arrest. Through use of RNA interaction by ligation and sequencing (RIL-seq), we observe that the Hfq-mediated RNA interactome undergoes extensive changes during N starvation, with the conserved SdsR sRNA making the most interactions with different mRNA targets exclusively in long-term N-starved *E. coli*. Taking a proteomics approach, we reveal that in growth-arrested cells SdsR influences gene expression far beyond its direct mRNA targets. We demonstrate that the absence of SdsR significantly compromises the ability of the mutant bacteria to recover growth competitively from the long-term N-starved state and uncover a conserved post-transcriptional regulatory axis which underpins this process.

Understanding the Physiological and Regulatory Systems of Community-Acquired *Staphylococcus aureus* in Response to Copper Exposure.

Sean Brennan[1], Inderpreet Kaur[2], Julie Morrissey[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

Copper is an essential co-factor for enzymes involved in various biological functions in bacteria. Its significance is highlighted by its dual nature as essential in appropriate concentrations, yet toxic in excess. This characteristic is utilised not only in medical settings but also by the host immune system to eliminate pathogens. Anthropogenic pollution introduces an additional dimension, potentially elevating copper exposure and influencing bacterial communities and phenotypes. Consequently, bacteria must finely regulate copper homeostasis and physiological systems to sustain growth, navigate toxic concentrations and persist within the host.

To determine the effect of copper on community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA), we defined the transcriptional profile and identified regulatory pathways using several mutants involved in copper homeostasis. We employed differential gene expression analysis using DESeq2, weighted correlation network analysis (WGCNA), as well as gene set enrichment analysis (GSEA) and KEGG pathway analyses. Additionally, we conducted phenotypic assays to validate hypotheses derived from these expression networks.

Our results reveal sub-inhibitory copper concentrations alter various physiological mechanisms including virulence and immune evasion. Intriguingly, copper also alters the metabolic dynamics of CA-MRSA through master regulators towards fermentation and towards the production of glutamate which may play a role in copper tolerance through chelation. Interestingly, WGCNA also highlights subtle alterations in metabolic networks across mutant samples further emphasising the importance of central metabolism in copper tolerance mechanisms. Overall, copper acts as a signal to alter central metabolism to induce tolerance which may influence persistence in harmful niches within the host.

Stress responses and tolerance mechanisms of Pseudomonas putida to the plastic industry monomers ethylbenzene, styrene and methacrylate ester.

Charles Begley[1], Nicholas Tucker ORCID iD[2]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Suffolk, Ipswich, United Kingdom

Abstract

The plastics industry is almost exclusively reliant on petrochemical feedstocks for the synthesis of large-volume commodity chemicals. Collectively, the amount used for plastic production is equivalent to approximately 1.3 billion barrels of crude oil per annum. To improve the sustainability of materials such as acrylic, the chemical industry is investing heavily in biological production methods.

Pseudomonas spp. are tolerant to a wide range of chemical stresses and we have demonstrated that this genus is significantly enriched in metagenomic experiments where complex populations of bacteria are challenged with toxic plastic monomers. In particular, *Pseudomonas putida* has gained traction as a synthetic biology chassis organism and is ideally suited to the sustainable production of plastic monomers because of its inherent solvent tolerance. To further understand and optimise *P. putida* for biosynthesis of plastic monomers we have taken a transcriptomic approach to understand its response to chemical stress associated with ethylbenzene, styrene and methacrylate esters. In addition to several efflux pumps, we identified TetR and MarR class transcriptional regulators that are strongly derepressed in the presence of all three solvents. These regulators are capable of binding diverse substrates and *in silico* docking experiments indicate the plausibility of solvent ligand binding. Deletion of each regulator facilitated assessment of their regulons by RNA-Seq revealing that the MarR type regulator may contribute to the generalised envelope stress response of *P. putida*. Applications of these regulators as biosensors for combinatorial synthetic biology will be discussed as well as recent insights gained from integrating RNA-Seq and Tn-Seq datasets.

Enhancing stress resistance of probiotic strains through co-culturing

Kosuke Oana, Kensuke Shimizu, Toshihiko Takada, Hiroshi Makino, Mikiko Yamazaki, Miyuki Katto, Minoru Ando, Takashi Kurakawa, Kenji Oishi

Yakult Central Institute, Tokyo, Japan

Abstract

Probiotic products are widely available and recognized for their health benefits. However, the viability of probiotic strains is often reduced by gastric acid and bile in the gastrointestinal tract, which limits the effectiveness of live probiotic strains. We found that *Lacticaseibacillus paracasei* strain Shirota (LcS*) beverages co-fermented with *Lactococcus lactis* subsp. *lactis* YIT 2027 (LL-1) had higher numbers of live LcS passing through the human gastrointestinal tract than monofermented LcS beverages. During the LcS co-culture with LL-1, the pH of the culture medium decreased earlier than in monoculture, and this early pH decrease was critical for improving stress tolerance. The improvement in stress tolerance by co-culturing with LL-1 was not observed in *L. helveticus* or *L. gasseri* but observed in *L. paracasei* strains other than LcS, suggesting that it is limited to *L. paracasei*. Transcriptome analysis revealed changes in energy metabolism, fatty acid synthesis, and molecular chaperones, suggesting potential mechanisms for enhancing stress resistance. The strains with improved stress tolerance had a higher percentage of dihydrosterculic acid in their fatty acid composition. Notably, a mutant strain lacking dihydrosterculic acid did not show improved stress tolerance when co-cultured. In conclusion, we found that stress tolerance in *L. paracasei* is enhanced by an early decrease in pH during culture, which increases dihydrosterculic acid in the membrane fatty acid.

Oana, K. et al. 2023. Manipulating the growth environment through co-culture to enhance stress tolerance and viability of probiotic strains in the gastrointestinal tract. *Appl. Environ. Microbiol.*

* Formerly *Lactobacillus casei* strain Shirota

SOS response helps Pseudomonas aeruginosa overcome phage infection.

Benoit J Pons ORCID iD[1], Urszula Łapińska[2], Matthew Chisnall[1], Bridget Watson[1], Edze Westra[1], Stefano Pagliara[2], Stineke van Houte[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

In their continuous battle against their phage foes, bacteria have developed a wide range of defence mechanisms, among which is the CRISPR-Cas system. CRISPR-Cas is an adaptive system relying on storage of phage genetic material from previous failed infections into CRISPR loci on the bacterial chromosome. Processed CRISPR transcripts are used to guide sequence-specific cleavage of phage genetic material in subsequent infections.

Recent studies have demonstrated that the associated costs of the CRISPR-Cas system depend on environmental factors. However, these population-level studies might not reflect heterogeneity of bacterial response at a single-cell level. Here, we use a novel microfluidics-based single-cell approach to directly monitor the effects of phage DMS3*vir* exposure on survival of its host *Pseudomonas aeruginosa*. With this method, we observed that phage exposure provokes time-delayed cell filamentation and activates bacterial SOS response independently of CRISPR-Cas system presence. While CRISPR deficient cell lyse in response to phage exposure, CRISPR resistant cells overcome phage infection and finally resume division. These results show that in *P. aeruginosa*, anti-phage defence systems and SOS response work together to defeat phage threat.

How the ESRT-III-like protein Vipp1 mitigates membrane stress and repairs membrane in cyanobacteria

Souvik Naskar[1], Andrea Merino[2], Javier Espadas[3], Jayanti Singh[4], Aurelien Roux[3], Adai Colom[5], Harry Low[1]

[1]Imperial College, London, United Kingdom. [2]Biofisika Institute, bilbao, Spain. [3]University of Geneva, Geneva, Switzerland. [4]imperial college, london, United Kingdom. [5]Biofisika Institute, Bilbao, Spain

Abstract

Stress induced membrane remodeling and repair are essential for all cells. Proteins that perform these functions include Vipp1 in cyanobacteria, PspA in bacteria, and ESCRT-III in eukaryotes. Here, using a combination of evolutionary and structural analyses, we show that these protein families are homologous and share a common ancient evolutionary origin that likely predates the last universal common ancestor. This homology is evident in cryo-electron microscopy structures of Vipp1 dome-shaped rings and helical filaments from the cyanobacterium Nostoc punctiforme. Using Fast Atomic Force Microscopy (F-AFM), we also show how Vipp1 functions as a sensor that scans and binds highly curved and perturbed membranes. Vipp1 small oligomers are recruited to the membrane edge where they nucleate into filaments that curl into spirals or rings. Additionally, we show how pre-assembled dome-shaped rings sense and stably bind membrane ruptures where they form cage-like sutures over the damaged region. Our data suggests a general mechanism for Vipp1 stress induced membrane biogenesis and repair in cyanobacteria and chloroplasts. Spirals encircle the damaged membrane region, which may incorporate protein complexes, ultimately assembling a central ring structure capable of membrane budding and repair. Our data also explains how other bacterial homologues such as PspA or the antibiotic induced LiaH may mitigate membrane stress and repair membrane in bacteria.

Microbes Moving Metals

John Helmann ORCID iD

Cornell Univ., Ithaca, USA

Abstract

Bacteria must adapt to both metal ion limitation and excess. In the model Gram-positive bacterium *Bacillus subtilis*, the MntR (manganese transport regulator) protein binds Mn(II), represses uptake genes, and activates two genes (*mneP*, *mneS*) encoding cation diffusion facilitator proteins that export Mn(II). Genetic studies of mutants dysregulated for Mn homeostasis (*mntR*) or lacking the major Mn efflux pumps (*mneP mneS*) revealed a class of mutations with increased expression or activity of two TerC family membrane proteins, MeeF(YceF) and MeeY(YkoY). MeeF and MeeY (metalation of exoenzymes) are Mn efflux proteins that function to support metalation of enzymes that function outside the cell membrane. Unlike MneP and MneS, the MeeF and MeeY proteins do not play a major role in resistance to elevated Mn, although mutations can increase their efflux activity. Mutant strains (FY mutants) with deletions of both *meeF* and *meeY* are slow growing due to defects in protein secretion and a reduced ability to metalate a key enzyme for cell wall synthesis, the Mn-dependent lipoteichoic acid synthase (LtaS). Consistent with a role in the metalation of Mn-requiring exoenzymes, the MeeY protein is regulated by a Mn-responsive riboswitch. We propose that MeeF and MeeY function, in part, in the co-translocational metalation of secreted enzymes. These findings highlight the challenge of properly metalating enzymes that function outside the cell and may prove relevant to studies of TerC homologs in plants and animals.

A key role for cysteine biosynthesis in *Mycobacterium tuberculosis* response to hypoxia and copper stress during infection

Wendy Le Mouëllic[1], Florence Levillain[1], Gerald Larrouy-Maumus[2], Ting-Di Wu[3,4], Yannick Poquet[1], Olivier Neyrolles[1]

[1]Institut de Pharmacologie et de Biologie Structurale, CNRS, Université de Toulouse, Toulouse, France. [2]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom. [3]Institut Curie, PSL Research University, Paris, France. [4]Multimodal Imaging Center, Paris Saclay University, CNRS, INSERM, Orsay, France

Abstract

During infection *Mycobacterium tuberculosis* (*Mtb*), the agent of tuberculosis, faces multiple adverse conditions, including a hypoxic microenvironment in the lungs and a burst of copper in the phagosome of infected macrophages. Screening of a *Mtb* transposon mutant library in murine macrophages cultivated in hypoxia *vs*. normoxia revealed that *Mtb* sulfur metabolism, and in particular the cysteine synthase CysK2, plays a key role in mycobacterial intracellular survival under hypoxia. Using NanoSIMS, we found that sulfate and cystine reach *Mtb*-containing phagosomes, and that cystine consumption increases in hypoxia, thus fuelling sulfur metabolism. Furthermore, a *subI-KO* strain, lacking the sulfate transporter, showed reduced survival in mice, reinforcing the key role for sulfur acquisition during infection.

Intracellular stresses may synergize as, for example, exposition of macrophages to hypoxia increases copper burst, thus leading to oxidative stress. Since sulfur metabolism sustains redox balance and since we found that CysK2 is the only cysteine synthase strongly induced by copper, we hypothesize that the CysK2 pathway is crucial for *Mtb* to maintain its redox equilibrium and mitigate copper-induced stress within macrophages under hypoxia. Thus, we generated CRISPR/Cas9-edited macrophages lacking copper transporters, and we will compare the redox status and survival of wild-type and *cysK2-KO* strains in these cells. In addition, we perform metabolomics analyses to characterize the metabolic adaptations of *Mtb* to copper.

Collectively, our findings suggest a fundamental role for sulfur metabolism and in particular the CysK2 cysteine synthase in the response of *Mtb* to hypoxia and copper overload, two host-imposed stresses that can synergize during infection.

Transcriptional Resilience of *T. pallidum* under Diverse Culturing Conditions

Linda Grillova, George Lacey, William Roberts-Sengier, Nicholas R. Thomson

Parasites and Microbes Programme, Wellcome Sanger Institute, Hinxton, United Kingdom

Abstract

Limited knowledge exists about the gene expression of *Treponema pallidum* subsp. *pallidum* (TPA), the causative agent of the escalating health concern, syphilis. Our research aims to bridge this gap by analyzing TPA's transcription profiles, correlating them with genome annotations, and exploring its response to challenging growth conditions.

As mammalian cells are essential for TPA's optimal growth, significant differences in cell number and motility were observed between standard culture conditions (with Sf1Ep cells) and axenic conditions (without Sf1Ep cells) after 7 days of incubation. In standard conditions, TPA cells multiplied as expected, maintaining high motility. However, in axenic conditions, many TPA cells were dying and the motility was significantly reduced. Post-incubation, RNA was extracted for directional RNAseq, and whole genome sequencing was performed to identify genetic variants.

Remarkably, the transcriptional profiles of TPA under both conditions exhibited a high degree of similarity. Most genes were active in all conditions and strains, including those for unknown proteins taxonomically restricted to *Treponema*. A limited number of genes exhibited differential expression between these two conditions (n = 9). The most notable was the ssrA gene, coding for tmRNA, which helps ribosomes during protein synthesis, especially under stress.

Our findings suggest that TPA relies on a vital, yet compact set of actively transcribed genes essential for its survival. While transcription is typically a central aspect of gene expression regulation in bacteria, our results prompt further research to confirm whether this holds true for TPA, considering its unique transcriptional resilience under different environmental conditions.

Unravelling Streptomyces Complex Regulatory Mechanisms That Underpin Cryptic Biosynthetic Gene Clusters.

Ainsley Beaton ORCID iD, Thomas McLean, Matt Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

Streptomyces produce around 50% of clinically used antibiotics. Understanding the regulation of these compounds could be key to the discovery of novel specialised metabolites. Two-component systems (2CS) are common bacterial mechanisms to sense and respond to environmental conditions.

The CutRS 2CS is highly conserved in *Streptomyces* and it regulates the production of specialised metabolites, with its deletion increasing the production of the redox-active antibiotic actinorhodin in *S. coelicolor* and chloramphenicol in *S. venezuelae*. We have evidence that CutR binds to the promoter of, and differentially regulates two of the four highly conserved HtrA-family foldases: HtrA3 and HtrB, which suggests a link to extracellular protein folding. The cognate sensor kinase, cutS, has two highly conserved cysteines which we believe to be important in sensing disulphide bond formation and correct folding of secreted proteins. Substitution of these leads to a change in function and phenotype under redox stress conditions suggesting they are essential for response to extracellular stress and CutRS functionality. Additionally, redox-active compounds such as dithiothreitol and pyocyanin reverse and recover the phenotype of Δ*cutRS* in *S. coelicolor.* q-RT-PCR experiments have revealed the effect of this change on *htrA3* and *htrB*. Our results suggest that CutS senses extracellular stress via conserved dual cysteine motif in the extracellular domain which leads to changes in the regulation of genes involved in secretion stress and specialised metabolite production. Understanding the mechanisms underpinning the regulation of conserved 2CS could allow us to induce the expression of previously “cryptic” biosynthetic gene clusters, potentially more widely within the genus.

Uncovering the role of metabolic adaptations in the emergence of pre-resistant bacterial populations of the WHO priority pathogens *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*

Lisa Juliane Kahl ORCID iD[1,2], Svenja Osta[1], Ludwig Roman Sinn ORCID iD[1], Daniela Ludwig[1], Craig MacLean ORCID iD[2], Markus Ralser ORCID iD[1,2]

[1]Charité University Medicine, Berlin, Germany. [2]Oxford University, Oxford, United Kingdom

Abstract

The escalating global health threat of antimicrobial resistance is characterised by pathogens acquiring resistance-conferring mutations, often disseminated through horizontal gene transfer. Despite this, the mechanisms underlying the survival and resilience of "pre-resistant" cells exposed to lethal antibacterial doses remain unclear. Recent discoveries highlight the overlooked role of the extracellular metabolic environment and metabolic adaptations in transiently buffering antibiotic lethality.

This study systematically investigates the mechanisms at play in populations of "pre-resistant" cells from three WHO priority pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*), capable of transiently withstanding antimicrobial treatment. Using a bacterial physiology screen, we assess resilience phenotypes against major clinically applied antimicrobials under both standard laboratory and clinically relevant conditions. While confirming known species-specific inherent resilience, our results reveal that susceptibility is substantially decreased in infection site-mimicking conditions compared to standard laboratory conditions.

By employing over 400 combinations of antimicrobial treatment and culturing conditions in a high-throughput proteomics, we further methodically explore the metabolic stress response to identify key metabolic and signalling processes supporting cell survival during sub-inhibitory antimicrobial exposure in *P. aeruginosa*. The data expectedly show an upregulation of virulence mechanisms such as iron scavenging and toxin production, it is a reshaping of central metabolic processes that enable survival and antimicrobial protection of “pre-resistant” cells in response to antimicrobial exposure. We posit that metabolite dynamics contribute to the emergence of antimicrobial tolerant and resistant phenotypes and, in future, aim to pinpoint the crucial metabolic effectors preceding resilience-conveying adaptations that contribute to the evasion of antimicrobial treatment.

Estimating Mutation Rates Under Heterogeneous Stress Responses

Lucy Lansch-Justen ORCID iD[1], Meriem El Karoui ORCID iD[2,3], Helen Alexander ORCID iD[1,3]

[1]Institute of Ecology and Evolution, University of Edinburgh, Edinburgh, United Kingdom. [2]Institute of Cell Biology, University of Edinburgh, Edinburgh, United Kingdom. [3]Centre for Engineering Biology, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Expression of stress responses is thought to increase mutagenesis in bacteria, thus potentially accelerating resistance evolution. Most studies that report such increased mutation rates under stress use the standard experimental approach of fluctuation assays. However, single-cell studies have revealed that many stress responses are heterogeneously expressed in bacterial populations, which is not addressed yet by existing estimation methods. We develop a population dynamic model that considers heterogeneous stress responses (subpopulations of cells with the response *off* or *on*). In our model, expression of the stress response impacts both mutation and cell division rates, inspired by the DNA-damage response in *Escherichia coli* (SOS response). We implement a computational method in R to estimate the mutation rate increase specifically associated with the expression of the stress response. Using this method, we analyse simulated mutant count data and experimental mutant count data gathered in a recent metastudy. In many cases, our model of heterogeneous stress responses and the standard model with mutant fitness cost reproduce fluctuation assay data equally well. This suggests that separate experiments are required to identify the true underlying process. Moreover, our simulations show that current methods still correctly infer the effective increase in population mean mutation rate when stress responses are heterogeneous. However, we provide a novel method to infer heterogeneity in stress-induced mutation rates.

The repair of single-strand DNA breaks requires a minimal set of proteins and culminates in reloading of the bacterial replisome

Charles Winterhalter ORCID iD, Heath Murray ORCID iD

Newcastle University, Newcastle-upon-Tyne, United Kingdom

Abstract

DNA damage repair mechanisms are universally essential to sustain life. To proliferate, cells must produce a complete blueprint of their genetic material; yet DNA damage can hinder this process and result in collapse of the replication machinery (replisome). Such DNA damage must be repaired to avoid genetic material and cellular degeneration but critically, the complex processes underlying these repair events are not fully understood in live cells. This is underpinned by problems of redundancy within repair pathways and unsuitable methods to investigate bespoke repair events, which together make the identification of specific factors involved in a particular pathway extremely difficult. Here, we overcame this issue by developing an innovative strategy using the CrispR-Cas system to introduce a single site-specific break on the bacterial chromosome. This approach enabled us to investigate the impact of unique single-stand DNA breaks (SSBs - the most numerous type of breaks) in live cells, and to identify the essential factors required for their repair. First, we describe the method established to introduce bespoke DNA breaks onto the chromosome of the model organism *Bacillus subtilis.* Then, we show that SSBs lead to the arrest of DNA replication. Importantly, we elucidated the fundamental mechanisms leading up to reloading of the helicase and the replisome, and we report the sequential events underpinning these processes in live cells. We demonstrate that SSB repair relies on a minimal set of proteins that are conserved across the different phyla of life, which significantly advances the universal understanding of DNA repair.

Single Cell Omics

Invited talk: Toward Comprehensive Analysis of the 3D Chemistry of *Pseudomonas aeruginosa* Biofilms

Anna Kotowska ORCID iD[1], Junting Zhang[2], Alessandro Carabelli[1], Julie Watts[1], Jonathan Aylott[1], Ian Gilmore[2], Paul Williams[1], David Scurr[1], Morgan Alexander[1]

[1]University of Nottingham, Nottingham, United Kingdom. [2]National Physical Laboratory, Teddington, United Kingdom

Abstract

Bacterial biofilms consist of cells enmeshed in a self-generated extracellular matrix containing diverse classes of molecules including polysaccharides, lipids, proteins, nucleic acids and diverse small organic molecules. *In situ* characterization of the chemical composition and structure of these complex systems is necessary to fully understand their development on surfaces relevant to biofouling in health, industry and the environment. In contrast to biological imaging methods such as fluorescence microscopy or matrix assisted laser desorption ionisation (MALDI), secondary ion mass spectrometry (SIMS) has the capability to map several groups of compounds simultaneously in an untargeted way, without labelling or matrix deposition.

This work presents Orbitrap secondary ion mass spectrometry (OrbiSIMS) as a label-free method for mapping the chemistry of biofilms in their native state. Here, we employ a recently developed untargeted chemical filtering process utilizing mass spectral databases to assign secondary ions to deconvolute the large number of fragments present in the SIMS spectra. To move towards comprehensive analysis of different chemistries in the sample, we apply a molecular formula prediction approach which putatively assigns 81% of peaks in the OrbiSIMS depth profile analysis. This enables us to catalogue over 1000 lipids and their fragments, 3500 protein fragments, 71 quorum sensing-related molecules (2-alkyl-4-quinolones and N-acylhomoserine lactones), 150 polysaccharide fragments, and glycolipids simultaneously from one data set and map these separated molecular classes spatially through a *Pseudomonas aeruginosa* biofilm. Assignment of different chemistries in this sample facilitates identification of differences between biofilms grown on biofilm-promoting and biofilm-resistant polymers.

Invited talk: The expression profile of a pre-existing drug tolerant population of Saccharomyces cerevisiae points to defects in mitochondrial function

Ivan Clark ORCID iD[1], Elizabeth Hughes[1], Delma Childers ORCID iD[2], Edward Wallace ORCID iD[1], Peter Swain ORCID iD[1]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]University of Aberdeen, Aberdeen, United Kingdom

Abstract

Resistance and tolerance to antifungal agents are substantial and increasing problems in medicine and agriculture. While resistance changes the minimal inhibitory concentration (MIC) of a culture with respect to a drug, tolerance reflects the presence of cells that continue to grow at concentrations above the population MIC. By observing single *Saccharomyces cerevisiae* cells in a microfluidic device, we have identified a subpopulation that continues to proliferate after the introduction of a commonly used fungistatic agent, fluconazole. In the conditions we use, the majority of cells arrest while around 10 per cent continue to divide. Conventionally tolerance is thought to arise through stress responses induced by drug exposure. In contrast, we can recognize the tolerant cohort before addition of drug as they express the ABC transporter Pdr5p at high levels. These high Pdr5-expressers grow slowly compared to the more sensitive majority in the absence of drug. We compared gene expression between the two populations. The tolerant population show higher expression of genes for TCA cycle enzymes and iron metabolism, suggesting defects in mitochondrial function. Indeed, although present when cells are grown in glucose, which can be metabolized by fermentation in the cytosol, high-expressers are absent when pyruvate is the only carbon source, which requires mitochondrial function. We are investigating stress responses of each subpopulation by monitoring the localization of key markers for different signaling pathways using our single cell imaging platform. We expect that these insights in to how cells are tolerant will help in choosing adjuvants to enhance standard antifungals.

Single particle – inductively coupled plasma – mass spectrometry (SP-ICP-MS): A powerful tool to study metals during host-pathogen cell interactions

Claire Davison ORCID iD, Jordan Pascoe ORCID iD, Melanie Bailey ORCID iD, Dany JV Beste ORCID iD, Mónica Felipe-Sotelo ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Trace metals are essential for intracellular pathogens to survive within their hosts however host cells use these metals as bacteriostatic/bactericidal weapons and therefore understanding metal homeostasis could lead to novel therapeutics such as micronutrient therapies. High sensitivity is required to accurately quantify trace metals in mammalian cells so bulk techniques have predominantly been used for these elemental measurements with the disadvantage that heterogeneity of metal composition is masked. Here we developed single particle - inductively coupled plasma - mass spectrometry (SP-ICP-MS) methods to study the levels of the trace metals calcium, magnesium and zinc in a macrophage model of TB. This work showed that calcium levels were tightly regulated and we measured very little heterogeneity in the amount of this metal in monocytes, macrophages and also within our TB macrophage model. Similarly for zinc there was also little variation between uninfected and infected macrophages whereas monocytes showed significant heterogeneity indicating that immune activation tightly regulates zinc levels. This work suggests that bulk measurements would not accurately represent what is occurring at a single cell level for these metal ions. However, mycobacterial infection led to significant heterogeneity in the levels of magnesium. This is significant as magnesium has a variety of biological functions in regulating energy metabolism, enzyme activity, signal transduction, nucleic acid and protein synthesis and is known to be immunomodulatory and shown to drive an anti-inflammatory response (M2 phenotype). This observed heterogeneity could therefore have significant effects on the course of mycobacterial infection and also impact on antimicrobial susceptibility.

Single-cell RNA sequencing suggests plasmids constrain bacterial heterogeneity and conjugation is subpopulation specific

Valentine Cyriaque ORCID iD[1], Rodrigo Ibarra-Chavez ORCID iD[1], Anna Kuchina[2], Georg Seelig[2], Joseph Nesme ORCID iD[1], Jonas Madsen ORCID iD[1]

[1]University of Copenhagen, Copenhagen, Denmark. [2]University of Washington, Seattle, USA

Abstract

Transcriptional heterogeneity is common within isogenic bacterial populations. Nonetheless, the interplay between conjugative plasmids and host subpopulation dynamics remains elusive. Here, the efficacy of microbial split-pool ligation transcriptomics in unraveling plasmid-host interactions was demonstrated through the generation of high-quality single-cell transcriptomic data. We found that transcription was impacted by growth state but also by plasmid carriage at the single-cell level, resulting in a reduction in subpopulations. Transcriptional heterogeneity among plasmid-encoded genes was also uncovered. This included core plasmid functions such as replication and maintenance fluctuations, likely associated with the cell cycle. Other key heterogeneously transcribed functions included tra genes revealing a subpopulation not engaging in conjugation and representing a possible plasmid strategy decreasing its burden at the population level. In sum, this study advances the understanding of plasmid-mediated subpopulation dynamics through a comprehensive single-cell transcriptomics approach, providing valuable insights into the intricate interplay between plasmids and bacteria.

Small Talk: Mechanisms of Sensing and Signalling at the Host-microbe and Microbe-microbe Interface

Invited talk: An intricate network of antagonistic interactions between phage and host factors governs the phage lysis-lysogeny decision

Nuria Quiles Puchalt ORCID iD

Department of Biomedical Sciences, Faculty of Health Sciences, Universidad CEU Cardenal Herrera, CEU Universities, Valencia, Spain

Abstract

Communication between organisms is essential when individuals need to coordinate their actions when living within communities. Viruses are not exempt from this behavior observed in prokaryotes and eukaryotes. Concretely, temperate bacteriophages of the SPβ-like family infecting *Bacillus subtilis* use a phage-encoded quorum sensing system named arbitrium that allows them to gather information about the surrounding phage population to decide which life cycle they will pursue; either the lytic cycle that will lead to cell lysis or the lysogenic cycle by integrating the phage genome into the bacterial chromosome. By using this peptide-based communication system, phages coordinate their lysis-lysogeny decision, but the underlying molecular mechanism controlling the phage genetic switch remains unknown. Here, we have uncovered that phage phi3T modulates via arbitrium system the bacterial toxin-antitoxin MazE-MazF system activity to regulate its life cycle. At the initial stages of the infection process, the phage produces two homologue proteins AimX and YosL that interact with toxin MazF. AimX interacts additionally with SroB, a phage protein that promotes lysogeny by binding to antitoxin MazE which will result in MazF release. Altogether these interactions suppress MazF activity, promoting the lytic cycle. After several rounds of infection, an increase in AimP peptide production inactivates the activity of phage antiterminator AimR, suppressing expression *aim*X, allowing MazF activity which will promote the phage lysogenic cycle. Overall, our study reveals an intricate network of mutually exclusive interactions between phage and host components controlling the phage lysis-lysogeny decision.

Invited talk: The Type VI secretion system and inter-bacterial arms races

Sarah Coulthurst

University of Dundee, Dundee, United Kingdom

Abstract

The Type VI secretion system (T6SS) is a key weapon in the competitiveness and virulence of many Gram-negative bacteria. Bacterial cells use the T6SS to deliver diverse toxins ('effectors') directly into neighbouring cells by a contraction-based firing mechanism. The majority of T6SSs have an anti-bacterial function, being used to efficiently kill rival bacterial cells and provide a competitive advantage in a variety of polymicrobial niches. These anti-bacterial T6SSs deliver a highly variable portfolio of broad-spectrum anti-bacterial effectors and are used against competitors of the same and different species. Specific immunity proteins provide recipient sibling cells with protection against the cognate effector. We have used the potent anti-bacterial T6SS of the opportunistic pathogen *Serratia marcescens* as a model to study the mechanisms and consequences of T6SS effector delivery into competitors. This has revealed a varied portfolio of effector toxins used for several forms of inter-microbial competition. We have recently characterised several new families of anti-bacterial effectors and examined the distribution of the T6SS across *Serratia*. We have also demonstrated how exchange of effector domains and protection by so-called 'orphan' immunity proteins facilitates competitive 'arms races' between members of the same species. Our findings add weight to the emerging picture of T6SSs representing a widespread and critical determinant of microbial community composition and dynamics.

Invited talk: A *Pseudomonas aeruginosa* small RNA regulates chronic and acute infection

Marvin Whiteley

Gerogia Institute Technology, Atlanta, USA

Abstract

Bacterial pathogens exhibit remarkable adaptability, enabling many of them to thrive in both chronic and acute lifestyles. However, the molecular understanding of their lifestyle changes within the human host has been a challenge. Through transcriptome analysis of the opportunistic pathogen *Pseudomonas aeruginosa* during chronic human infection, we identified a gene, termed *sicX*, as the most highly expressed gene in human chronic infections but poorly expressed under most in vitro conditions. Genetic studies revealed that *sicX* encodes a small RNA that post-transcriptionally controls anaerobic ubiquinone biosynthesis, and deletion of *sicX* leads to reduced growth of *P. aeruginosa* in low oxygen conditions. Using a recently developed quantitative framework developed in our lab, we identified a murine preclinical model highly accurate for studying SicX function in vivo. Studies of WT *P. aeruginosa* and a *sicX* mutant in this murine surgical wound model revealed that SicX controls the chronic-to-acute transition in *P. aeruginosa*, with control of anaerobic ubiquinone synthesis being essential for SicX-mediated chronic infection. Additionally, SicX also serves as a biomarker for the chronic-to-acute transition, being the most downregulated gene when a chronic infection transforms into acute septicemia. This study resolves a longstanding question regarding the molecular basis of the chronic-to-acute switch in *P. aeruginosa*, pointing to oxygen as a primary environmental driver of acute lethality.

Invited talk: Epigenetic Reprogramming in Host-Parasite Coevolution: The Toxoplasma Paradigm

Mohamed-Ali HAKIMI ORCID iD

INSERM, GRENOBLE, France

Abstract

Like many intracellular pathogens, the protozoan parasite *Toxoplasma gondii* has evolved sophisticated mechanisms to promote its transmission and persistence in a variety of hosts by injecting effector proteins that manipulate many processes in the cells it invades. Specifically, the parasite diverts host epigenetic modulators and modifiers from their native functions to rewire host gene expression, thereby counteracting the innate immune response and limiting its strength. The arms race between the parasite and its hosts has led to accelerated adaptive evolution of effector proteins and the unconventional secretion routes they use. I will report on the findings from a decade of research by our team, focusing on how *Toxoplasma* effectors that have evolved disordered regions form extensive complexes and mimic host molecules that specifically target key host transcription factors and chromatin-modifying enzymes. I will specifically particularly spotlight recently discovered effectors and how they dictate the behavior of the infected cell and ultimately influence the outcome of infection.

Invited talk: Understanding mechanisms of cell-cell communication in African trypanosomes

Keith Matthews ORCID iD

University of Edinburgh, Edinburgh, United Kingdom

Abstract

African trypanosomes are protozoan parasites, spread by tsetse flies, that cause sleeping sickness in humans and the livestock disease 'Nagana'. To prepare for transmission to tsetse, trypanosomes generate a transmission-adapted developmental form in mammalian hosts - the stumpy form. Stumpy forms accumulate in response to a quorum sensing (QS)-type mechanism whereby parasites release peptidases into their environment that generate oligopeptides. Consequently, the oligopeptides provide a proxy for parasite density, with signal reception achieved via a TbGPR89 surface transporter with the subsequent signal transduction pathway comprising protein kinases, protein phosphatases, gene expression regulators and molecules of unknown function. To provide coherent understanding of the pathway we have exploited genome-wide gene silencing screens in the laboratory to assemble the pathway hierarchy. However, we have also exploited natural field isolates of trypanosomes which have reduced their capacity for QS, such that they are no longer transmitted by tsetse flies but instead exploit transmission by other biting flies (Trypanosoma evansi, causing 'surra' in animals), or are transmitted sexually (Trypanosoma equiperdum causing 'dourine' in horses). Analysis of the genomes of over 80 isolates has identified novel mutations that have been functionally validated using CRISPR for their role in QS. Complementing this, we have selected multiple parasite lines in the laboratory that exhibit reduced QS and have identified how QS becomes reversibly lost by the down regulation of key gene expression regulators. Our experiments provide new insight into the molecular control of trypanosome QS and identifies diagnostic markers for its loss in the field.

Invited talk: Virus-virus interactions: the good, the bad, and the unexpected

Pablo Murcia ORCID iD

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Respiratory viruses are responsible for major disease burdens, including seasonal epidemics and pandemics. Multiple viruses can cause respiratory infections, including influenza viruses, coronaviruses, respiratory syncytial virus, rhinovirus, human metapneumovirus, and parainfluenza viruses. Importantly, these viruses interact through co-infections which account for ~10% of respiratory virus detections, 80% of which are detected in children <5 years of age. Despite this, respiratory viruses have been traditionally studied individually, using a one virus-one disease approach.

I will present a series of studies from our group where we adopt a multi-virus and multi-scale approach that combines epidemiology, mathematical modelling, laboratory experiments , and structural biology. We find evidence of both positive and negative interactions among respiratory viruses, which indicate that deterministic (e.g. host responses to infection) and stochastic (e.g. timing of secondary viral infection) processes influence the outcome of co-infections. Cellular co-infections can also lead to the generation of hybrid virus particles which may play a significant role in virulence and virus evolution. Our work highlights the importance of incorporating ecological principles in experimental virology and suggest future avenues of investigation to shed light on the role of virus-virus interactions on host susceptibility to infection, virus pathogenesis, and disease presentation.

Invited talk: Unravelling the role of the gut microbiota in barrier development and function across the gut-brain axis

Emily G. Knox[1,2], Valentine Turpin[1,2], Alexandre Cergneux[1,2], Jennifer Morael[1,2], Lorena Morales[1,2], Hugo Blair[1,2], Carmen Tessier[1,2], Elisa Cintado[3], Jennifer Shearer[1,2], Anna Ratsika[1,2], Caoimhe M.K. Lynch[1,2], Michael L. Harvey[4], Jonathan Swann[4], John F. Cryan[1,2], María R. Aburto ORCID iD[1,2]

[1]Department of Anatomy and Neuroscience, School of Medicine,University College Cork, Cork, Ireland. [2]APC Microbiome Ireland, Cork, Ireland. [3]Instituto Cajal, CSIC, Madrid, Spain. [4]University of Southampton, Southampton, United Kingdom

Abstract

The intestinal microbiota plays a fundamental role in host physiology; however, many of the communication mechanisms between them are still unknown. In recent decades, our understanding of the significant influence of the intestinal microbiota on brain function has increased. This bidirectional communication is referred to as the gut-brain-microbiota axis. Along this axis, there are several cellular barriers whose function is essential for organismal homeostasis. Among these barriers are the intestinal barrier, the blood-brain barrier, and the blood-cerebrospinal fluid barrier. The influence of the microbiota on these barriers is crucial for the proper functioning of the gut-brain-microbiota axis.

In this work, we focus on understanding the importance of the intestinal microbiota in the establishment and maintenance of these barriers using in vitro cellular models and different in vivo models of microbiota disruption. Using a mouse model of cesarean birth, where the initial acquisition of microbiota at birth is altered, we observed a disruption in intestinal and brain barriers in the postnatal stage. Similarly, adult mice completely devoid of microbiota exhibit a disruption in the structure of the blood-cerebrospinal fluid barrier, revealing an essential role of the microbiota in the homeostasis of the barriers and, consequently, in communication along the gut-brain-microbiota axis.

Invited talk: Using defective viral genomes as a lens into influenza virus population dynamics and evolution

Katia Koelle

Emory University, Atlanta, USA

Abstract

Defective viral genomes (DVGs) are ubiquitous across many RNA viral species. The functional role of these DVGs in viral infections (if any) is still an area of lively discussion. Separate from this, however, the diversity of DVGs and their complex population dynamics alongside “wild-type” virus makes them potentially useful for informing our understanding of viral dynamics more generally. Here, I will focus on influenza virus DVGs, presenting our recent work that shows how DVG population dynamics can shed light on processes at both the intracellular level and the within-host level. In our first analysis, we show that complex cycling dynamics of DVGs and wild-type virus arise under a very limited set of viral input-output rules for infected cells. By statistically fitting models to cell culture measurements, we then show that these rules are empirically supported. In our second analysis, we re-examine the respective roles that stochastic processes and selection play in shaping within-host influenza virus dynamics that have been previously characterized using low-diversity patterns of genetic variation. Our complementary analysis of high-diversity DVG dynamics corroborate these previous findings as well as point towards narrow transmission bottlenecks.

Infection-induced membrane ruffling initiates danger and immune signaling via the mechanosensor PIEZO1

Lalitha Tadala[1], Dorothee Langenbach[1], Mirjam Dannborg[1], Ramon Cervantes-Rivera[1], Atin Sharma[1], Kevin Vieth[1], Lisa M. Rieckmann[1], Alkwin Wanders[2], David A. Cisneros ORCID iD[3,4], Andrea Puhar ORCID iD[1,4]

[1]The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umea University, Umea, Sweden. [2]Department of Pathology, Aalborg University Hospital, Aalborg, Denmark. [3]Umea University, Umea, Sweden. [4]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom

Abstract

Microorganisms are generally sensed by receptors recognizing microbial molecules, which evoke changes in non-transcriptional cellular activities and in gene expression programmes upon binding of the cognate ligand. Intestinal epithelial cells (IECs) are a barrier to gut microbes and important immune sentinels. During infection of IECs bacterial pathogens induce secretion of the danger signal ATP as an early alert response, initiating overt inflammation. However, what triggers ATP secretion during infection was unclear. We show that the inherently mechanosensitive plasma membrane channel PIEZO1 acts as a sensor for bacterial entry. PIEZO1 is mechanically activated by invasion-induced membrane ruffles upstream of Ca^{2+} influx and ATP secretion. Moreover, chemical or mechanical PIEZO1 activation evokes gene expression in immune and barrier pathways. Hence, PIEZO1-dependent detection of infection is driven by physical signals instead of chemical ligands. Given that pathogens, but not the microbiota, encode the ability to invade host cells, we propose that PIEZO1 differentiates invasive from non-invasive or commensal bacteria.

A novel viral signalling and antiviral effector pathway revealed by spatial proteomics

Marisa Oliveira[1,2], Ben Ravenhill[1,2], George Wood[3], Ying Di[1,2], Robin Antrobus[1,2], Nerea Irigoyen[3], David J Hughes[4], Youngxu Lu[3,5], Gill Elliott[6], Betty Chung[3], Michael P Weekes[1,2]

[1]Cambridge Institute for Medical Research, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [3]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [4]University of St Andrews, St. Andrews, United Kingdom. [5]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. [6]Department of Microbial Sciences, School of Biosciences, University of Surrey, Guilford, United Kingdom

Abstract

Cellular sensors and their signalling components form a crucial first step in the recognition of intracellular pathogens, determining the outcome of infection by orchestrating effective immunity. Cytoplasmic-to-nuclear transition of signalling components IRF3 and NF-kB underpin sensing pathways for viruses, bacteria, fungi and parasites. The discovery of related, novel signalling pathways will provide key insights into intrinsic immunity and transform our understanding of how pathogen recognition triggers immune gene expression. A detailed mechanistic understanding of known sensing/signalling components has led to the development of vaccine adjuvants, insights into autoimmunity and treatments for chronic inflammation and cancer. Many viral signalling proteins exhibit subcellular relocalisation upon activation, triggering expression of interferon and antiviral genes. To identify novel signalling components, we have developed innovative proteomic methodologies to globally quantify subcellular protein redistribution in virus-infected cells. Using Sendai virus infection of human fibroblasts, we identified CREB-Regulated Transcription Coactivators-2 and 3 (CRTC2/3) as novel 'hits' that translocate from cytoplasm to nucleus in addition to IRF3/NF-kB. CRTC2/3 also translocate during DNA virus infection, suggesting a common, novel cytosolic nucleic acid response. We have now identified a subset of CRTC2/3-dependent genes induced by viral infection. These include IL-11, an understudied fibrogenic cytokine acting via the same intracellular pathway as IL-6. CRTC-dependent IL-11 stimulation was dependent on COX2, an inducible isozyme essential in the prostaglandin biosynthesis pathway. By combining advanced proteomics with transcriptomics and cell biology, we have thus identified a new arm of the signalling and effector response to foreign nucleic acids, which may be therapeutically tractable.

The Consequences of Congenital Virus Infections

Invited talk: Zika viruses encode multiple 5’ upstream open reading frames with a role in neurotropism

Charlotte Lefèvre[1], Georgia Cook[1], Adam Dinan[1], Shiho Torii[2], Hazel Stewart[1], George Gibbons[1], Alex Nicholson[3], Liliana Echavarría-Consuegra[1], Luke Meredith[1], Valeria Lulla[1], Julia Kenyon[1], Naomi McGovern[1], Ian Goodfellow[1], Janet Deane[3], Stephen Graham[1], Andras Lakatos[1], Louis Lambrechts[2], Ian Brierley[1], Nerea Irigoyen ORCID iD[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Institut Pasteur, Paris, France. [3]Cambridge Institute for Medical Research, Cambridge, United Kingdom

Abstract

Zika virus (ZIKV), an emerging, mosquito-borne flavivirus, is associated with congenital neurological complications. Here, we investigated potential pathological correlates of virus gene expression in representative ZIKV strains through RNA sequencing and ribosome profiling. In addition to the single long polyprotein found in all flaviviruses, we identified the translation of multiple unrecognised upstream open reading frames (uORFs) in the genomic 5’ region. In Asian/American strains, ribosomes translate uORF1 and uORF2, whereas in African strains, the two uORFs are fused into one (African uORF). We used reverse genetics to examine the impact on ZIKV fitness of different uORFs mutant viruses. We found that expression of the African uORF and the Asian/American uORF1 modulates virus growth and tropism in human cortical neurons and cerebral organoids, indicating their role in ZIKV neurotropism. Although the uORFs are expressed in mosquito cells, we did not see a measurable effect on transmission by the mosquito vector *in vivo*.

The discovery of ZIKV uORFs sheds new light on ZIKV-induced neuropathogenesis and raises the question of their existence in other neurotropic flaviviruses.

Invited talk: Homologous prime-boost with a novel MVA-vectored Zika vaccine confers protection in A129 mice

Krishanthi Subramaniam[1], Gareth Platt[1], James Austin[1], Karen Buttigieg[2], Miles Carroll[3], Tom Blanchard[1,4], Lance Turtle[1,4], Neil French[1,4]

[1]University of Liverpool, Liverpool, United Kingdom. [2]UKHSA, Salisbury, United Kingdom. [3]University of Oxford, Oxford, United Kingdom. [4]Royal Liverpool Hospital, Liverpool, United Kingdom

Abstract

Background: There is no licensed vaccine for Zika virus despite ongoing transmission since the 2016 epidemic. There is significant potential for Zika to spread with global warming. Zika usually causes a mild, self-limiting infection but may severely affect a developing foetus and occasionally causes Guillain-Barré syndrome in adults. Modified vaccinia Ankara (MVA) has an excellent safety record in human use and is expected to be equally safe in pregnancy.

Methods: Recombinant MVA was constructed incorporating Zika prME, ubiquitinated NS5 and truncated NS3 (MVA-Zika). A129 mice were employed because they lack type 1 interferon receptors, making them susceptible to Zika. Immunogenicity and protection was assessed in homologous prime-boost immunised A129 mice challenged with a subcutaneous dose of Zika.

Results: MVA-Zika immunisation resulted in clearance of virus from tissues including blood, heart, liver, spleen, ovaries, uterus, kidneys and brain. Over 80% of immunised mice seroconverted and mounted a robust IFNγ T cell response to encoded Zika components. Vaccination induced neutralising antibodies and a strong memory response. Furthermore toxicity studies demonstrated that MVA-Zika did not cause any significant adverse events.

Conclusions: Our results show that the MVA-Zika vaccine is safe and immunogenic to use in a vertebrate host. The vaccine elicits robust antiviral responses to mediate clearance in multiple tissues. MVA-Zika is currently in phase I human clinical trial and hopefully provides a promising option for the prevention of future epidemics of congenital Zika syndrome.

Invited talk: iciHHV-6: prevalence, ancestral lineages, and phenotypic associations

Ruth Jarrett ORCID iD[1], Michael Wood[1], Annette Lake[1], Natasha Jesudason[1], Kostas Papageorgiou[1], Shona Kerr[2], Caroline Hayward[2]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]MRC Human Genetics Unit, Edinburgh, United Kingdom

Abstract

Human herpesvirus 6A and 6B (HHV-6A and 6B, or collectively HHV-6) are closely related herpesviruses that have the unusual ability to integrate into the telomeres of host chromosomes. Most individuals are infected by exogenous HHV-6 in childhood; however, a significant minority inherit the virus as a result of germline integration of the viral genome. This is referred to as inherited chromosomally integrated HHV-6 (iciHHV-6). Individuals with iciHHV-6 have one or occasionally more viral genomes in every cell, and the virus retains the ability to reactivate. To understand the epidemiology and clinical impact of iciHHV-6, we analysed iciHHV-6 in large cohort studies including the Generation Scotland study and UK Biobank.

The overall prevalence of iciHHV-6 in the UK is 1.4%, with a higher prevalence of iciHHV-6B (1.08%) compared to iciHHV-6A (0.33%). We observed significant regional variation with the highest iciHHV-6B prevalence in Ireland and Scotland (3%), and the highest iciHHV-6A prevalence in Wales and England. Although almost 90% of all iciHHV-6B was explained by four ancestral viral lineages, we identified over 25 different viral lineages with many distinct geographical distributions. The data suggest that establishment of an iciHHV-6 lineage is rare but ongoing.

Analysis of HHV-6 serology results from almost 9,000 individuals in the UK Biobank showed a significant association between iciHHV-6B and both HHV-6B seropositivity and antibody level, suggesting that viral reactivation is more frequent in iciHHV-6B+ve individuals compared to those with exogenous infection. Analysis of disease associations is ongoing but challenging due to the complex nature of iciHHV-6 epidemiology.

Invited talk: Characterization of Humoral and Cellular Immunologic Responses to an mRNA-Based Human Cytomegalovirus Vaccine From a Phase 1 Trial of Healthy Adults

Kai Wu, Yixuan Jacob Hou, Dan Makrinos, Runxia Liu, Alex Zhu, Matthew Koch, Wen-Han Yu, Yamuna Paila, Sumana Chandramouli, Lori Panther, Carole Henry, Anthony DiPiazza, Andrea Carfi

Moderna, Cambridge, USA

Abstract

Human cytomegalovirus (HCMV) is a widespread pathogen that can cause serious complications in immunocompromised individuals and is a leading infectious cause of birth defects. No prophylactic vaccine is currently approved . mRNA-1647 is an investigational mRNA-based vaccine against HCMV that contains sequences encoding the viral proteins glycoprotein B and pentamer. Humoral and cellular immune responses were evaluated in blood samples collected from healthy HCMV-seropositive and HCMV-seronegative adults who participated in a phase 1 trial of a 3-dose series of mRNA-1647 (NCT03382405). Neutralizing antibody (nAb) titers against fibroblast and epithelial cell infection in sera from HCMV-seronegative mRNA-1647 recipients were higher than those in sera from control HCMV-seropositive samples and remained elevated up to 12 months after dose 3. nAb responses elicited by mRNA-1647 were comparable across 14 HCMV strains. Frequencies of antigen-specific memory B cells increased in HCMV-seropositive and HCMV-seronegative participants after each mRNA-1647 dose and remained elevated for up to 6 months after dose 3. mRNA-1647 elicited robust increases in frequencies and polyfunctionality of $CD4^+$ T helper type 1 and effector $CD8^+$ T cells in samples from HCMV-seronegative and HCMV-seropositive participants after stimulation with HCMV-specific peptides. Administration of 3 doses of mRNA-1647 to healthy adults elicited high nAb titers with wide-breadth, long-lasting memory B cells, and strong polyfunctional T-cell responses. These findings support further clinical development of the mRNA-1647 vaccine against HCMV.

Invited talk: In utero human cytomegalovirus infection expands NK cell-like FcγRIII-expressing CD8+ T cells that mediate antibody-dependent functions

Eleanor Semmes[1], Danielle Nettere[1], Ashley Nelson[2], Jillian Hurst[1], Derek Cain[1], Trevor Burt[1], Joanne Kurtzberg[1], Keith Reeves[1], Carolyn Coyne[1], Genevieve Fouda[2], Justin Pollara[1], Sallie Permar[2], Kyle Walsh[1]

[1]Duke University, Durham, USA. [2]Weill Cornell, New York, USA

Abstract

Human cytomegalovirus (HCMV) profoundly modulates host T and natural killer (NK) cells across the lifespan, expanding unique effector cells bridging innate and adaptive immunity. Though HCMV is the most common congenital infection worldwide, how this ubiquitous herpesvirus impacts developing fetal T and NK cells remains unclear. Using computational flow cytometry and transcriptomic profiling of cord blood from neonates with and without congenital HCMV (cCMV) infection, we identify major shifts in fetal cellular immunity. This was marked by an expansion of Fcγ receptor III (FcγRIII)-expressing CD8+ T cells (FcRT) following HCMV infection in utero. FcRT cells from cCMV-infected neonates expressed a cytotoxic NK cell-like transcriptome and were able to mediate antigen-specific antibody-dependent functions including degranulation and IFNγ production, the hallmarks of NK cell antibody-dependent cellular cytotoxicity (ADCC). FcRT cells may represent a previously unappreciated effector population with innate-like functions that could be harnessed for maternal-infant vaccination strategies and antibody-based therapeutics in early life.

Invited talk: Impact of fetal immune sex differences on susceptibility to in utero HIV infection and on HIV cure/remission potential

Philip Goulder ORCID iD

University of Oxford, Oxford, United Kingdom

Abstract

-

Zika virus and the interferon response: A tale of two mechanisms

Duncan R. Smith ORCID iD, Oradee Khammaneejan, Wannapa Sornjai ORCID iD, Suwipa Ramphan ORCID iD

Mahidol University, Bangkok, Thailand

Abstract

When a pregnant woman becomes infected with the mosquito transmitted Zika virus (ZIKV) in the first or second trimester, the virus can pass across the placenta to infect the developing fetus. While this can have a number of consequences on fetal development, the most significant is microcephaly, or abnormally small brain development. The strain of ZIKV circulating in Thailand has been shown to cause microcephaly as evidenced by a number of cases. Our laboratory has been able to isolate the virus from autopsy tissues of a fetus medically terminated for reasons of fetal defects including microcephaly. Analysis showed that this virus differs from the endemic fever associated virus at only a nine amino acids, but that it has a distinctly different replication profile. Examination of the induction of the interferon response in both a cell line and in human neural cells differentiated from induced pluripotent stem cells showed that the fever virus triggered significantly greater interferon β expression, as well as greater expression of a number of interferon stimulated genes. In a polyIC assay, it was shown that the two NS5 proteins significantly differed in their ability to dampen the innate immune response. Further dissection of NS5 is currently underway. However, the study clearly shows that even relatively small changes can have a significant impact on the interaction of closely related viruses with the innate immune system.

Controlling Human Cytomegalovirus (HCMV) through NK-dependent ADCC-inducing Immunotherapies.

Hannah Preston, Zack Saud, Virginia-Maria Vlahava, Andreas Zaragkoulias, Edward Wang, Richard Stanton

Cardiff University, Cardiff, United Kingdom

Abstract

Human Cytomegalovirus (HCMV) has a global seroprevalence of approximately 80% and causes lifelong infection. It can result in severe disease in immunocompromised individuals and following congenital infection. Therapeutics are limited by virus resistance and undesirable side effects, and no vaccines are licensed. Vaccines have focused on the induction of neutralising antibodies, however HCMV spreads cell-to-cell within the host, limiting the efficacy of this response. There is therefore a need for strategies that target the infected cell. We investigated the capacity of antibodies to bind infected cells and activate NK-dependent antibody-dependent cellular cytotoxicity (ADCC). Despite encoding multiple immune-evasins, HCMV infected cells were susceptible to ADCC. However, this was not mediated by antibodies targeting the structural glycoproteins that are commonly used as vaccine and immunotherapeutic targets. Profiling of the infected cell-surface via quantitative proteomics identified 15 non-structural viral proteins on the cell surface during early stages of infection, of which three (UL16, UL141, US28) activated ADCC. Human monoclonal antibodies (mAbs) targeting these antigens efficiently controlled virus spread through ADCC, when used as a mix of 5. To determine whether it was possible to reduce the number of mAbs needed to activate ADCC, they were converted into antibody-like constructs, including Bispecific Killer Engagers (BiKEs), Trispecific Killer Engagers (TriKEs) and Redirected Optimised Cell Killers (ROCKs). These constructs demonstrated a superior capacity to mediate ADCC. Our findings reveal a novel immunotherapeutic approach against HCMV and demonstrate ways of maximising antiviral activity by exploiting engineered antibody-like molecules.

A protective multiple gene-deleted African swine fever virus genotype II, Georgia 2007/1, expressing a modified non-haemadsorbing CD2v protein

Anusyah Rathakrishnan ORCID iD[1], Ana-Luisa Reis ORCID iD[1], Vlad Petrovan ORCID iD[1], Lynnette Goatley ORCID iD[1], Katy Moffat ORCID iD[1], Lui Yuan ORCID iD[2], Mai T Vuong ORCID iD[2], Shinzi Ikemizu ORCID iD[3], Simon J Davis ORCID iD[2], Linda Dixon ORCID iD[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]John Radcliffe Hospital, Oxford, United Kingdom. [3]Kumamoto University, Kumamoto, Japan

Abstract

African swine fever virus is a complex DNA virus that causes high fatality in pigs and wild boar and has a great socio-economic impact. The continuing spread of ASFV threatens global food security. Modified live vaccines, attenuated by rational gene deletions or modifications, are currently the most promising approach. We constructed ASFV vaccine candidates based on the current pandemic genotype II strain. To improve safety of these candidates we aimed to reduce virus levels and persistence in blood but retain high levels of protection. We modified the gene for the wildtype ASFV CD2v transmembrane protein to introduce single or double amino acid substitutions which reduced or abrogated the binding to red blood cells (HAD) to infected cells and virions and reduce virus persistence in blood. The mutant CD2v proteins were expressed at similar levels to the wildtype of protein on the surface of infected cells. Additional gene combinations were deleted to further attenuate the virus. Immunisation and challenge experiments in pigs compared clinical signs and levels of protection induced by three recombinant ASF viruses. The results showed that, as predicted, the partially HAD virus with a single mutation in CD2v induced moderate levels of replication compared to low levels induced by viruses expressing non-HAD CD2v. All viruses induced high levels of protection against challenge with virulent virus. Further The results confirm that modification of CD2v in combination with other gene deletions can improve vaccine safety and maintain efficacy.

T cell responses to diverse adenovirus genotypes are widespread in adults, and are boosted in ChAdOx1 recipients

Arnold Lambisia[1], Benjamin Krishna[2], Charlotte Houldcroft[3]

[1]Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya. [2]Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), Cambridge, United Kingdom. [3]Department of Genetics, University of Cambridge, Cambridge, United Kingdom

Abstract

Human adenoviruses (AdVs) are a diverse collection of seven species and 100+ genotypes which cause 5-20% of coughs, colds and severe lower respiratory tract infections year-round. CD3+ T cells have been recognised as key to viral clearance during adenovirus infection, and pre-existing T cell responses are known to blunt the effectiveness of adenovirus vectors for vaccines and gene therapy, even in the absence of genotype-specific neutralising antibody responses. Most studies to date have focused on the T cell response to the common respiratory genotype C5.

In this study, we used FluoroSpot measurement of IFNγ and IL2 to show that T cell responses to species A and F (enteric), C (respiratory) and Y25 (chimpanzee AdV similar to species E, respiratory) AdVs are widespread in healthy adult blood donors. However, the frequency of the T cell responses, as a percentage of T cells producing IFNγ in response to stimulation, is highly variable. The frequency of IFNγ and IL2 responses to each AdV species do not correlate with each other within donors, suggesting both a cross-species and species-specific component to the T cell response to AdV infection.

This is also seen in recipients of adenovirus-vectored SARS-CoV-2 vaccine, ChAdOx1. They have higher mean IFNγ T cell responses to both to the ChAdOx1 vector Y25 and to C5, compared to temporally matched mRNA vaccinees. This suggests a cross-genotype boosting effect in the ~50% of the UK adult population who received ChAdOx1, with unknown consequences for population immunity, future adenovirus vaccine usage and viral evolution.

A novel IFNγ-stimulated gene with anti-HIV-1 activity

Hollie Jackson Ireland ORCID iD, Elena Sugrue, Vojtech Pavelka, Arthur Wickenhagen, Quan Gu, Adam Fletcher, Suzannah Rihn

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Over the last several decades, HIV-1 infection has had a substantial impact on global health worldwide, with over 40 million people having died from AIDS-related diseases, and over 39 million currently living with HIV-1. Interferons (IFNs) are a key barrier to the establishment of infection in the host as they upregulate hundreds of interferon-stimulated genes (ISGs) which interfere with viral replication, creating an 'antiviral state'. Although ISGs with antiretroviral activity have previously been identified, the inhibitory effects of these well-characterised ISGs cannot wholly explain the antiretroviral activity of IFN. We have identified a member of the Wnt/β-catenin pathway as an IFN**γ**-stimulated ISG with anti-HIV-1 activity. This ISG exhibits differential antiviral activity depending on the HIV-1 group the virus belongs to, and the host co-receptor to which it binds. We explore the virus specificity of the ISG in addition to the possible determinants of antiviral activity by the ISG, and of the rescue of inhibition by a GSK-3 inhibitor. This research aims to enhance our understanding of the complex interplay between HIV-1 and host ISGs, which may advise the development of targeted strategies in fighting this global health challenge.

Comprehensive vaccine protection against congenital cytomegalovirus by targeting key components of the virus life cycle and inducing both antibody and T cell immunity

Alistair McGregor, Nadia El-Hamdi, K.Yeon Choi

Texas A&M University, Bryan/College Station, USA

Abstract

Congenital cytomegalovirus (cCMV) is a leading cause of hearing loss/cognitive impairment in newborns. A vaccine is complicated by the existence of multiple CMV strains enabling re-infection and a vaccine must exceed convalescent natural immunity. Guinea pig cytomegalovirus (GPCMV) is the only small animal model for cCMV. A GPCMV non-replication competent DISC vaccine strain demonstrated the importance of neutralizing antibodies to viral glycoproteins for protection. The fusogenic gB glycoprotein is essential for entry into all cell types. A trimeric gB complex based adenovirus (Ad) vaccine (AdgB) induced potent neutralizing antibodies, unlike a gB monomer. However, cell associated virus was able to disseminate and cause cCMV. In convalescent HCMV, T cell responses are considered important against latent infection. GPCMV encodes functional homologs of IE1 and pp65 (GP83) pathogenicity factors targeting PML bodies and IFI16/cGAS-STING pathway respectively. Ad based vaccine candidates (AdIE1/AdGP83) induced T cell responses in vaccinated animals and reduced viral load in target organs but lacked complete protection. A combination strategy of AdgB and AdIE1 (targeting humoral and cell-mediated responses) against mixed wild type strains of GPCMV in a congenital protection study was evaluated. 4 groups of female animals (N=10/group) were vaccinated with either AdgB, AdIE1, AdgB+AdIE1, or unvaccinated before mating. During 2nd trimester, animals were challenged with GPCMV. Pup cCMV transmission in unvaccinated was 91% but the AdgB+AdIE1 group had no detectable virus in pup tissues unlike single antigen vaccines. Results demonstrate the importance of immune response to IE1 and gB for a potent cross strain protection against cCMV.

Therapeutics: the Use of Bacteriophage, Viruses, and Viral Components

Invited talk: The state of play of phage biology in the UK

Martha Clokie ORCID iD

University of Leicester, Leicester, United Kingdom

Abstract

Bacteriophages, viruses that target and kill bacteria are the most dominant and genetically diverse biological entities on Earth. They drive bacterial dynamics and evolution and as natural predators of bacteria they can be studied and developed as new tools to treat antibiotic bacterial infections. This huge potential to be developed as novel therapeutics is increasingly recognised by bodies that monitor global infection such as the World Health Organisation. The importance, strengths and shortcomings of bacteriophage research and translation in the UK was recently highlighted by the UK House of Commons Committee report on the antimicrobial potential of bacteriophages that was released in January 2024.

Evidence for interest in fundamental and translational aspects of bacteriophages can be seen from multiple streams: 1) the recent UK Viruses of Microbes Society meeting, held in Newcastle that was full to capacity; 2) Together with colleagues at Leicester I established the UK's first Centre for Phage Research in 2023 to provide a critical mass of multidisciplinary phage researchers, and to systematically curate our collections of bacteriophages; 3) A new Innovate UK KTN, the *Phage Innovation Network* which matches bacteriophage researchers and innovators with Industrial partners where phages may provide solutions; 4) Several efforts to establish networks to unite UK clinical researchers and start to build an infrastructure where phages can be used to treat patients; 5) Increased interactions between phage biologists, biological production specialists, regulators and policy makers. I will review these developments and provide an update of bacteriophage research in the UK.

Characterisation of a novel flagellotropic phage targeting *Burkholderia cenocepacia*

Jessica Lewis ORCID iD[1], Thomas Naderer ORCID iD[2], Jeremy Barr ORCID iD[2], Trevor Lithgow ORCID iD[2], Yin Chen ORCID iD[3], Antonia Sagona ORCID iD[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Monash University, Clayton, Australia. [3]University of Birmingham, Birmingham, United Kingdom

Abstract

The *Burkholderia cepacia* complex is responsible for severe respiratory infections in individuals afflicted with Cystic Fibrosis, resulting in high levels of morbidity and recurrent infections. The ability of *Burkholderia* to hide within human cells further complicates treating these infections, as many treatments are unable to bypass the human cell membrane. While personalised bacteriophage therapy has been applied for the treatment of extracellular bacteria, treatment of intracellular bacteria remains a challenge. Despite recent advances showing that bacteriophages naturally enter human cells at low levels, genetic engineering is required to enhance this ability. We aimed to tackle this challenge using *Burkholderia cenocepacia* as a model and a recently isolated bacteriophage from wastewater. Testing of the phage against mutants of *B. cenocepacia* with a paralysed or completely absent flagella revealed loss of activity, suggesting the flagella is a crucial receptor of phage. Transmission electron microscopy revealed a large phage (Matilda) with a head diameter of 107 nm, belonging to the family of *Myoviridae*. Accompanying the large phage was a much smaller *Myoviridae* phage, which sequencing revealed to be the previously characterised prophage KS10. The Matilda phage encodes for 390 genes, with 314 of unknown function. Using AlphaFold and FoldSeek, we have begun to characterise these hypothetical proteins. By understanding the genetics of this novel phage, we enter a realm of possibilities to engineer a more weaponised and useful therapeutic agent.

Diverse and predictable evolutionary responses to phage therapy across multiple case studies

Meaghan Castledine ORCID iD[1], Jesica Soria Pascual[1], Pawel Sierocinski[1], Chloe Esom[1], Maya Merabishvili[2], Jean-Paul Pirnay[2], Daniel De Vos[2], Angus Buckling[1]

[1]University of Exeter, Penryn, United Kingdom. [2]Queen Astrid Military Hospital, Brussels, Belgium

Abstract

With rising antibiotic resistance, modern medicine needs new approaches for tackling bacterial infections. Phage therapy uses the viruses of pathogenic bacteria to clear the infection. Unlike antibiotics, phage can evolve if bacteria become resistant to maintain or even increase their infectivity (coevolution). Understanding how bacteria evolve resistance in vivo, and whether phages reciprocally evolve is critical for assessing whether evolutionary changes drive treatment success. Despite concerns of phage resistance, understanding of bacteria-phage (co)evolution in vivo is limited. We analyse how bacteria and phages have evolved in vivo across three phage therapy case studies, including the bacteria Pseudomonas aeruginosa (sepsis), Staphylococcus aureus (necrotising fasciitis) and Klebsiella pneumoniae (surgical infection). Our results show, for the first time, rapid bacteria and phage coevolution during clinical phage therapy. Interestingly after treatment stopped, resistance reached fixation showing the importance of continual phage application to give phage an advantage in vivo. Furthermore, we find diverse responses of pathogens to phage including emergence of persister cells and induction of biofilm formation to resist phage infection across our case studies. Importantly, responses to phages were predictable using rapid in vitro experiments, showing the applicability of laboratory studies to predict how bacterial infections may respond to treatment. Predicting bacterial responses is crucial for designing treatments to aid bacterial clearance, for instance in predicting the emergence of persister cells and applying antibiotics that can work synergistically with phage to clear these populations. Our results highlight the diversity and predictability of bacterial evolution in vivo, and how this can be exploited to maximise treatment success.

Phage SEP1 activates and replicates efficiently in *S. epidermidis* stationary phase cells

Maria Daniela Silva ORCID iD[1,2], Graça Pinto[1,2], Ângela França[1,2], Joana Azeredo[1,2], Luís D. R. Melo[1,2]

[1]CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal. [2]LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Abstract

Most bacteriophages are not able to infect stationary cells. Nevertheless, it was previously shown that the *Staphylococcus epidermidis* SEP1 phage has this rare capability, reducing the number of cells in this lower metabolic state. In this study, both exponential and stationary cultures were infected with SEP1, and their response was investigated by total RNA-seq. An increase in the number of reads that mapped to SEP1 genome was observed over the 30 min of infection in both conditions, indicating that SEP1 successfully took over the transcriptional resources of the exponential and stationary cells necessary for its replication. However, in stationary cells, SEP1 transcription was delayed comparatively to exponential cells, with a high expression of genes within the gp142-gp154 module, particularly at 5 min post-infection, leading to the hypothesis that these are the "very early" genes that are necessary to start the replication process. The host responded to SEP1 infection by upregulating three genes involved in a DNA modification system, with this being observed already 5 min after infection in exponential cells and later in stationary cells. In stationary cells, the number of upregulated genes increased from 29 at 5 min-post infection to 894 at 15 min, and 1319 at 30 min. Functional analysis showed that genes involved in translation and RNA metabolic and biosynthetic processes were significantly enriched. These results are promising since phages like SEP1 could be more effective in the treatment of recalcitrant infections. Moreover, the activation of stationary cells feature could be used to resensitize bacteria to antibiotics.

Antibacterial synergy between a phage endolysin and citric acid against the Gram-negative kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*

Hazel Sisson[1,2], Robert Fagerlund[1,2,3,4], Simon Jackson[1,2,3,4], Yves Briers[5], Suzanne Warring[1], Peter Fineran[1,2,3,4]

[1]University of Otago, Dunedin, New Zealand. [2]Bioprotection Aotearoa, Dunedin, New Zealand. [3]Genetics Otago, Dunedin, New Zealand. [4]Maurice Wilkins Centre for Molecular Biodiscovery, Dunedin, New Zealand. [5]Ghent University, Gent, Belgium

Abstract

Horticultural diseases caused by bacterial pathogens provide an obstacle to crop production globally. Management of the infection of kiwifruit by the Gram-negative phytopathogen Pseudomonas syringae pv. actinidiae (Psa) currently includes copper and antibiotics. However, emergence of bacterial resistance and a changing regulatory landscape are providing the impetus to develop environmentally sustainable antimicrobials. One potential strategy is the use of bacteriophage endolysins, which degrade peptidoglycan during normal phage replication, causing cell lysis and the release of new viral progeny. Exogenous use of endolysins as antimicrobials is impaired by the outer membrane of Gram-negative bacteria that provides an impermeable barrier and prevents endolysins from accessing their target peptidoglycan. Here, we describe the synergy between citric acid and a phage endolysin, which results in a reduction of viable Psa below detection. We show that citric acid drives destabilisation of the outer membrane via acidification and sequestration of divalent cations from the lipopolysaccharide, which is followed by degradation of the peptidoglycan by the endolysin. Scanning electron microscopy revealed clear morphological differences, indicating cell lysis following the endolysin-citric acid treatment. These results show the potential for citric acid - endolysin combinations as a possible antimicrobial approach in agricultural applications.

Protein-protein interactions at the interface of serine integrases and their recombination directionality factors

Abdulrazak Alsaleh[1], Alexandria Holland ORCID iD[1], Oluwateniola Taiwo-Aiyerin[1], Tania Reyes[2], Ying Pigli[2], Heewhan Shin[2], Phoebe Rice[2], Femi Olorunniji ORCID iD[1]

[1]Liverpool John Moores University, Liverpool, United Kingdom. [2]Univeristy of Chicago, Chicago, USA

Abstract

Large serine integrases (LSIs) are bacteriophage derived DNA recombinases capable of recombining DNA at specific attachment sites on phage DNA (attP) and bacterial host genome (attB), known as PxB recombination. The resulting DNA sites after recombination has taken place are known as attL and attR. LSIs are highly specific, directional and have no requirement of host machinery or additional factors to carry out recombination. The reverse reaction, excising viral DNA from the host genome, requires an additional protein molecule known as the recombination directionality factor (RDF). In the presence of an LSIs RDF, the reverse reaction between attL and attR takes place excising the viral DNA and resulting in attP and attB being reformed. As a consequence of this capability, specificity and convenience, LSIs are receiving much attention as molecular tools for synthetic biology. However, little is understood about the interaction of LSIs and their RDFs, and for thousands of known integrases there are only a handful of identified RDFs. Using structure prediction tools, we have modelled this interaction between an LSI and it's RDF and narrowed the interface to a few key residues. Then, experimental analysis using mutational and biochemical assays confirmed these findings, elucidating for the first time the location and key residues involved in LSI and RDF communication. The findings provide unexpected insights into serine integrase mechanism, and simplifies their development into more versatile tools for synthetic biology applications.

Isolation and characterization of bacteriophages infecting *Serratia marcescens* as a novel treatment for antibiotic resistant infections

Mia Horton, Lesley Hoyles ORCID iD, Yashwani Malik, David Negus ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Serratia marcescens has been responsible for a notable increase in multidrug resistant infections in healthcare and community settings. In the UK, the independent emergence of multidrug resistant clones suggests that future treatment of *S. marcescens* with antibiotics will become increasingly difficult. We aimed to isolate lytic bacteriophages specific to *S. marcescens* and to identify encoded depolymerases. Such depolymerases could be used as anti-virulence agents and alternatives to antibiotics. Phages were isolated from river water sampled from around Nottingham using three clinical strains of *Serratia marcescens* as hosts. 11 morphologically distinct plaques from different water sources were picked to purity. Genomic DNA was purified and sequenced in-house using the Illumina MiSeq. Genome assembly and comparative genomics revealed four genetically distinct phages; 1a, 6a, 10b1 and 12. Host range analysis of the four phages was performed using a range of 15 clinically relevant *Serratia* strains. Phage morphology was visualised by transmission electron microscopy. Three of the phages displayed halos on host lawns indicative of capsule-degrading enzymes. Four candidate depolymerase genes were identified using the machine learning depolymerase prediction tools PhageDPO and DePP. Candidate depolymerase genes were cloned and expressed within *E. coli.* A halo on spot tests indicated the soluble fraction of the crude lysate containing the expressed minor tail protein from phage 12 possessed potential depolymerase activity. This research contributes to the limited range of *S. marcescens* phage genomes published. Furthermore, the identified depolymerases have the potential to be used therapeutically to mitigate the effects of multidrug resistance in *S. marcescens.*

Understanding the Contribution of Prophages to *S. uberis* Pathogenicity

Thomas Smith-Zaitlik ORCID iD, Logan Newstead, David Gally, Gavin Paterson

University of Edinburgh, Edinburgh, United Kingdom

Abstract

We are elucidating the relationships between *Streptococcus uberis* and its prophage content. *S. uberis* is a Gram-stain positive coccus and a major cause of bovine mastitis. In the UK alone, bovine mastitis (inflammation of the mammary gland, usually caused by infection) is estimated to cost the beef and dairy industry up to £450 million annually. Bacteriophages are viruses which can infect and kill bacteria. While lytic bacteriophages are promising antimicrobials, it is also important to understand the role of prophages in bacterial diversity and pathogenicity.

We analysed 661 *S. uberis* genomes from the UK, New Zealand, USA, Italy, Bangladesh, and Switzerland. Of these, 345 were sequenced by our laboratory and 316 were publicly available. Using PhiSpy we identified 1,054 prophage regions in this dataset. Prokka annotated 34,835 genes, 55 % of which were annotated as hypothetical proteins. A wide variety of metabolic and virulence-associated genes were identified, as well as 735 bacteriophage lysins. PADLOC and ABRicate were used to identify numerous anti-bacteriophage and antimicrobial resistance mechanisms. Analysis of host strain metadata and prophage gene content using Scoary highlighted the presence of antimicrobial resistance genes carried by the prophages of UK *S. uberis* isolates. This highlights the contribution of prophages to the fitness and pathogenicity of *S. uberis*. Understanding the prophage content of *S. uberis* is important to developing effective phage therapeutics to treat bovine mastitis infections.

Using Bacterial Impedance to Test Phage Susceptibility for Fast Infection Profiling

Libby Duignan[1], Jo Fothergill[1], Rachel Floyd[1], Lucy Bock[2], Bethany Martin[2], Charlotte Hind[2], Mark Sutton[2]

[1]University of Liverpool, Liverpool, United Kingdom. [2]UK Health Security Agency, Salisbury, United Kingdom

Abstract

Phage therapy is an alternative treatment for bacterial infections which is rapidly gaining interest through compassionate use cases and clinical trials; phage therapy is already widely used in Eastern Europe. This suggests that phage therapy will become a treatment used in the UK, as a frontline alternative to certain antibiotics and to treat highly drug resistant infections. The current gold standard methods used to test the susceptibility of a bacterial strain to a phage are time consuming usually taking a minimum of 12-24hrs, similar to antibiotic susceptibility tests (ASTs). Faster methods assess the antimicrobial susceptibility of bacteria, to allow informed prescription are needed, and this applies to phages also. This is especially important with phages being very specific to their host bacteria, so it is important to know the infection profile before treatment.

This study demonstrates how a new bacterial impedance-based test known as the i-FAST, originally designed for ASTs, can also be used to rapidly test for phage susceptibility, producing data within 1-2 hours. Clinical *E. coli* isolates from UTI patients were tested using the i-FAST system, which measures the single bacteria at high speed and distinguishes between susceptible and resistant strains, based on changes in the electrical phenotype after exposure to a certain phage. The susceptibility results correlated with results from plaque assays for the same combination of bacteria and phage. This system would be extremely beneficial in hospital settings allowing fast, informed prescription of either antibiotics and/or phage, and enabling rapid matching of treatment to infection.

The complicated relationship between phage resistance and antibiotic susceptibility in *S. aureus*

Janine Bowring ORCID iD, Roshni Haider, Hanne Ingmer ORCID iD

University of Copenhagen, Copenhagen, Denmark

Abstract

Combination or sequential treatment using lytic bacteriophages and antibiotics is a promising strategy against antibiotic resistant bacteria. The use of phages can instigate an evolutionary trade off, in which bacterial antibiotic susceptibility can be increased. In *Staphylococcus aureus* the wall teichoic acid of the cell wall functions as the main phage receptor, and wall-related mutations have been identified in phage-resistant strains. Thus, phage-resistance might come at the cost of changed susceptibility to wall-targeting antibiotics. To investigate, we evolved the *S. aureus* USA300 strain JE2 against different lytic staphylococcal phages and assessed the impact of phage-resistance on susceptibility to wall-targeting antibiotics. We found that while the majority of phage-resistant clones were more susceptible to a range of wall-targeting antibiotics, a small subset showed increased resistance to these antibiotics. In some cases, these divergent changes in susceptibility correlated with mutations in genes relating to cell wall synthesis, while in other cases mutations were solely linked to cytoplasmic processes. Additionally, most phage-resistant clones exhibited increased delta-hemolysis, although there was no common mutation to explain this. Our results indicate varied paths to phage-resistance and divergent effects of phage resistance on antibiotic susceptibility and other phenotypes. The complexity of phenotypic changes implicates potential expression- or epigenetic-based alterations outside of the detected mutations, which should be further explored in future work.

Challenges to phage interventions for cattle colonised with *Escherichia coli* O157:H7

David Gally ORCID iD[1], Asim Ullah[1], Stephen Fitzgerald[2], Marianne Keith[1], Sean McAteer[1], Alison Low[1]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Moredun Research Institute, Edinburgh, United Kingdom

Abstract

E. coli O157 is a zoonotic pathogen that colonises the terminal rectum of cattle and poses a risk to human health with disease ranging from mild diarrhoea to life-threatening systemic infection. Several control interventions have been trialled in cattle including treatment with phage but to date these have had limited success. We have taken the approach of trying to understand phage predation and bacterial resistance under conditions more representative of those encountered in the host, specifically while *E. coli* O157 is intimately attached to bovine epithelial cells via the activity of its type 3 secretion system. Our work shows that escape populations have multiple strategies to evade phage infection, some permanent and others transient, helping inform the composition of phage cocktails. Interplay between surface polysaccharides; LPS, colanic acid and group 4 capsule contribute to phage evasion and so phage targeting each of these moieties as well as protein receptors are required to predate the major sub-populations at the terminal rectum. Our on-going research is aimed at understanding the dynamics and genetics of transient resistance as well as training additional phage for cocktails. The most effective combinations will be tested on *ex vivo* samples from colonised cattle and the most effective trialled in cattle colonised with *E. coli* O157.

Engineering third-generation lysins as enzybiotics for *Acinetobacter baumannii*-infected burn wounds

Vincent De Maesschalck ORCID iD[1], Sybren Van Ginneken ORCID iD[1], Greetje Vande Velde ORCID iD[1], Jan Paeshuyse ORCID iD[1], Yves Briers ORCID iD[2], Rob Lavigne ORCID iD[1]

[1]KU Leuven, Leuven, Belgium. [2]Ghent University, Ghent, Belgium

Abstract

The World Health Organization (WHO) estimates 11 million burn injuries occur annually. For these complicated injuries to heal properly, a delicate balance between a pro- and anti-inflammatory response is required, making them challenging to treat. This delicate balance is frequently disrupted by opportunistic pathogens such as *Acinetobacter baumannii*¸ and can lead to life-threatening infections. In 2015, over 50% of *A. baumannii* isolates in both Europe (56%) and the USA (45-65%) were reported to be carbapenem-resistant. Therefore, the WHO highlighted in 2017 carbapenem-resistant *A. baumannii* as a top priority for the discovery and development of new antibiotics. Bacteriophages have been engaging in this evolutionary arms race for ages and hence form a promising reservoir for new antibacterial strategies of which endolysins have been explored the most. However, several challenges in lysin engineering remain. Therefore, it is interesting to tailor the lysin to address challenges in its final clinical application. We further engineered a potent *A. baumannii* lysin, 1D10, to enhance its performance in burn wounds. Using the VersaTile platform, a library of fusions was made between anti-inflammatory peptides and 1D10. Screening identified three lead variants, which were characterized for their antibacterial properties and structural integrity. The lead variant, BZAb1, achieved a 4.2 ± 0.21 log killing against *A. baumannii* NCTC13423 in PBS at 1x MIC in only 30 minutes. Furthermore, this lead lysin was characterized in a bioluminescent *ex vivo* burn wound model, and a proof-of-concept for its potential anti-inflammatory activity was delivered by demonstrating *in vitro* LPS-binding.

Improving Wastewater Treatment in Low-Middle Income Countries Through Bacteriophage Bioremediation.

Hannah V. Pye[1], Jeroen Wagemans[2], Goncalo Macedo[3], Lucia Galben[4], Oana-Simina Iaconi[5], Greta Balan[5], Mihail Todiras[5], Alina Ferdohleb[5], Heike Schmitt[3], David G. Weissbrodt[6], Rob Lavigne[2], Evelien M. Adriaenssens[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Laboratory of Gene Technology, KU Leuven, Leuven, Belgium. [3]Dept. Biotechnology, Delft University of Technology, Delft, Netherlands. [4]Timofei Mosnega Republican Clinical Hospital, Chișinău, Moldova, Republic of. [5]Nicolae Testemitanu State University of Medicine and Pharmacy, Chișinău, Moldova, Republic of. [6]Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway

Abstract

Wastewater is a reservoir for antimicrobial-resistant bacteria (ARB), including the ESKAPE pathogens, and antimicrobial resistance gene (ARG) accumulation and dissemination. Traditional wastewater treatment plants (WWTPs) reduce overall bacterial load, however, ARBs, ARGs, and antibiotic residues are still detected in resulting effluent. Constructed wetlands are a more economical and sustainable alternative to WWTPs, especially in low-middle income countries such as Moldova, where the incidence of deaths associated with antimicrobial resistance is high. Bacteriophages (phages) are predators of bacteria with the ability to lyse bacterial cells and are promising alternatives to antibiotics. Phages have the potential to be implemented as a novel intervention strategy to prevent the spread of antibiotic resistance from wastewater to surface water, by combining their specificity to kill bacteria with constructed wetland technology to eradicate ARBs from wetlands. More than 90 phages targeting ESKAPE pathogens were isolated from wastewater and were genotypically and phenotypically characterised. Host range was assessed against a panel of patient derived ESKAPE isolates from the Timofei Moșneaga Republican Clinical Hospital in Moldova. A subset of polyvalent phages were identified as low-temperature and UV-irradiation tolerant and were combined into a phage cocktail for use in microcosm experiments emulating constructed wetland conditions. The efficacy in removing multidrug-resistant pathogens from wastewater using the phage-wetland technology is being evaluated in a pilot-scale constructed wetland, alongside the effect of these phages on the flora and fauna inhabiting the natural wetlands. This alternative technology will be particularly valuable in low-middle income countries, where WWTPs are expensive to establish and resource-demanding.

Multi-omics analysis of a patient with hepatitis following adeno-associated virus gene therapy

Sarah Buddle ORCID iD[1], Oscar Enrique Torres Montaguth[1], Li-An Kiri Brown[1], Riccardo Zennezini Chiozzi[2], Mahdi Moradi Marjaneh[3,4], Laura Atkinson[5], Nathaniel Storey[5], John Counsell[6], Simon Waddington[7,8], Hannah Macpherson[9], Jasmaine Lee[9], Michael Levin[3], Julianne Brown[10], Sofia Morfopoulou[1,3], Judith Breuer[1,5]

[1]Infection, Immunity and Inflammation Department, Great Ormond Street Institute of Child Health, University College London, London, United Kingdom. [2]University College London Mass Spectrometry Science Technology Platform, Division of Biosciences, University College London, London, United Kingdom. [3]Section for Paediatrics, Department of Infectious Diseases, Faculty of Medicine, Imperial College London, London, United Kingdom. [4]Section of Virology, Department of Infectious Diseases, Faculty of Medicine, Imperial College London, London, United Kingdom. [5]Department of Microbiology, Virology and Infection Control, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom. [6]Research Department of Targeted Intervention, Division of Surgery and Interventional Science, University College London, London, United Kingdom. [7]Gene Transfer Technology Group, EGA-Institute for Women's Health, University College London, London, United Kingdom. [8]Medical Research Council Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witswatersrand, Johannesburg, South Africa. [9]Department of Neurodegenerative Disease, Queen Square Institute of Neurology, University College London, London, United Kingdom. [10]Department of Microbiology, Virology and Infection Control, Great Ormond Street Hospital for Children NHS Foundation Trus, London, United Kingdom

Abstract

Adeno-associated viruses (AAVs) are important vectors in gene therapy, yet the occurrence of hepatitis remains a significant complication associated with this treatment. Despite its prevalence, the underlying mechanism of this adverse effect remains poorly understood, posing challenges for effective prevention and intervention. The identification of adeno-associated virus 2 (AAV2) as a potential culprit in the 2022 outbreak of unexplained hepatitis in children raises intriguing parallels, hinting at a shared mechanism in these forms of AAV-related hepatitis.

We present a detailed case analysis of a patient who developed hepatitis after an AAV-based gene therapy treatment. Initially, we conducted long and short read metagenomic sequencing of a liver biopsy from the patient. We found evidence of multiple gene therapy plasmids, characterized by elevated levels of complex structures and concatemerization. Notably, we also identified the presence of human betaherpesvirus 6B in the liver sample, a finding associated with the 2022 paediatric hepatitis outbreak. Additionally, we employed proteomic techniques to investigate the presence of viral and plasmid-related proteins. Transcriptomic and proteomic analyses of host responses unveiled multiple immune pathways implicated in the hepatitis. Comparison with liver samples obtained during the 2022 paediatric hepatitis outbreak suggested some similarities between the two forms of hepatitis. This investigation provides insights into the mechanisms underpinning hepatitis following AAV gene therapy, paving the way for improved therapeutic strategies and mitigation of adverse effects.

Predictive phage therapy for *Escherichia coli* urinary tract infections: cocktail selection for therapy based on machine learning models

Alison Low ORCID iD[1], Marianne Keith[1], Alba Park de la Torriente[1], Antonia Chalka[1], Sean McAteer[1], Gavin Paterson[1,2], David Gally[1]

[1]Roslin Institute, Edinburgh, United Kingdom. [2]R(D)SVS, University of Edinburgh, Edinburgh, United Kingdom

Abstract

We have developed a pipeline for predictive bacteriophage (phage) therapy enabling the selection of phage to treat a bacterial infection using machine learning models (MLM). MLMs were trained with a database of interactions between a panel of phage and sequenced bacterial isolates. This helps to overcome the hurdle of quickly being able to identify phage(s) to treat specific infections. For this proof of concept study we focused on *Escherichia coli* (*E. coli*) associated with urinary tract infections, a common infection in humans and companion animals from which multi-drug resistant (MDR) bloodstream infections can originate. The activity of 31 phage were measured on 314 isolates with growth curves in artificial urine, a media reflective of the *in vivo* conditions where the phage ultimately need to be active. Random Forest models were built for each phage from bacterial genome features and the more generalist phage, acting on over 20% of the bacterial population, exhibited F1 scores of >0.6 and could be used to predict active. We compared our best general phage cocktail and two bespoke cocktails selected using observed or predicted data, against previously untested strains. The bespoke cocktails performed better than the general cocktail with the predicted cocktail performing similarly to the cocktail designed from observed data. The study demonstrates the potential of predictive models which integrate bacterial genomics with phage activity datasets allowing their use on bacterial sequence data directly derived from clinical samples to inform rapid and effective phage therapy.

A unique immunotherapy combining uv-inactivated reovirus and the targeted agent sorafenib for the treatment of hepatocellular carcinoma

Russell Hughes, Adel Samson, Amy Moran, Stephen Griffin

University of Leeds, Leeds, United Kingdom

Abstract

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and has a 5-year survival rate <20%. Until recently, treatment options for patients with advanced HCC were limited to such targeted therapies as sorafenib, which has significant toxicity and limited survival benefit. Therefore, new treatments are needed that improve survival outcomes for HCC patients and immunotherapies are of growing interest.

Reovirus has been studied predominantly as an oncolytic agent but has potent immune-stimulating properties, highlight its potential as an immunotherapy. Reovirus-induced immune activation occurs independent of viral replication and *uv*-inactivated reovirus elicited a more robust interferon response from HCC cells compared to live virus. Therefore, we hypothesised that *uv*-reo could induce anti-tumour immunity and improve survival outcomes in HCC.

In vivo, we found that *uv*-reo/sorafenib therapy extended the survival of mice bearing HCC tumours. This combination therapy significantly increased the abundance of $CD4^+$ T_H1-cells, but not $CD8^+$ CTLs, and elevated expression of T_H1-associated chemokines/cytokines, including CCL5, IFNG and TNFA, alongside the immunomodulatory cytokine IFNB.

T_H1-mediated suppression of HCCs required a complex antigen-independent mechanism that began with the release of TNFA by T_H1-cells, to which HCCs were further sensitised by both IFNG and sorafenib. The crucial element of this response involved IFNB-induced upregulation and secretion of granzyme-B by T_H1-cells leading to the perforin-independent, caspase-dependent killing of HCCs.

Taken together, these data illustrate for the first time how an inactivated reovirus can be used to improve the efficacy of the targeted agent sorafenib in the treatment of HCC.

Staphylococcus aureus adaptation to vancomycin drives changes in phage susceptibility

Jack Abrahamsson[1], Esther Lehmann ORCID iD[1], Anaëlle Fait[2,1], Hanne Ingmer[1]

[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark. [2]Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland

Abstract

The rise in hard-to-treat infections caused by antibiotic resistant bacteria is one of the major health challenges of our century. Bacteriophages are increasingly being considered as alternative therapeutics. As for antibiotic resistance, the potential of bacteria to develop resistance to phages has been widely acknowledged. However, the possible drivers for phage resistance in patients, rather than under laboratory conditions, remain severely understudied. As phages are currently only used on a case-by-case basis for particularly severe infections after all standard clinical procedures have failed, we studied how exposure to cell wall targeting antibiotics may impact phage susceptibility through alteration of key cell wall structures recognized by phages. To this end, we evolved vancomycin-intermediate isolates of the highly endemic *Staphylococcus aureus* USA300 lineage (VISA). These isolates were genetically, transcriptionally and phenotypically characterized. Our results showed epistasis as an underlying cause for altered phage susceptibility, i.e. interplay between genetic mutations favoring antibiotic resistance that indirectly also affect phage susceptibility. Changes in gene expression of key transcriptional regulators can be driven by treatment with cell wall targeting antibiotics, providing a link between antibiotic- and phage resistance. This has implications for future phage therapy as well as for the potential of combination therapy with phages and antibiotics.

Understanding Phenotypes in the Omics Era

Invited talk: Functional genomics of *Pseudomonas aeruginosa*

Susanne Häussler ORCID iD

Helmholtz Centre for Infection Research, Braunschweig, Germany

Abstract

Amidst the escalating numbers of infections attributed to multi-drug resistant bacterial pathogens, we are working on a comprehensive genome sequencing project targeting bacterial isolates of selected problematic pathogens - namely, *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae.* The resultant sequence data will be aligned with antibiotic resistance data using traditional culture-based methodologies in medical microbiology, alongside an examination of virulence-associated traits. Our overarching vision is that Whole Genome Sequencing (WGS) of bacterial pathogens will provide detailed insights not only into the bacterial resistance profile, but also shed light on pathogenicity potential and the phylogenetic relatedness of nosocomial pathogens. This, in turn, holds the promise of serving as the foundation for a more precisely targeted treatment approach and the effective implementation of infection control measures. To fulfil this vision, the WGS data will be harnessed to achieve the following objectives: i) establish a pan-genomic database encompassing all conceivable sequence variations within a bacterial species. This forms a crucial prerequisite for the robust and automated extraction of sequence information, ii) correlate phenotypically determined resistance with molecular resistance markers, with the ultimate aim of genotypically predicting resistance, and iii) develop a user-friendly tool for visualizing the outcomes of genotyping, facilitating a clearer and more accessible understanding of the results. In essence, our undertaking seeks to harness the power of WGS to not only comprehensively document bacterial genetic variations but also to enhance our ability to predict and combat antibiotic resistance, all while advancing our understanding of the intricacies of nosocomial pathogen dynamics.

Invited talk: Genetic modulators of N. gonorrhoeae drug resistance and physiology

Yonatan Grad

Harvard T.H. Chan School of Public Health, Boston, USA

Abstract

The evolution of the obligate human pathogen *Neisseria gonorrhoeae* has been shaped by selective pressures from diverse host niche environments and antibiotics. Here, we used a combination of molecular microbiology and population genomics to investigate two observations. First, the varying prevalence of antibiotic resistance across *N. gonorrhoeae* lineages suggests that underlying metabolic differences may influence the likelihood of acquisition of specific resistance mutations. We hypothesized that the requirement for supplemental CO_2, present in approximately half of isolates, reflects one such example of metabolic variation. We showed that CO_2 dependence is attributable to a single substitution in a *β*-carbonic anhydrase, CanB. CanB^{19E} is necessary and sufficient for growth in the absence of CO_2, and the hypomorphic CanB^{19G} variant confers CO_2 dependence. Furthermore, ciprofloxacin resistance is correlated with CanB^{19G} in clinical isolates, and the presence of CanB^{19G} increases the likelihood of acquisition of ciprofloxacin resistance. Second, in contrast with the trend of increasing antibiotic resistance, clinical isolates that have reverted to susceptibility regularly appear, prompting questions about which pressures compete with antibiotics to shape gonococcal evolution. We showed that mutations in the efflux pump *mtrCDE* operon increased antibiotic susceptibility and demonstrated that these mutations are overrepresented in cervical relative to urethral isolates. Overall, our findings highlight the impact of integrating microbial population genomics with host metadata and molecular microbiology in defining the patterns of adaptation to selective pressures.

Invited talk: Finding chinks in the armour: high throughput genetic screens to identify therapeutic targets in bacteria.

Ian Henderson

University of Queensland, Brisbane, Australia

Abstract

Genetic screens are a key tool for linking phenotype and genotype. Transposon mutagenesis was one of the first genetic methodologies to associate genetic loci with phenotypes. The advent of next-generation sequencing transformed the use of this technique allowing rapid interrogation of whole genomes for genes that correlate with phenotype. Here we describe the use of transposon directed insertion-site sequencing (TraDIS), the latest developments in the technique, and how it reveals new information about cell envelope biogenesis and antibiotic resistance.

Invited talk: Stepwise pathogenic evolution of bacteria

Andres Floto

Heart and Lung Research Institute, Cambridge, United Kingdom

Abstract

I will describe how opportunistic bacteria evolve into specialised lung pathogens through discrete steps revealed through population genomics, transcriptomics, and multi-dimensional phenotyping. I will use *Mycobacterium abscessus* and *Pseudomonas aeruginosa* as case studies.

Invited talk: Using genomics to identify novel aspects to the pathogenicity of *Staphylococcus aureus*.

Ruth Massey

UCC, Cork, Ireland

Abstract

Bloodstream infections are a global public health concern with mortality rates as high as 30%. The bacterium *Staphylococcus aureus* is one of the leading causes of fatal bloodstream infection, causing ~290,000 deaths per year globally, with antimicrobial resistant (AMR) strains, such as methicillin resistant *S. aureus* (MRSA), responsible for the highest number of AMR attributable deaths in high income countries. Since mandatory reporting of *S. aureus* bacteraemia (SAB) began in 2011 there has been a 30% increase, largely attributed to methicillin sensitive *S. aureus* (MSSA), the reasons for which are unknown. Given that morbidity and mortality associated with MSSA bacteraemia are comparable to MRSA bacteraemia, this is a grave concern. To address this problem, we have developed a multi-disciplinary, genomics based approach and applied this to collections of SAB isolates. In doing so we have identified previously unknown aspects to the pathogenicity of *S. aureus* and here two of these, namely the MspA and the TcaA proteins, and the role they play in the development of SAB will be presented.

Bacterial H-NS is a transposon capture protein that drives phenotypic diversity in bacterial populations

Charles Cooper, Simon Legood, James Haycocks, Rachel Hoare, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

We present a new mechanism, common to many bacteria, that controls genome-wide transposition and associated phenotypic change. Interest in transposons is resurgent because they create diversity in bacterial populations. The bacterium *Acinetobacter baumannii* is a fascinating case study for the topic. The microbe tops the World Health Organisation's list of "priority pathogens", thrives in hospitals, resists most antibiotics, and frequently rearranges its genome. In the latter respect, transposons are a key driver of genome plasticity. How this relates to the organism's success is unknown. When culturing the bacterium, we found occasional atypical colonies. We refer to these as "grey" derivatives. The variant has altered motility, ability to form biofilms, acquire exogenous DNA and interact with the human immune system. Intrigued, we sought the genetic basis by whole genome sequencing. We discovered a transposon, Insertion Sequence *A. baumannii* 13 (ISAba13) at a new site in grey variants. This site, the K-locus, encodes genes important for cell surface biology. By tracking the movement of IS*Aba*13 through entire *A. buamannii* populations, we identified a new function for the histone-like nucleoid structuring (H-NS) protein: capture of transposable DNA elements. We show that H-NS captures transposons using a DNA bridging mechanism and is almost entirely responsible for determining patterns of transposition in many bacteria. Our findings have major implications for understanding interactions between bacterial chromosomes, the different types of mobile genetic element they contain, and resulting phenotypic diversity.

Determinants of the spread of plasmid-mediated beta-lactamase resistance in *N. gonorrhoeae*

Tabea Elsener ORCID iD, Anna Cehovin, Connor Philp, Keith Jolley, Martin Maiden, Christoph Tang

University of Oxford, Oxford, United Kingdom

Abstract

Neisseria gonorrhoeae (gonococcus) has acquired resistance to most available antibiotics. The gonococcal beta-lactamase plasmid p*bla* encodes the TEM beta-lactamase, which could become an extended-spectrum beta-lactamase, rendering the last recommended treatment ineffective. p*bla* is mobilisable by the gonococcal conjugative plasmid pConj, enabling the spread of beta-lactamase-mediated resistance.

Several p*bla* variants have been reported, which can differ in the TEM allele they carry and their ability to be spread by pConj. However, large-scale analyses of the distribution of p*bla* variants are lacking. Previously, we implemented the p*bla* typing scheme, Ng_p*bla*ST, on the PubMLST database to assess the spread of p*bla* variants across 15 532 gonococcal isolates. This revealed distinct associations of three major p*bla* variants with TEM alleles, gonococcal lineages, and variants of pConj.

To gain insights into the molecular basis of plasmid distribution, we investigated the mobilisation of p*bla* and its interplay with pConj. We observed specific mobilisation patterns of p*bla* variants due to characteristic deletions. Furthermore, pConj variants differ in their ability to mobilise p*bla*, consistent with the co-occurrence of certain plasmid variants. We identified interactions between the plasmid backbone, TEM allele and strain background, leading to different resistance levels of p*bla* variants and plasmid-carrying strains. This suggests antibiotic selection pressure could be responsible for the success of particular p*bla*-carrying lineages.

Linking the molecular epidemiology of p*bla* variants with their ability to be transferred and confer resistance should provide insights into the spread and co-existence of certain plasmid variants and help predict the emergence and success of gonococcal resistance plasmids.

Mutations associated with adaptation to chlorhexidine are prevalent in clinical *Proteus mirabilis* isolates, and indicate reduced susceptibility to other biocides used in infection control procedures

Vicky Bennett[1], Ocean Clarke[2,1], Anthony Slate[3,1], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]University of Glasgow, Glasgow, United Kingdom

Abstract

Infection control in healthcare settings relies on biocide efficacy; however many nosocomial pathogens adapt to these agents and tolerate concentrations far higher than those used in many products. Using a combined approach of adaptive evolution studies in conjunction with genomic, transcriptomic, and phenotypic analysis, we have explored mechanisms through which *Proteus mirabilis* adapts to chlorhexidine (CHD), the most widely used biocide.

Population screening of 78 clinical isolates demonstrated that many survive at CHD concentrations of >512µg/mL, with genomic analysis showing mutations that occur after CHD adaptation in the laboratory were enriched in highly tolerant clinical isolates. Additional screening of 500 whole-genome sequences demonstrated these mutations arise in high-prevalence in real-world settings. These included truncation in the *smvR* repressor, which results in over expression of the SmvA efflux pump. Adaptation associated changes were also significantly associated with high tolerance to other clinically used biocides such as octenidine.

Transcriptomic analysis of CHD adapted populations substantiated these findings, revealing constitutively overexpressed *smvA*, and upregulation of genes involved in stress responses. Chemotaxis and motility pathways were predominantly downregulated following CHD adaptation. Phenotypic analysis confirmed CHD induced changes in motility predicted by transcriptomic analysis and demonstrated that initiation of swarming was delayed in the presence of CHD in adapted populations. Conversely, they displayed enhanced swimming migration consistent with reduced expression of chemotaxis related genes.

These findings demonstrate how integrating omics datasets with molecular and phenotypic analysis can advance understanding of phenotype behaviour patterns, and the evolution of tolerance to antimicrobial agents in clinically relevant pathogens.

Functional genomics reveals mechanisms of dairy niche adaptation by *Staphylococcus aureus*

Amy Pickering ORCID iD, Jamie Gorzynski ORCID iD, Grace Taylor-Joyce, Gonzalo Yebra ORCID iD, Ross Fitzgerald ORCID iD

Roslin Institute, Edinburgh, United Kingdom

Abstract

Staphylococcus aureus is a leading cause of bovine mastitis, a disease with major welfare and economic impacts to the global dairy industry. We have discovered that when cultured in bovine milk, most *S. aureus* isolates from bovine-associated lineages mediate clotting, produce robust milk-associated biofilms, and exhibit enhanced growth compared to *S. aureus* isolates from human infection. To investigate the genetic basis of these bovine adaptive phenotypes, we employed a population genomic approach that indicated convergent evolution in different lineages. Furthermore, transcriptomic analysis revealed that bovine strains exhibited increased expression of genes for lactose utilisation, amino acid biosynthesis and extracellular proteases in comparison to human strains, which exhibit increased urea metabolism. Using molecular microbiology and functional analysis, we have discovered that upregulation of aureolysin expression is required for milk clotting by bovine *S. aureus* which leads to enhanced growth and biofilm formation in milk. Conversely, the overexpression of proteases in human-adapted strains is sufficient to promote milk clotting but does not confer an enhanced growth phenotype suggesting additional metabolic adaptations are required for adaptation to the bovine niche. Taken together, we have utilised multiple omic and functional approaches to dissect key niche-adaptive traits of *S. aureus*. Future work will focus on understanding if this knowledge can be exploited therapeutically.

Identifying a regulatory switch for the Type VI secretion system linked to clinical adaptation across *Serratia marcescens*

David Williams ORCID iD[1], Giuseppina Mariano[1], Ruth Hernandez[1], Katharine Mathers[1], Grant Buchanan[1], Alexandra Hawkins[1], Barnaby Stonier[1], Nicholas Thomson[2], Matthew Holden[3], Sarah Coulthurst[1]

[1]School of Life Sciences, University of Dundee, Dundee, United Kingdom. [2]Parasites and Microbes, Wellcome Sanger Institute, Hinxton, United Kingdom. [3]School of Medicine, University of St. Andrews, St. Andrews, United Kingdom

Abstract

Serratia marcescens is separated into multiple lineages associated with different environmental niches, including clinically-associated lineages with rising levels of multi-drug resistance. The insect pathogen S. marcescens Db10 has been used as a model to study the Type VI Secretion System (T6SS), which it uses as a potent weapon to inhibit the growth of rival Gram-negative bacteria and microbial fungi in microbial communities. However, the importance of this anti-microbial behaviour for clinical success of S. marcescens, particularly in the context of polymicrobial infection, is not yet known. Here, we have adopted a multidisciplinary approach, using a combination of next-generation sequencing, genomics, genetics, in vivo phenotypic assays, and transcriptomics to investigate conservation and variation in the T6SS and anti-bacterial activity across clinically-derived S. marcescens.

We show that the Db10-like T6SS is conserved across S. marcescens. However, frequently-observed disruptive mutations in a conserved regulatory locus result in a loss in T6SS firing and resulting anti-bacterial activity, which is restored upon repair to the intact regulator. Mutations in this regulator are enriched compared to neighbouring loci, and are only observed in clinically-derived isolates of S. marcescens. Transcriptomic analyses comparing “regulator-intact” and “regulator-disrupted” isolates reveals that this system involves a pleiotropic regulator promoting motile, aggressive and antibiotic-producing phenotypes, and repressing static, silent and adhesive phenotypes. These findings, subsequently validated in the lab, indicate the presence of selection pressure in clinical environments for a regulated lifestyle switch in S. marcescens. This study also illustrates the power of bi-directional integration of genomics and experimental molecular microbiology.

The (small) data gap in high-throughput functional screening

Emily Goodall ORCID iD, Weine Kok, Freya Hodges, Jerry Yang, Ian Henderson

Institute for Molecular Bioscience, Brisbane, Australia

Abstract

In the last decade there has been a rapid expansion in sequenced bacterial genomes, genome annotations and high-throughput screens to identify gene functions. Many functional screens to link genotype and phenotype hinge upon the underlying reference genome annotation. But does a computed annotation capture the entire coding potential of a single organism?

One class of overlooked genes are small genes, typically <150 nucleotides. Small genes have been identified within most species of bacteria with functions ranging from antibiotic efflux pump regulation to cell-cell communication. Despite their important roles, small proteins are systematically overlooked: in part because of size thresholds for the classification of a gene and in part because they can be challenging to identify both physiologically and computationally. This presents a significant data gap for "whole genome" screens; however, there are few methods available to empirically identify protein coding sequences at scale.

Here, we applied a high-throughput screen that couples a reporter system with transposon mutagenesis to identify protein coding genes on a whole cell scale. We generated a pool of ~1 million mutants in the model organism *Escherichia coli* K-12 and identified >300,000 unique insertions, equivalent to an insertion every 15 bp, or 5 codons. We screened the transposon library for successful translation-fusion events, validating many known protein coding genes in addition to identifying new genes, including small genes and those with non-canonical start codons. Overall, our results suggest that the annotation of even one of the best studied organisms, *E. coli* K-12, is still lacking.

Deciphering AMR phenotype predictions from functional genes: a machine learning approach.

Lucy Dillon ORCID iD[1], Nicholas Dimonaco ORCID iD[1,2], Christopher Creevey ORCID iD[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]McMaster University, Hamilton, Canada

Abstract

Background:

AMR is a global burden that requires a deeper understanding of the relationship between microbial genotype and AMR phenotype. Laboratory-based methods are the gold standard for the identification of AMR phenotype, yet issues exist such as the time required to establish cultures. Computational approaches such as ML have the potential to solve some of these issues and elucidate the role of multi-gene interactions in the resistances observed.

Methods:

To evaluate the role of multi-gene interactions, we applied two machine learning models: decision trees and convolutional neural networks (CNNs) to 5,990 genomes across 19 Genera, with corresponding MIC values. We built 23 antibiotic-specific models to predict the AMR phenotype from functional genes identified from eggNOG-mapper.

Results:

The average accuracy of decision tree models was 92.2%, compared to the CNNs which was 90.5%. However, the precision and recall of the models were higher on average in the CNN models. The results show machine learning techniques can be used to build accurate models to predict AMR phenotype from genotype and highlight the importance of multi-gene interactions and the role of non-AMR associated genes AMR phenotype. These models will be publicly available as part of a web application to predict AMR phenotype and can also be used locally on the command line as a Snakemake workflow. This work aims to be the first step in providing a rapid computational method to support laboratory-based AMR phenotype identification.

Understanding cooperative interactions of antiphage systems in *Pseudomonas aeruginosa*

Aleksei Agapov ORCID iD, Anna Olina, Edze Westra, Stineke van Houte

Environment and Sustainability Institute, Centre for Ecology and Conservation, University of Exeter, Penryn, United Kingdom

Abstract

Prokaryotes have evolved various immune mechanisms to protect against bacteriophages. Beyond restriction-modification and CRISPR-Cas, dozens of novel prokaryotic defence systems (DSs) have been discovered in the past few years, and often multiple DSs are found within individual prokaryotic genomes. However, it is unclear if and how these defences interact with each other. To answer this question, we conducted a large-scale bioinformatic search for the DSs in 300 genomes of clinical isolates of the opportunistic pathogen Pseudomonas aeruginosa. We found that DSs are distributed unevenly across the genomes, and that some DSs co-occur more frequently than expected by chance, suggesting potential cooperation between them. This work aims to explore how DSs combinations affect bacterial fitness in the presence of phages. To directly test the role of DSs in providing protection against phage infection, we created a P. aeruginosa strain lacking all known DSs, to which we can introduce DSs of interest, either individually or in combination. Current work uses a diverse phage collection to test the protection range of those individual and combined DSs. This work paves the way to a comprehensive understanding of interactions between immunity mechanisms in bacteria.

Virus Workshop: Molecular Basis of the Host:pathogen Interaction

How is CFTR required during BK polyomavirus entry in kidney and urothelial cells?

Gemma Swinscoe ORCID iD[1], Margarita-Maria Panou[1], Michelle Antoni[1], Eleni-Anna Loundras[1], Matthew Welberry-Smith[2,1], Simon Baker[3], Andrew Macdonald[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Leeds Teaching Hospital NHS Trust, Leeds, United Kingdom. [3]University of York, York, United Kingdom

Abstract

BK Polyomavirus (BKPyV) is a widespread opportunistic pathogen, and the causative agent in a multitude of diseases both in transplant and immunosuppressed patients. Despite its frequent occurrence, predominantly in renal transplant recipient, many aspects of its life cycle remain under-characterised. In particular, the subject of BKPyV entry into host cells has become a complex discussion of multiple cell-type dependent pathways.

Our previous work investigating viral entry in primary renal proximal tubule epithelial (RPTE) cells, identified Glibenclamide as a potent inhibitor of infection. From this we utilised other ion channel inhibitors to determine that cystic fibrosis transmembrane regulator (CFTR) channel activity is essential during early stages (~2hr post-infection) of BKPyV entry. We have since identified that CFTR function is also critical for JCPyV and SV40 infection, in SVGA and RPTE cells respectively, but not required for BKPyV or SV40 entry into vero cells. This again suggests subtle cell-dependent differences in polyomavirus entry. To explore these subtle differences further, in the context of human physiology, we are beginning to investigate whether CFTR is requirement for BKPyV entry into urothelial cells.

Furthermore, we are beginning to unravel the exact nature of CFTR involvement within polyomavirus entry. This work is being guided by global kinase protein arrays, which identified kinases associated with cAMP signalling are activated during BKPyV entry. Kinases which are also important for the regulation and recycling of CFTR within a normal cellular environment. Gaining this mechanistic detail will further clarify CFTR-dependent polyomavirus entry mechanism in the context of different cellular tropisms.

Feeling the chill: Regulation of influenza A virus mRNA by cold-inducible RBM3.

Hannah Coutts ORCID iD, Stefano Bonazza ORCID iD, Swathi Sukumar ORCID iD, Hannah Turkington, David G. Courtney ORCID iD

Queen's University Belfast, Belfast, United Kingdom

Abstract

Temperature is an important aspect of the normal regulation of cellular processes. In mammals, several genes are characterised as cold-induced, most notably, CIRP and RBM3. The latter is an RNA-binding protein that plays a crucial role in neuroprotection during hypothermic conditions and has been implicated in several aspects of mRNA processing and regulation. Interestingly, the respiratory tract is a unique environment regarding temperature regulation: the lower tract is stable at 37°C, while the upper tract sits constantly at a cooler temperature around 33°C. Adapting to this temperature differential is essential for respiratory viruses. Pathology is generally caused by lower tract replication, while transmission predominantly arises from upper tract replication. Currently, we have a poor understanding of how molecular mechanisms of IAV infection differ between 33°C and 37°C. Unsurprisingly, RBM3 protein levels are found at their highest in nasopharyngeal tissue. Manipulation of RBM3 expression significantly alters IAV replication kinetics, indicating a proviral role for RBM3 during IAV infection. Measuring viral RNA half-life, we have shown that RBM3 extends the half-life of viral NP transcripts, suggesting RBM3 stabilises these mRNAs. These data suggest a positive role for RBM3 in IAV replication in the colder nasopharyngeal tract. Understanding IAV replication in this environment could provide fundamental insights into mechanisms of flu transmission and reassortment.

Structural characterization of the interactions between Herpes Simplex Virus Glycoprotein C and the Complement Protein C3b

Moises Rojas Rechy ORCID iD[1], Gary Cohen[2], Harvey Friedman[2], Juan Fontana[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Pennsylvania, Pennsylvania, USA

Abstract

Herpes simplex virus (HSV) is a ubiquitous virus that can result in oral and ocular inflammation and even in fatal encephalitis (HSV-1), or genital infections (HSV-2). HSV has developed several mechanisms to evade the immune response of the host. One of these mechanisms evades the humoral innate immune response by utilizing the glycoprotein gC, which binds to the complement protein C3b, which is key to the complement response. However, the molecular details of how gC binds to C3b, and the structure of gC remain unknown. To address this, soluble versions of glycoproteins gC1 from HSV-1 and gC2 from HSV-2 were incubated with C3b to create a complex. Complex formation was monitored using mass photometry. Both gC1 and gC2 rapidly formed a 1:1 complex with C3b and these complexes were stable for at least 30 minutes. Subsequently, the interaction of the proteins was visualized by cryo-EM, and we obtained a 3D map of C3b and C3b in a complex with gC2. Comparing both maps will allow us to model how gC2 inhibits the innate immune system by binding the C3b protein. We are also working on crosslinking the complex and purifying it by size exclusion chromatography, to obtain high-resolution three-dimensional cryo-EM maps. On the other hand, we have found that HSV virions incubated with C3b have a reduced attachment to host cells. This has led us to the hypothesis that gC1 and gC2 capture C3b by direct binding, and we are in the process of visualizing this interaction by cryo-electron tomography.

Characterising the activity of the Marek's Disease Virus Virion Host Shutoff Protein.

Sophie Cutts ORCID iD[1,2], Alistair Tweedie[1], Yongxiu Yao[2], Gill Elliott[1]

[1]University of Surrey, Guildford, United Kingdom. [2]The Pirbright Institute, Woking, United Kingdom

Abstract

Marek's Disease Virus (MDV) is a cell-associated alphaherpesvirus that infects chickens, and is within the same subfamily as herpes simplex virus type 1 (HSV- 1). HSV-1 has been shown to alter the mRNA landscape of the cell through the activity of the virion host shutoff (vhs) protein, a well-characterised endoribonuclease involved in degrading mRNA and leading to host translational shutoff. MDV expresses a homologue of vhs (vhsM), but mRNA degradation has not been determined before during MDV infection. Nonetheless, previous work has shown that deletion of the vhsM gene in MDV has little effect on pathogenesis. To develop our understanding of the activity of vhsM, we expressed it in chicken DF-1 fibroblast cells and showed that the poly-A binding protein (PABPC1) was relocalised to the nucleus, a property that correlates with endoribonuclease activity. Co-expression of vhsM with the Gaussia Luciferase (GLuc) reporter protein also demonstrated a reduction in GLuc activity and a reduction in the GLuc mRNA, while single-cell puromycin labelling revealed a block to translation in cells expressing vhsM. Taken together, these data suggest that vhsM is active when expressed by transient transfection and functions in a manner similar to HSV-1 vhs. In order to study the effect of vhsM on the RNA landscape of MDV infected cells, we have now used CRISPR-Cas9 to generate an MDV-ΔvhsM virus in the highly virulent virus strain RB1B. This virus will be used to determine if vhsM functions during infection of primary chicken cells to alter the infected cell transcriptome.

Investigating the role of Vpr in HIV-1 replication

Lucy Ahern ORCID iD, Ross Hall, Megan Burley, Kate Bishop

Francis Crick Institute, London, United Kingdom

Abstract

HIV-1 contains four accessory proteins that function to enhance infection *in vivo*. Whilst three of these proteins have been well-characterised, the role of viral protein R (Vpr) during viral replication is still unknown, although it is conserved in all primate lentiviruses. The most well-documented Vpr-induced phenotype is cell cycle arrest of infected cells in G2. This has been linked to ATR activation, but the mechanistic details of this block are limited and contentious. A paralogue of Vpr found in HIV-2, called Vpx, targets the cellular protein SAMHD1 for degradation by the host proteosome. This results in increased dNTP levels, which facilitates reverse transcription of the HIV-1 genome. HIV-1 Vpr does not target SAMHD1 for degradation but does bind the host Cullin4/DDB1 E3 ubiquitin ligase to target cellular proteins for degradation, resulting in global changes to the host proteome.

We have shown that Vpr expression causes an increase in dNTP levels, via activation of ATR, and that virion-incorporated Vpr is sufficient to elevate dNTP levels during the first 12 hours of infection when reverse transcription takes place, thus providing a potential benefit to replication. We have also conducted a proteomic screen to identify proteins targeted for degradation by Vpr during HIV-1 infection. Gene ontology analysis suggests that chromatin-modifying proteins are significantly downregulated in the presence of Vpr, and we are investigating how this may be linked to cell cycle arrest and HIV replication.

Interferon-stimulated gene expression screening reveals genes with candidate antiviral activity against HCoV-OC43

Emma Davies[1], Arthur Wickenhagen[1], Matthew Turnbull[1], Douglas Stewart[1], Suzannah Rihn[1,2], Massimo Palmarini[1], Adam Fletcher[1], Sam Wilson[1,2]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]Cambridge Institute of Therapeutic Immunology & Infectious Disease, University of Cambridge, Cambridge, United Kingdom

Abstract

The COVID-19 pandemic, in addition to the SARS and MERS outbreaks, highlight the consequences of coronavirus transmission within the human population. The presence and timing of a functional interferon (IFN) response is important in controlling coronavirus infection. Thus, identifying interferon-stimulated genes (ISGs) with antiviral activity can provide insights into genetic risk factors associated with coronavirus disease severity and into the barriers to coronavirus zoonosis. To identify ISGs that inhibit wild-type coronaviruses, we optimised an arrayed ISG expression screening protocol that involves immunostaining of the dsRNA replication intermediate with quantification of virus infection by image cytometry. The endemic coronavirus HCoV-OC43 was screened against a library of >500 human, >300 macaque and >250 bovine genes encoded into lentiviral vectors. This revealed ISGs with known antiviral activity against coronaviruses, including HCoV-OC43, as well as ISGs not previously shown to be involved in HCoV-OC43 infection. The ability of these hits to cause cytotoxicity and/or stimulation of IFN-stimulated response elements was determined to exclude non-specific inhibitors of HCoV-OC43. Further investigation into the specificity and mechanism of action of ISGs is being carried out. Understanding the mechanisms by which ISGs specifically inhibit coronaviruses could provide new information on interactions of both endemic and emerging coronaviruses with the innate immune system and can help inform future therapeutic approaches.

The HSV-1 UL13 protein kinase is required for optimal late gene transcription and counteraction of antiviral responses.

Natasha Preston ORCID iD, Gill Elliott

University of Surrey, Guildford, United Kingdom

Abstract

Phosphorylation by the HSV-1 protein kinase UL13 has been implicated in a number of pathways, including type I interferon responses; activity of the viral endoribonuclease vhs; and modification of the RNA polymerase II large subunit. However, such diverse results could be a consequence of the different virus strains and cell types used in these earlier studies. To reconcile this, we have now characterised an HSV-1 strain 17 UL13 deletion virus (ΔUL13) in interferon-competent human fibroblasts, and have shown that ΔUL13 spreads poorly in these cells, and that interferon b and interferon stimulated gene (ISG) transcripts are increased compared to Wt infection. Nonetheless, interferon upregulation did not correlate with enhanced nuclear translocation of IRF3, suggesting that UL13 acts downstream in the innate virus sensing pathway.

Given the apparent general effect of UL13 on antiviral transcripts, we further measured vhs activity and found that it was reduced in the absence of UL13. However, this reduction in activity reflected reduced vhs protein and transcript. Moreover, despite the vhs transcript being susceptible to degradation, this reduction was not a consequence of enhanced instability but rather of reduced vhs transcription. Intriguingly, despite reduced virus production in ΔUL13-infected cells, genome replication was unaltered. Nonetheless, a subset of additional late transcripts were also shown to be reduced. Hence, we propose that the properties attributed to UL13 are an indirect consequence of a failed transition from early to late virus gene expression resulting in reduced vhs expression amongst others, and a combined deficiency in counteracting antiviral responses.

Haematopoietic landscape dynamics during human cytomegalovirus latency establishment and maintenance.

Ines Hofer ORCID iD[1], Matthew Reeves[2], Nicolae Zabet[1]

[1]Queen Mary University of London, London, United Kingdom. [2]University College London, London, United Kingdom

Abstract

Haematopoiesis involves stringent genetically and epigenetically dictated patterns of gene expression that is only partially understood. Human cytomegalovirus (HCMV) establishes latency in CD34+ haematopoietic progenitors (HPCs) but preferentially persists in the myeloid lineage where it ultimately reactivates. Hypothetically, understanding how HCMV re-programmes CD34+ cells could reveal new insight into myelopoiesis.

Human CD34+ HPCs were infected with HCMV and cultured in homeostatic conditions for a total of 17 days post infection – with infection confirmed by qPCR for viral transcription which persisted throughout the culture period. Cells were harvested at day0 (baseline), day10 and day17 along with uninfected controls. Samples were subjected to single cell multiome (RNAseq and ATACseq) analyses to concomitantly define transcription and epigenetic profiles with the aim of defining virus specific changes in the host transcriptome. Our results show a temporal differentiation trajectory between the sampling points, with 21 cell types annotated, and distinct clusters between myeloid and lymphoid/erythroid populations. We detect viral transcripts in approximately 7% of the cells in infected samples. Most importantly, we identify changes in the different subpopulation of cells in the infected samples compared to uninfected ones. These changes in cell populations are characterised by cell specific gene expression programmes which are linked with distinct epigenetic states. In this work, we are defining virally induced changes in CD34+ HPCs with a view to identifying the specific changes required for myelopoiesis. Overall, our results add to the growing understanding of how HCMV manipulates HPC identity and illuminate the mechanisms governing haematopoietic differentiation.

Systems biology approaches define the landscape of host defences to dengue virus

Laura Martin-Sancho[1], Jeffrey Johnson[2], Eun-Young Kim[3], Dabeiba Bernal[2], Dexter Pratt[4], Christopher Churas[4], Paul de Jesus[5], Trey Ideker[4], Steven Wolinsky[3], Eva Harris[6], Ana Fernandez-Sesma[2], Sumit Chanda[7]

[1]Imperial College London, London, United Kingdom. [2]Icahn School of Medicine at Mount Sinai, New York, USA. [3]Northwestern University Feinberg School of Medicine, Chicago, USA. [4]University of California San Diego, San Diego, USA. [5]The Scripps Research Institute, La Jolla, USA. [6]University of California at Berkeley, Berkeley, USA. [7]The Scripps Research Institute, San Diego, USA

Abstract

Dengue virus (DENV) is the most prevalent mosquito-borne human virus. Due to climate change and urbanization DENV has emerged beyond tropical areas currently causing 390 million annual infections. Yet, we lack systematic understanding of the front-line host defences that restrict DENV replication. To define the landscape of host factors and networks that execute DENV restriction, we adopted a systems biology approach, using primary dendritic cells and DENV clinical isolates as relevant models of infection. First, we carried out a genome-wide siRNA screen to identify host factors that inhibit DENV, and then profiled the impact of DENV infection on the transcriptome and proteome. These three datasets were integrated with published DENV-host protein-protein interaction (PPI) datasets to identify DENV restriction factors that are regulated over the course of infection and interact with DENV proteins. These analyses revealed 264 DENV restriction factors, of which 125 were identified in two or more datasets. Gene ontology analyses revealed enrichment of interferon signalling regulators and effectors, including novel and previously identified interferon stimulated genes (ISGs) such as IFITM3, as well as enrichment of interferon-independent pathways, including xenophagy, mitochondria homeostasis and other pathways not previous linked to viral restriction, which represent exciting avenues to uncover novel biology. Overall, these meta-analyses have provided a systematic understanding of the poorly characterized mechanisms of host control against DENV and defined restriction factors that may inform virus- and host-directed antiviral therapies.

N6-methyladenosine (m^6A) regulates interferon-stimulated gene expression in influenza A virus infection.

Piotr Kwiatkowski, Michelle Holland, Chad Swanson

King's College London, London, United Kingdom

Abstract

N^6-methyladenosine (m^6A) is the most abundant internal modification on mRNA in higher eukaryotes. It acts as a regulatory mechanism that controls gene expression by influencing the fate of modified mRNAs. m^6A is also found on RNA genomes, antigenomes, and transcripts of numerous viruses including influenza A virus (IAV), the causative agent of the seasonal flu. It has been shown that depletion of the cellular m^6A writer METTL3/14 decreases IAV replication, however the direct mechanism by which m^6A exerts its effect on IAV activity remains unclear. Here we show that replication of IAV is potently restricted by a small molecule inhibitor of METTL3/14. Importantly, the antiviral effect of the METTL3/14 inhibitor against IAV is alleviated by blocking the type I interferon (IFN) response, indicating that it is regulated by m^6A and contributes to restricting IAV. We further determine that global suppression of m^6A addition leads to an increase in the levels of IFN-induced transmembrane (IFITM) proteins 2 and 3, which are IFN-stimulated genes (ISGs) known to inhibit entry of many enveloped viruses. Consequently, targeted depletion of IFITM2/3 rescues IAV replication in the presence of the METTL3/14 inhibitor. Collectively, our findings reveal that post-transcriptional control of ISG expression by m^6A modulates viral replication.

Early HCMV Downregulation of the cGAMP Exporter MRP1 Increases Activation of the cGAS-STING Pathway

Joanne Kite[1,2], Samuel Gavronski[1,2], Abraham Tolley[1], Robin Robin Antrobus[1], Ceri Fielding[3], Richard J Stanton[3], Paul J Lehner[4], Michael P Weekes[1,2]

[1]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [3]School of Medicine, Cardiff University, Cardiff, United Kingdom. [4]Cambridge Institute for Therapeutic Immunology and Infectious Disease, Department of Medicine, University of Cambridge, Cambridge, United Kingdom

Abstract

Human cytomegalovirus (HCMV) is a dsDNA virus that causes persistent infection in 60-90% of people worldwide, with significant morbidity and mortality in the immunocompromised and congenital infection in 1/100 pregnancies. The cGAS-STING pathway is a crucial cellular pathway that couples dsDNA sensing to the activation of innate immunity, and as such is perturbed by multiple viruses. HCMV pUL138 is known to downregulate multidrug resistance protein 1 (MRP1, ABCC1) from the cell surface during infection via lysosomal degradation, however the reason has hitherto been unclear. MRP1 has recently been established as a c-GAMP exporter, suggesting that by degrading MRP1 HCMV may increase intracellular cGAMP.

To investigate the role of cGAMP in HCMV infection we used human fibroblasts knocked out for MRP1 or overexpressing UL138 to orthogonally recapitulate MRP1 degradation. Intracellular cGAMP was significantly increased in the absence of MRP1. cGAS-STING pathway activation, as measured by phosphorylation of STING, IRF3 and TBK1 increased during HCMV infection in the absence of MRP1. Modified vaccinia Ankara (MVA), another dsDNA virus, induced a similar MRP1-dependent response, although could not antagonise MRP1. Utilising HCMV WT and ΔUL138 recombinants expressing GFP, we are now investigating how increased intracellular cGAMP levels affects HCMV spread through cell monolayers.

Overall, we have identified that HCMV degradation of a cell surface protein MRP1 acts to increase dsDNA sensing and activation of the cGAS-STING pathway. We propose that this acts to prevent extracellular release of cGAMP and therefore facilitates the spread of progeny virus.

Investigating the antiviral response to HPIV3 using genome wide CRISPR knockout screens

Chloe Jones[1], Wenfang S Tan[2], Lynsey West[1], Eleanor Gaunt[2], Finn Grey[2], Paul Digard[2], David Hughes[1]

[1]University of St Andrews, St Andrews, United Kingdom. [2]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom

Abstract

The cytokine interferon (IFN), whose principal role is to limit viral replication and spread, is expressed as a consequence of infection. IFN-mediated signalling induces several hundred IFN-stimulated genes (ISGs) and affects the expression of thousands of additional genes. A prevailing model suggests that multiple ISGs are required to prevent infection as they have low to moderate activity when expressed in isolation, yet ISGs that are antiviral for one virus, may have no activity against another, indicative of highly redundancy response. Therefore, genome wide approaches lend themselves to untangling these complex responses. We have applied genome wide CRISPR knockout screens to investigate the antiviral response to human parainfluenza virus 3 (HPIV3). We pretreated CRISPR-library-transduced A549 cells with IFN-alpha followed by infection with HPIV3-GFP. Using FACS, we sorted cells capable of supporting infection (and therefore knocked out for an important antiviral factor) followed by sequencing to identify knockouts. This analysis, consistent with current models, showed only a limited number of ISGs were antiviral against HPIV3, including ZAP and IFIT1. Surprisingly, while independent knockout of ZAP validated our screen, independent knockout of IFIT1 suggested it had limited antiviral activity. However, when we adapted our well-characterised ISG15.KO cell line, that due to the over-amplification of antiviral ISGs greatly enhances the identification of antiviral factors, we showed that IFIT1 was indeed a HPIV3 restriction factor, albeit with low activity. We shall discuss these data that suggests current screens, that rely on moderate-large phenotypes, likely miss important factors leading to an incomplete picture of antiviral responses.

A druggable genome RNAi screen for host factors across the entire replication cycle identifies a Rab11a-mediated exit strategy and novel inhibitor of SARS-CoV-2.

Holly Kerr[1], Alison Daniels[1], Sarah Fletcher[1], Nicky Craig[1], Marie O'Shea[1], Samantha Griffiths[2], Juergen Haas[2], Christine Tait-Burkard[1]

[1]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, United Kingdom. [2]Division of Infection Medicine, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Host factors are recruited and repressed by SARS-CoV-2 throughout the entire viral replication cycle. Elucidating these interactions will aid in identification of candidates to develop host-directed antiviral therapies, and can be done through *in vitro* host factor screens. However, many studies utilising genome-wide knock out screening have biased identification of factors in the early stages of the viral replication cycle. In contrast, arrayed RNAi screening can identify genes involved at all stages of replication and essential genes. Here, we used direct lysis RT-qPCR quantification of viral RNA from cell supernatant following siRNA-mediated gene knockdown allowing a comprehensive analysis of the full replication cycle, including viral release.

siRNA-mediated knockdowns of a druggable genome siRNA library (~7000 genes) were performed in human Caki-1 cells using a clinical isolate of SARS-CoV-2 EDB-2(B.1). Pro- and anti-viral host factors with strong Robust Z scores were identified at 24hpi and 48hpi. Pathway and network analysis identified high confidence interactions in expected pro-viral and anti-viral gene clusters, spanning viral entry and endocytosis, replication transcription complex formation, and viral release. A cluster of pro-viral hits involved in vesicle-mediated transport were validated in EDB-2 and variants Delta and Omicron. We identify Rab11a as a key host factor in SARS-CoV-2 viral release which can be targeted using a novel inhibitor of Rab11a cargo delivery, CDKI-73. By identifying both pro- and anti-viral host factors across the entire replication cycle, we highlight the power of RNAi screening to elucidate host-pathogen interactions and targets for novel anti-SARS-CoV-2 and potentially pan-anti-coronavirus therapies.

Delineating the interaction of Influenza A virus Matrix protein 1 with host Transportins during virus entry

Carl Winn[1,2], Katja Klein[2], Othmar Engelhardt[1], Jason Long[1], Yohei Yamauchi[3]

[1]MHRA, Potters Bar, United Kingdom. [2]University of Bristol, Bristol, United Kingdom. [3]ETH Zurich, Zurich, Switzerland

Abstract

Influenza A viruses (IAV) remain a global health threat , causing a significant disease and economic burden during seasonal epidemics in addition to potential future pandemics emerging from novel zoonotic Influenza viruses. Various host factors are exploited by IAV during its replication cycle, including a viral uncoating host factor Transportin-1 (TNPO1) which interacts with viral protein Matrix protein 1 (M1) during virus entry. TNPO1, along with TNPO2 and TNPO3 function as nuclear import proteins in the host cell, however the dependency and interaction between TNPO2, TNPO3 and IAV remains unclear. Here, we aimed to delineate the breadth of dependency IAV has on the various TNPOs, utilsing an siRNA-targeting approach in qPCR-based and immunofluorescence microcrosopy-based viral entry assays. As expected, in TNPO1-depleted cells, viral ribonucleoprotein (vRNP) nuclear import was reduced when measuring primary transcription of NP and import of incoming NP by qPCR and confocal microscopy, respectively. In contrast, TNPO3-depleted cells exhibited an increase in IAV infection and vRNP nuclear import compared to the non-targeting control. Similarly, M1 uncoating during virus entry is reduced in TNPO1-depleted cells but increased in TNPO3-depleted cells compared to the non-targeting control, suggesting a potential anti-viral role of TNPO3. Work is ongoing to determine the mechanisms of this phenotype in TNPO3-depleted cells along with a proximity-based confocal microscopy approach to determine the in-vitro interaction between M1 and the various TNPOs. Furthering our understanding of viral-host protein interactions and the dependence of influenza virus on its host may in turn lead to novel therapeutic strategies.

Proteolytic cleavage orchestrates the formation and function of the astrovirus replication complex

David Noyvert ORCID iD[1], Leandro X. Neves[2], Gemma Lindsey[1], Aleksei Lulla[1], Ed Emmott[2], Valeria Lulla[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Astroviruses are positive-sense, single stranded RNA viruses infecting both avian and mammalian species. Classical human astroviruses are common enteric pathogens, affecting young children and presenting mild gastroenteritis symptoms. Recently, two novel stains of human astroviruses, MLB and VA, were shown to possess extraintestinal pathogenicity and demonstrate neurotropic features, with reported infections of the central nervous system. The replication cycle of astroviruses begins from the translation of a non-structural polyprotein (nsP1a) produced from the VPg-linked genomic RNA. The viral serine-like protease cleaves the polyprotein to release proteins for genomic and subgenomic RNA replication to initiate structural protein synthesis. Cleavage sites for nsP1a have been predicted but not experimentally confirmed and the subsequent processing products have not been characterised. We aim to map the cleavage sites to study the processing dynamics in HAstV1- and MLB2-infected cells. Employing N-terminomics mass spectrometry, we identify processing sites during viral infection. To experimentally validate these processing sites, we have detected full-length virus-specific products at different stages of infection. Additionally, intermediate processing products were detected using mammalian overexpression of astrovirus (poly)proteins. Furthermore, we have identified cellular proteases that are involved in astrovirus polyprotein processing. To investigate the importance of the identified functional elements in the context of viral replication, we use astrovirus dual luciferase replicon and reverse genetics systems. This work advances our understanding of astrovirus non-structural polyprotein cleavage and may inform on future drug targets and vaccine candidates.

The differential antiviral activities of IFITM2/3 against SARS-CoV-2 are regulated by their N-terminal domain

Alisha Reid, Helena Winstone, Maria Lista Brotos, Harry Wilson, Rui Pedro Galao, Stuart Neil

King's College London, London, United Kingdom

Abstract

IFN-induced transmembrane proteins (IFITMs) are a family of small membrane proteins comprised of four domains; N-terminal domain (NTD), intramembrane domain (IMD), transmembrane domain (TMD), and a short extracellular C-terminal domain (CTD). They modulate the entry of diverse enveloped viruses and localise predominantly to either the plasma membrane (IFITM1) or endosomal membranes (IFITM2/3). Despite high sequence homology, we have found IFITM2/3 can differentially modulate SARS-CoV-2 entry. Whilst IFITM2 restricts the ancestral Wuhan strain (with or without D614G), the alpha variant (B.117) is resistant to IFITM2 and enhanced by IFITM3.

To understand the molecular mechanism and identify the structural domain mediating this differential modulation, we generated several IFITM mutants. Each IFITM domain was independently swapped between IFITM2 and IFITM3, stably expressed in A549 cells, and infected with both pseudotyped lentiviral vectors (PLVs) and full-length SARS-CoV-2.

IFITM2-mediated restriction of both PLVs and full-length virus was only abolished if the NTD was replaced with that of IFITM3. Furthermore, insertion of the NTD of IFITM2 into IFITM3 sensitized both PLVs and full-length SARS-CoV-2, indicating the NTD is a major determinant of IFITM2 restriction. By contrast, using SARS-CoV-2 PLVs, no domain swap was found to effect IFITM3, indicating enhancement may not be modulated by a single domain.

In conclusion, we found the IFITM2 NTD is the major determinant of SARS-CoV-2 restriction. We are carrying out additional studies to identify the molecular determinant within the NTD of IFITM2 that mediates restriction and uncover the biological basis for the phenotypical distinction between IFITM2 and IFITM3.

ZNFX1 is an RNA-regulated E3 ubiquitin ligase involved in antiviral responses

Eilidh Rivers[1], Daniel Squair[2], Arda Balci[1], Hanna Sowar[1], Roosa Harmo[1], Satpal Virdee[2], Adam Fletcher[1]

[1]MRC–University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]MRC Protein Phosphorylation & Ubiquitylation Unit, Dundee, United Kingdom

Abstract

ZNFX1 (Zinc finger NFX1-type containing 1) is a highly conserved interferon stimulated RNA helicase, distantly related to RIG-I-like receptors. ZNFX1 is described to bind to viral RNA and promote IFN production following viral infections. Recently three cohorts of patients carrying mutations in ZNFX1 have been described. These patients have very severe and recurrent viral infections, mycobacterial disease, and other systemic inflammatory disease. They are affected very early in life, with a mean age of death in one study of 3.6 years. Here we discover that ZNFX1 is also an E3 ubiquitin ligase, a class of enzymes that add ubiquitin modifications to proteins or even non-proteinaceous targets. Additionally, we have identified an RNA-dependent activation mechanism of ZNFX1. In cells, the stability of the protein is sensitive to its ability to recruit an E2 conjugation enzyme and bind RNA. We are now investigating the role of ZNFX1 E3 ligase activity during infection, and how interactions with RNA influence this process.

Antagonism of a novel CRTC-mediated signalling pathway by human cytomegalovirus

Xinyue Wang[1,2], Marisa Oliveira[1,2], Benjamin Ravenhill[1,2], Ceri Fielding[3], Robin Antrobus[1,2], Richard Stanton[3], Michael P. Weekes[1,2]

[1]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [3]School of Medicine, Cardiff University, Cardiff, United Kingdom

Abstract

Human cytomegalovirus (HCMV) persistently infects 60-90% of all people worldwide. In immunocompromised patients, reactivation of latent HCMV results in significant morbidity and mortality, and congenital infection affects 1/100 pregnancies. There is presently no vaccine and available drugs exhibit toxicity and suffer from drug resistance. Novel therapies require an improved understanding of HCMV's biology and capacity for evasion of all arms of innate and adaptive immunity.

Our ongoing research has unveiled a novel cellular signalling pathway triggered by infection with diverse viruses and mediated by nuclear translocation of CREB-regulated transcriptional co-activators CRTC2 and CRTC3. This pathway stimulates production of interleukin 11 (IL-11), a fibrogenic cytokine and IL-6 family member. We have now identified two novel HCMV-encoded CRTC pathway antagonists. The ribonucleoside diphosphate reductase UL45 can influence CRTC activation. HCMV US15 targets the IL-11 signal transducer IL6ST for lysosomal degradation, inhibiting receptor-mediated signalling. A detailed characterization of the mechanisms of viral antagonism mediated by US15 and UL45 may provide insights into the function of this novel signalling pathway and ultimately facilitate the development of novel antiviral therapeutics that prevent US15/UL45-host interactions.

Myoferlin is a key player in Influenza vRNP trafficking along the Rab11 recycling pathway

Stefano Bonazza ORCID iD, Hannah Coutts, Swathi Sukumar, Hannah Turkington, David Courtney

Queen's University, Belfast, United Kingdom

Abstract

Due to its segmented nature, influenza A virus (IAV) evolved sophisticated mechanisms to sort and traffic replicated segments (vRNPs). It is well known that IAV exploits and remodels the endocytic recycling pathway, forming vRNP-clad, Rab11-positive vesicles, or influenza-trafficking vesicles (ITVs). However, it is unclear which other host factors are recruited to ITVs.

We sought to identify host determinants of trafficking by immunoprecipitation of the vRNP interactome. Among the most enriched hits, we identified Myoferlin (MYOF), a transmembrane protein involved in membrane dynamics and receptor recycling. We confirmed previous studies associating Rab11 and MYOF in uninfected cells. MYOF localisation was dependent on Rab11 function, while recycling was disrupted in the absence of Myoferlin.

Importantly, viral replication of multiple IAV strains was diminished in the absence of MYOF. This was also observed for other Rab11-dependent viruses: respiratory syncytial virus (RSV), Sendai virus (SeV) and SARS-CoV-2. This highlights the importance of MYOF across a broad range of viruses.

During infection, MYOF extensively colocalised with vRNPs and Rab11. We employed Nucleozin, which causes selective aggregation of Nucleoprotein-coated (NP) vesicles, to prove Myoferlin's inclusion in ITVs. Nucleozin treatment generated NP, Rab11 and MYOF aggregates, leaving other compartments unaffected.

EHD2 is a known interactor of MYOF, and EHD members are involved in the recruitment of motor proteins to vesicles undergoing fission. Nucleozin treatment revealed the presence of EHD2 in ITVs, thus we hypothesise that MYOF recruits EHD2 to ITVs, facilitating their maturation and transport to the plasma membrane. Further research is needed to elucidate this pathway.

A novel role of Dead box helicase 1 (DDX1) in viral translation

Natasha Palmalux ORCID iD[1], Marko Noerenberg[1], Wael Kamel[1], Louisa Iselin[2,1], Vincenzo Ruscica[1], Zaydah de Roland Laurent[1], Max Graf[3], Stefan Weitzer[4], Shabaz Mohammed[5,2], Nerea Irigoyen[6], Ana Da Silva Filipe[1], Javier Martinez[4], Alfredo Castello[1]

[1]MRC - Centre for Virus Research - University of Glasgow, Glasgow, United Kingdom. [2]Biochemistry - University of Oxford, Oxford, United Kingdom. [3]University of Tuebingen, Tuebingen, Germany. [4]Vienna Biocenter - Medical University of Vienna, Vienna, Austria. [5]Rosalind Franklin Institute, Oxford, United Kingdom. [6]University of Cambridge, Cambridge, United Kingdom

Abstract

Several host RNA helicases have been shown to play regulatory roles in viral infection. Our lab reported that Dead box helicase 1 (DDX1), a component of the tRNA ligase complex, is important in the lifecycle of a wide range of viruses. I showed that knocking down DDX1 substantially reduces the expression of cytoplasmic RNA viruses such us SARS-CoV-2, Zika virus and Sindbis virus (SINV). Although all proteins of the tightly assembled tRNA ligase complex relocate to viral replication factories, only DDX1 shows a regulatory role in infection. Using iCLIP2, I revealed the footprints of DDX1 on viral and cellular RNAs. Strikingly, my data shows that DDX1 switches from cellular to viral RNA upon infection, interacting primarily with the 5' UTR and the start of CDS. Viral RNA and protein levels indicates that the effect of DDX1 knock-down manifests at an early stage of infection. This suggests either its involvement in synthesis of the RNA-dependent viral RNA polymerase, or in replication of the viral genome. Using an alphavirus replicon system, I was able to disentangle replication from translation, showing that DDX1 is critical for viral RNA translation. This is further strengthened by our observation through polysome profiling, that DDX1 is present in the 40S fractions suggesting interaction with translation initiation components. Moreover, protein-protein interaction analysis reveals a switch of DDX1 interaction partners from the spliceosome to the ribosome in infected cells. Taken together, our data suggests DDX1 is recruited during viral infection to promote viral translation initiation.

Regulation of HSV-1 induced PKR activation by the cGAS-STING DNA sensing pathway

Hannah Burgess ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Multiple innate immune pathways exist in mammalian cells which sense and respond to pathogen associated molecular patterns (PAMPs). In one such pathway the eIF2α kinase PKR is directly activated by binding to double-stranded (ds) RNA, leading to a global inhibition of translation. In a separate pathway, cytosolic sensors detect nucleic acid PAMPs and activate a complex signaling cascade resulting in type I interferon (IFN) expression. During infection with HSV-1, the virus-encoded endoribonuclease virion host shutoff (vhs) suppresses accumulation of dsRNA preventing PKR activation and PKR–dependent stress granule formation. Meanwhile, IFN is induced by sensing of dsDNA by cGAS and signaling through the adaptor protein STING. We explored the possibility of crosstalk between these pathways using a vhs-deficient virus and discovered that cGAS-STING signaling regulates the activation of PKR in this context. When either protein was siRNA-depleted in primary human fibroblasts PKR was activated more poorly and fewer PKR–dependent stress granules were formed. Active signaling was required since pharmacological inhibition of cGAS or STING had the same effect, as did inhibition of downstream kinase TBK1. Importantly the regulation of PKR by cGAS-STING was not observed following transfection of a dsRNA analog poly(I:C), suggesting that the accumulation of PKR-activating dsRNA during herpes infection is cGAS-STING dependent. These data posit co-regulation of cGAS-STING and PKR as an unexpected mode of crosstalk between dsDNA-dependent IFN signaling and a dsRNA-responsive cell intrinsic innate immune effector that regulates mRNA translation.

Virus Workshop: Translating Knowledge - Understanding and Preventing Disease

Propylene glycol inactivates respiratory viruses and prevents airborne transmission

Christine Major-Styles[1], Jie Zhou[1], Katie Flight[1,2], Jonathan Brown[1], Charlotte Lewis[3], Xinyu Wang[4], Michael Vanden Oever[1,5], Thomas Peacock[1], Ziyin Wang[6], Rosie Millns[1], John O'Neill[7], Alexander Borodavka[4], Joe Grove[3], Wendy Barclay[1], John Tregoning[1], Rachel Edgar[1]

[1]Department of Infectious Disease, Imperial College London, London, United Kingdom. [2]UCL, London, United Kingdom. [3]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [4]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom. [5]Life Edit Therapeutics, Morrisville, NC, USA. [6]Imperial College London, London, United Kingdom. [7]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Abstract

Viruses are vulnerable as they transmit between hosts, and we aimed to exploit this critical window. We found that the widely used, safe, inexpensive and biodegradable small molecule propylene glycol (PG) has robust virucidal activity. Propylene glycol rapidly inactivates a broad range of enveloped viruses including influenza A virus (IAV), SARS-CoV-2, Epstein-Barr virus (EBV) and lentiviral-based pseudotype viruses expressing glycoproteins from different viruses including Ebola and MERS. PG permeabilised viral envelopes but also inactivated non-enveloped rotavirus, indicating that this small molecule can act via direct biophysical disruption of either lipid membranes or capsid proteins, presenting a substantial barrier to evolutionary escape. Concomitant inhalation of IAV with PG reduced disease burden in mice compared to IAV alone, suggesting PG is protective *in vivo* and acts rapidly to limit infection within the nose and respiratory tract. Most critically, using *in vitro* transmission models, we demonstrated that safe concentrations of vaporised PG efficiently neutralise airborne SARS-CoV-2 and IAV, potently preventing infection at levels well below those tolerated by mammals, and additionally inactivated virus deposited on different surfaces. We present PG vapour as a first-in-class non-toxic airborne virucide that can prevent transmission of a diverse range of existing and emergent viral pathogens, with clear and immediate implications for public health.

(Styles et al, EMBO Molecular Medicine, 2023)

Correlation between pseudotyped virus and authentic virus neutralisation assays

Nigel Temperton ORCID iD

University of Kent, Chatham, United Kingdom

Abstract

Background: The virus neutralization assay is a principal method to assess the efficacy of antibodies in blocking viral entry. Due to biosafety handling requirements of viruses classified as hazard group 3 or 4, pseudotyped viruses can be used as a safer alternative. However, it is often queried how well the results derived from pseudotyped viruses correlate with authentic virus. This systematic review and meta-analysis was designed to comprehensively evaluate the correlation between the two assays.

Methods: Using PubMed and Google Scholar, reports that incorporated neutralisation assays with both pseudotyped virus, authentic virus, and the application of a mathematical formula to assess the relationship between the results, were selected for review. Our searches identified 67 reports, of which 22 underwent a three-level meta-analysis.

Results: The three-level meta-analysis revealed a high level of correlation between pseudotyped viruses and authentic viruses when used in an neutralisation assay. Reports that were not included in the meta-analysis also showed a high degree of correlation, with the exception of lentiviral-based pseudotyped Ebola viruses.

Conclusion: Pseudotyped viruses identified in this report can be used as a surrogate for authentic virus, though care must be taken in considering which pseudotype core to use when generating new uncharacterised pseudotyped viruses.

This data has implications for pre-clinical evaluation through to licensure of vaccines and mAbs for current and emerging RNA viruses.

Antibody response to HCMV gB vaccine in the context of gB protein sequence and structure

Anastasia Lankina[1], Marco Thomas[2], Judith Breuer[1], Matthew Reeves[1]

[1]University College London, London, United Kingdom. [2]Universitätsklinikum Erlangen, Erlangen, Germany

Abstract

A vaccine to prevent human cytomegalovirus (HCMV) disease in transplant patients and congenitally infected infants remains the highest priority. The most successful vaccine to date is based on a recombinant form of glycoprotein B (gB) – the viral fusion protein essential for entry. Surprisingly, protection was not explained by neutralising antibodies or responses against known antigenic domains of gB (AD1-5). Instead, we identified a novel antigenic domain (AD6) as a potential mechanistic correlate of protection.

Recently, we developed a temperature-dependent neutralisation assay that likely selects for high-affinity antibodies and revealed the presence of low levels of neutralising antibodies in sera from gB vaccine recipients. Using an agnostic peptide-oriented approach we identified a 70aa segment of the larger AD5 conformational antibody target (further dubbed AD5N) that was enriched as a response in the “neutraliser” vaccine population and in individuals who control HCMV in vivo. Interestingly, a concomitant in silico analysis of gB suggested AD5N to include potent B-cell epitopes. Furthermore, we observe that AD5N is spatially located in the proximity of the fusion loops on the pre-fusion structure of gB – congruent with an antibody that would block HCMV entry. Finally, although AD5N is highly conserved across HCMV sequences the greatest variation was observed in the region of AD5N on the surface of gB.

Although antibodies against AD5 are well-characterised, those directed specifically against AD5N are less so. These data suggest strategies that promote AD5N antibody responses could potentially contribute to the better control of HCMV infection in vivo.

Investigating the virome of mosquitoes in Kangerlussuaq, Greenland

Mirjam Schilling ORCID iD[1], Madhujot Jagdev[1], Huw Thomas[2], Nicholas Johnson[1]

[1]Animal and Plant Health Agency, Addlestone, United Kingdom. [2]Animal and Plant Health Agency, Nympsfield, United Kingdom

Abstract

Mosquitoes are major vectors of emerging zoonotic diseases and occur in all climatic zones, including the Arctic. The rapid global environmental changes occurring are already affecting the distribution and population dynamics of many different mosquito species, and with them the viruses they carry. The climate in northern regions is affected faster than the global average. However, very little is known about the viruses that are circulating in arctic mosquito populations. Critically, as arctic mosquitoes expand their range into more temperate areas this may increase the risk of mosquito-borne disease in naïve hosts.

Therefore, we collected mosquitos at lake Sanningasoq, approximately 11.5km northeast from Kangerlussuaq in central-western Greenland in July 2022 and 2023 to screen for viruses and assess their potential as disease vectors. The mosquitoes were identified to species level using a combination of morphology and DNA barcoding. We identified mosquitoes of the *Aedes impiger* and *Aedes nigripes* species. Individually homogenized mosquitoes were pooled for RNA extraction and next generation sequencing. The majority of reads mapped to bacterial genomes and bacteriophages. Additionally, contigs mapping to a variety of different DNA and RNA virus families could be assembled that phylogenetically cluster with well-known pathogens. Although it is unclear what risk these viruses represent to plants, animals, or people, it suggests that many may have some zoonotic potential.

Our results highlight, that arctic mosquitoes are likely to contain viruses which may impact animal and public health, and in light of a changing climate should be considered in future risk management plans.

Fundamental and applied studies on lytic and persistent parainfluenza virus type 5 infections.

Richard Randall[1], Dan Young[1], Maria Pisliakova[1], Jelena Andrejeva[1], Elizabeth Wignall-Fleming[1], Lynsey Murray[1], David Hughes[1], Steve Goodbourn[2]

[1]University of St Andrews, St Andrews, United Kingdom. [2]St George's, University of London, London, United Kingdom

Abstract

Different strains of parainfluenza virus type 5 (PIV5) cause either persistent or lytic infections in a wide range of mammalian tissue culture cells. Persistently infected cells exhibit little or no cytopathic effect (CPE) and can readily be passaged. In persistently infected cells the virus fluxes between active and repressed states. We have shown the phosphorylation state of the PIV5-P protein determines whether a particular strain of PIV5 establishes a persistent or lytic infection. Using PIV5 vectors expressing either mCherry or GFP with persistent or lytic phenotypes, we show that in co-infections the persistent phenotype is dominant. Furthermore, 10-20% of virus released from dually infected cells contain both genotypes, indicating that PIV5 particles can package more than one genome. Our understanding of PIV5 persistence has facilitated the development a suite of PIV5-based vectors for the expression of recombinant proteins both *in vitro* and *in vivo,* including single cycle vectors in which the F-gene has been deleted. As proof of principle that these F-deleted single-cycle viruses, which grow to high titres in F-expressing helper cells, can be used as expression vectors, we have cloned and expressed a humanized (Hu) anti-V5 tag antibody. Suspension CHO cells grown to a density of 2 x 10^6 cells/ml and infected with PIV5DF.Hu.anti-V5 produce approximately 20 -50 mg of antibody/litre after 4 days infection. Fundamental and applied aspect of PIV5 persistence will be highlighted.

Influenza A packaging and protein expression: *in silico* improvements in molecular characterisation

Emma Beniston, Jordan Skittrall ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

All viruses must solve the problem of ensuring their genetic material is recognised and packaged by a viral capsid. This problem is especially intricate in viruses with segmented genomes, such as influenza viruses. Interchangeability of packaging signals in different influenza A subtypes remains a key unanswered question in determining strains' pandemic potential. The packaging signals' specificity makes them candidates for drug targets with low risk of host cross-reactivity.

We have recently refined a computational technique for finding regions of high conservation in RNA virus coding regions. We applied this technique to eight different haemagglutinin/neuraminidase/host combinations of influenza A (983,552 gene sequences analysed). We then used the predicted high conservation regions as input to a RNA structural prediction algorithm.

Many of our predictions better delineate regions of RNA crucial to influenza A packaging. Our technique also predicts regions of conservation beyond packaging signals. Other predictions include a region near the influenza A PA/PA-X frameshift whose conformational switch would allow regulation of relative protein abundances, a region in the NS intron with the potential to inhibit spliceosome activity, and association between avian strains and conservation around the initiation site of an N-terminal truncated form of PB1.

Our work provides computational insights into RNA structure and function across the influenza A genome. The resultant structural predictions explain a number of the functional results of previous packaging signal mutational analyses. They naturally lead to predictions for experiments that would confirm the structural bases of some packaging signals and other functional elements.

How the virus spread: Deep-sequence phylogenetics to track the spread of HIV infections in a universal test and treat trial.

Lerato Magosi ORCID iD[1], Victor De Gruttola[2], Marc Lipsitch[2]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]Harvard T.H. Chan School of Public Health, Harvard University, Boston, USA

Abstract

Presented on behalf of the Botswana Combination Prevention Project (BCPP / Ya Tsie trial) and the PANGEA consortium

The human immunodeficiency virus (HIV), is a virus that attacks the body's immune system leaving an individual vulnerable to infections (e.g. pneumonia) and certain cancers (e.g AIDS-related Kaposi Sarcoma). Importantly, the end-stage of HIV, acquired immunodeficiency syndrome (AIDS) claims nearly a million lives globally each year, thus, limiting the occurrence of new HIV infections is a major public health concern in infectious disease. In the absence of a successful HIV vaccine or widely administrable cure, we show here, for the first time in a community-randomized trial of HIV prevention in sub-Saharan Africa, that deep-sequenced HIV viral genomes can be used to track the directional spread of HIV infections between communities to identify sub-epidemics and groups of individuals that could be prioritized to target interventions where the need is greatest. Specifically, we deep-sequenced whole genome HIV viral sequences from 5,114 trial participants in the 30-community Ya Tsie trial in Botswana to infer where people were infected and by whom (gender, age). Deep-sequence phylogenetic analyses revealed the following factors: geographic proximity, age, gender and randomized-HIV-intervention as contributors to the spread of infection among trial communities. Our findings suggest that population movement patterns intertwine with how infections spread and are important to the impact on intervention strategies.

Detection of alpha and beta coronaviruses in bats in England during 2021-2023

Matthew Bagley, Hooman Goharriz, Victoria Gould, Shweta Shukla, Jessica Lavery, Amanda Seekings, Guanghui Wu, Lorraine McElhinney

UK Animal and Plant Health Agency, Weybridge, United Kingdom

Abstract

Coronavirus surveillance in bat species is important to determine virus diversity currently circulating in bat populations in the UK and assess potential for spillover into human or other animal populations. Throughout 2021-2023, oral and rectal swabs were collected from bat carcasses submitted to the Animal and Plant Health Agency (APHA) as part of a passive bat surveillance program. Bat guano samples were submitted by bat rehabilitators and from bat roosts. A smaller number of swabs were also obtained from live captured bats as part of an active surveillance study and tested in the same way. Extracted RNA was tested by pan-coronavirus RT-PCR targeting a conserved region of the RdRp gene. In total 545 bats from 13 different species were screened for coronaviruses, of which 18 bats (3.3%) tested positive. These bat species were, Common Pipistrelle *(Pipistrellus pipistrellus)*, Soprano Pipistrelle (*Pipistrellus pygmaeus)*, Natterer's (*Myotis nattereri)* and Brown Long-Eared (*Plecotus auritus)*. Sanger sequencing has identified a range of alpha and beta coronaviruses with high (95% to 100%) similarity to coronaviruses previously detected in bats in the UK during 2007-2021. Furthermore, bat carcasses have been retained and will be tested for the presence of viral RNA in various organs, to investigate tissue tropism and potentially allow the isolation of live virus. Coronavirus surveillance in UK bats will continue into 2024 and the results will be presented. This study provides insight into the diversity of coronaviruses among UK bat populations, contributing to our understanding of the potential for zoonotic risk.

A protein-based molecular toolkit to study, detect, prevent and treat Oropouche fever, a neglected tropical viral disease

Monique Merchant[1], Juliano de Paula Souza[2], Sahar Abdelkarim[3,4], Jared Rudd[2], Lauren Eyssen[3], Luis da Silva[2], Ray Owens[3,4], Eurico Arruda[2], Stephen Graham[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]University of São Paulo, Ribeirão Preto, Brazil. [3]Rosalind Franklin Institute, Didcot, United Kingdom. [4]University of Oxford, Oxford, United Kingdom

Abstract

Oropouche fever is a neglected tropical viral disease endemic to Latin America and the Caribbean with potential to cause future pandemics. Oropouche fever is caused by Oropouche virus (OROV), an orthobunyavirus with a tri-segmented (-)ssRNA genome and a propensity for genetic reassortment. OROV commonly infects sloths, birds, monkeys and rodents but has been known to spill over into human populations *via* transmission from biting insects. After Dengue fever, Oropouche fever is the second-most prevalent insect-borne disease in Brazil. Changes in climate and land use are increasingly bringing humans into contact with OROV reservoir and vector species, but currently there is no readily available point-of-care diagnostic test for OROV infection.

We have generated high-quality recombinant OROV antigens and have utilized these to detect seroconversion following experimental infection of animals or historical infection of humans, confirming their antigenic authenticity. These antigens stimulate the production of high neutralizing antibody titers in animals, highlighting their promise as immunogens for vaccination. We have also used the antigens to generate camelid-derived variable heavy chain (VHH) antibodies (a.k.a. nanobodies). These VHH antibodies recognize both recombinant and infection-derived viral antigens with high affinity, and some of the VHH antibodies potently neutralize OROV in cell-based models of infection. We will discuss the use of these antigens and VHH antibodies to develop new low-cost diagnostics for acute and historical OROV infection, for potential prevention or treatment of Oropouche fever, and as research reagents to study the biology of this poorly characterized virus.

Evaluation of virus transmission blockers in an in vivo pig influenza challenge model

Katie Hatton[1], Bhawna Sharma[1], Alice Guan[1,2], Emily Briggs[1,3], Ehsan Sedaghat-Rostami[1,3], Elliot Moorhouse[1], Basu Paudyal[1], Elma Tchilian[1,3]

[1]The Pirbright Institute, Pirbright, United Kingdom. [2]University of Manchester, Manchester, United Kingdom. [3]University of Surrey, Guildford, United Kingdom

Abstract

The public health burden of respiratory infections, such as influenza and COVID-19, is not adequately addressed by existing vaccines and antivirals as they primarily prevent severe disease. Therefore, identifying therapeutics that interfere with human-to-human transmission of respiratory viruses remains a pressing need. We developed an in vivo porcine model to assess the ability of novel antivirals and innate immune stimulators, previously identified in small animal models, to block viral transmission. The pig is physiologically, immunologically and genetically more similar to humans, and is a natural influenza host, increasing the applicability of results to humans. Our optimised models enable assessment of transmission blockers following direct infection with influenza virus (strain H1N1pdm09) or in a contact challenge model where the animals were given a transmission blocker 24 hours prior to contact with H1N1pdm09 infected donor pigs. The severity of disease, lung pathology and viral load were assessed to determine transmission blocker efficacy.

We first assessed the efficacy of human recombinant monoclonal antibody, 2-12C, delivered by aerosol (AE) or intravenously (IV), as well as intranasally delivered innate immune stimulant, poly(I:C), in the direct challenge model. Both IV and AE delivery of 2-12C significantly reduced viral shedding, lung viral load and pathology, while poly(I:C) showed no effect. 2-12C IV pre-treatment also prevented H1N1pdm09 infection and abolished viral shedding in the contact challenge model. Taken together, we propose that the pig influenza contact challenge model is useful for testing candidate novel antiviral and emerging delivery platforms prior to human trials.

Assessing serological exposure to H5 avian influenza in humans in Malaysian Borneo, along the East Asian - Australasian Flyway

Hannah Klim ORCID iD[1], Timothy William[2,3,4], Tock H Chua[5], Giri S Rajahram[4], Helena Brazal Monzó[6], Caolann Brady[1], Jecelyn Leaslie John[7], Mohammad Saffree Jeffree[8], Jack Mellors[9], Tom Tipton[9], Craig P Thompson[10], Kamruddin Ahmed[11,7,12], Chris J Drakeley[13], Miles W Carroll[1], Kimberly M Fornace[14,15]

[1]Wellcome Centre for Human Genetics and Pandemic Sciences Institute, Oxford, United Kingdom. [2]Infectious Diseases Society Sabah-Menzies School of Health Research Clinical Research Unit, Kota Kinabalu, Malaysia. [3]Gleneagles Hospital, Kota Kinabalu, Malaysia. [4]Clinical Research Centre, Queen Elizabeth II Hospital, Kota Kinabalu, Malaysia. [5]Faculty of Medicine and Health Sciences, University of Malaysia Sabah, Kota Kinabalu, Malaysia. [6]Department of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, London, United Kingdom. [7]Borneo Medical and Health Research Centre, Faculty of Medicine and Health Sciences, University Malaysia Sabah, Kota Kinabalu, Malaysia. [8]Dept. of Public Health Medicine, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, United Kingdom. [9]Wellcome Centre for Human Genetics and Pandemic Sciences Institute, Oxford, Malaysia. [10]Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom. [11]Dept. of Pathology and Microbiology, Faculty of Medicine and Health Sciences, University Malaysia Sabah, Kota Kinabalu, Malaysia. [12]Research Center for Global and Local Infectious Disease, Oita University, Oita, Japan. [13]Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom. [14]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom. [15]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore

Abstract

Cases of highly pathogenic H5 avian influenzas are on the rise globally. H5N1 viruses are estimated to have a mortality rate in humans of nearly 60%. Malaysian Borneo is a stopover site for shorebirds migrating between East Asia and Australia. Although no human cases of H5 avian influenzas have been reported in Malaysian Borneo to date, highly pathogenic H5N1 has circulated in poultry and migratory avian species transiting through this region. Malaysian Borneo has seen recent deforestation and destruction of natural shorebird habitats, which may be increasing the proximity between humans and avian species. We hypothesize that higher rates of human-animal contact, caused by this habitat destruction, will increase the likelihood of a potential zoonotic spillover event. In 2015, an environmentally stratified cross-sectional survey was conducted collecting geolocated questionnaire data on potential risk factors for emerging zoonotic disease in 10,100 individuals. We performed a serological survey of these individuals with influenza antigens for ELISAs, pseudotyped neutralization assays, and a cross-reactivity depletion assay. We find evidence of high H5 responses that persist upon depletion of the plasma. The presence of these antibodies suggests that some individuals living near these migratory sites may have been exposed to H5 influenzas. There is a significant spatial correlation between individuals displaying high H5 binding and the distributions of these migratory species. These serological profiles highlight the urgent need to study interactions and increase surveillance in these migratory zones to decrease the risk of future spillover events.

Development of a Bovine coronavirus mRNA vaccine representative of circulating isolates collected from Ireland in 2022/23

Tristan Russell ORCID iD[1], Jose Maria Lozano[2], Cailyn Challinor[1], Gerald Barry ORCID iD[1]

[1]University College Dublin, Dublin, Ireland. [2]Department of Agriculture, Food and Marine, Cellbridge, Ireland

Abstract

Bovine coronavirus (BoCoV) is a pneumoenteric pathogen causing winter dysentery, calf diarrhoea and is one of several pathogens associated with the bovine respiratory disease complex. Inactivated virus and a recently developed modified live virus vaccine are administered to protect against disease, but comparisons between the vaccine strains and natural isolates showed they are highly divergent. Phylogenetic analysis of natural isolates also demonstrated divergence based on location suggesting different regions may require location-specific vaccines. Predicted BoCoV immune epitopes combined with selective pressure analysis identified the surface proteins, spike and haemagglutinin esterase (HE), as the viral proteins most likely recognised by the adaptive immune response. As such, mRNA vaccines expressing BoCoV spike and HE were developed. Spike and HE sequences of Irish isolates collected during 2022 and 2023 were used to design the mRNA vaccine so it expressed proteins representative of BoCoV currently circulating in Ireland. Conventional mRNA vaccines were generated and tested to determine their functionality. Attempts were also made to generate self-amplifying mRNA vaccines by inserting spike and HE genes downstream of the Semliki Forest virus replicase. This work demonstrated how surveillance of currently circulating animal viruses and targeted sequencing of immunogenic proteins can be used to quickly develop or update a vaccine.

Knowledge, Attitudes, and Practice of Dual Use Research of Concern

Abigail A. O'Malley, Christopher J. Finnegan, Anthony R. Fooks ORCID iD, Sharon M. Brookes ORCID iD

Animal and Plant Health Agency (APHA), Weybridge, United Kingdom

Abstract

An understanding and knowledge of the attitudes and practices related to Dual Use Research of Concern (DURC) and the related 'Gain-of-Function' (GoF) studies are inconsistent throughout the UK and globally. There is not any consensus regarding either institutional oversight or training of researchers, especially early career scientists. The aim of this survey was to address DURC issues and its training requirements. It is a self-administered, anonymous, online survey created using *Microsoft Forms*. The pilot survey "*Awareness towards GOF research and DURC and its training needs*" consisted of 43 questions and was distributed to colleagues at APHA. After refinement, an updated version, was renamed *"Attitudes and awareness of Dual Use Research of Concern (DURC) and its training needs"* and shortened to 18 questions. This version was distributed to members of the European Virus Archive – Global (EVAg) consortium. In total, there were 49 responses to both surveys, 96% being educated to post-graduate level (MSc and PhD). Of these respondents, 71% had not received any formal training on the potential for misuse of research. Notably, 87% considered that institutions should provide scientists with educational support and mandatory training on DURC. The results from these initial surveys indicated the need for formal training of researchers in DURC. These data from existing and future surveys will be used to develop a training module, to enhance knowledge on DURC and GoF issues, with an overall outcome to improve the consistency of health and safety working practices, translating knowledge, and reducing disease risks.

Ubiquitin variants as antivirals agents against avian infectious bronchitis virus

Vera van Vliet[1,2], Olivia Roscow[3], Marjolein Kikkert[1], Christine Tait-Burkard[2]

[1]Leiden University Medical Center, Leiden, Netherlands. [2]The Roslin Institute, the University of Edinburgh, Edinburgh, United Kingdom. [3]University of Guelph, Guelph, Canada

Abstract

Infectious bronchitis virus (IBV) is an avian coronavirus causing respiratory disease in domestic fowl, primarily chicken. Isolated in the 1930's, IBV causes great economic losses to the poultry industry. Even though vaccines are available, they are poorly cross reactive to other and new variants of the virus, which are always on the cusp of emerging. Other antiviral strategies, such as cost-effective antivirals or the production of genome edited or transgenic animals immune to IBV infection, are therefore sorely needed.

All coronaviruses encode a papain-like protease (PLpro) as part of their non-structural proteins. PLpro performs many activities, including cleaving the viral non-structural polyproteins (pp1a and pp1ab) into protein subunits and affecting the innate immune response. PLpro is crucial for viral replication and an attractive target for potent antiviral agents. Furthermore, PLpro has deubiquitinating activity and therefore binds and cleaves ubiquitin. Here, we have applied a novel strategy of selecting sequence variants of ubiquitin (UbVs) from a phage-displayed library that bind with high affinity to the IBV PLpro. A selection of UbVs were found to bind to two different strains of IBV, M41 and Beaudette. These UbVs might inhibit viral replication and the viruses' ability to evade the innate immune response in the host cell, as we have found to be true for UbVs against other (human) coronaviruses. Next to the antiviral ability, we are currently investigating delivery options and development of resistance against IBV in chicken.

Using serological responses to establish a correlate of protection against Chikungunya virus.

Daniel Yara ORCID iD[1], Sarah Kempster ORCID iD[1], Deborah Ferguson[1], Joanna Hall[1], Claire Ham[1], Adrian Jenkins[1], Neil Berry[1], Sally Baylis[2], Pierre Roques[3], Roger LeGrand[3], Raphaël Zellweger[4], Sushant Sahastrabuddhe[4], Sumathy K[5], Bob Small[6], Tim Endy[6], Amy Shurtleff[6], Neil Almond[1]

[1]Medicines and Healthcare products Regulatory Agency, Potters Bar, United Kingdom. [2]Paul-Ehrlich-Institut, Langen, Germany. [3]Commissariat à l'énergie atomique et aux énergies alternatives, Fontenay aux Roses, France. [4]International Vaccines Institute, Seoul, Korea, Republic of. [5]Bharat Biotech International Ltd., Hyderabad, India. [6]Coalition for Epidemic Preparedness Innovations, Oslo, Norway

Abstract

Vaccine development for protection against Chikungunya virus (CHIKV) faces many challenges such as carrying out efficacy endpoint-driven clinical trials due to the sporadic nature of CHIKV outbreaks. Consequently, regulators acknowledge that a CHIKV vaccine may be licensed utilising immunogenicity data and serological efficacy studies in animal models.

Here, studies were carried out to evaluate whether pools of (i) convalescence plasma or (ii) serum from volunteers administered with a candidate CHIKV vaccine would protect cynomolgus macaques (*Macaca fascicularis*) against CHIKV elicited disease. All animals were challenged intra-dermally with 10e5 infectious units of CHIKV ESCA strain LR2006 – OPY1. Naïve control macaques clearly became infected, with viremia being detected through RT-qPCR over 14 days post-challenge, along with fever developing over the first week of infection. In addition, *de-novo* synthesis of anti-CHIKV IgM and IgG were detected in the blood using commercially available ELISA kits.

In contrast, cynomolgus macaques exhibited no indication of infection when administered with convalescent plasma or serum isolated from recipients of a candidate vaccine. Overall, a distinct titratable level of protection was exhibited, where a correlate of serological protection can be identified. These data suggest that similar human anti-CHIKV antibodies levels are needed for protection in macaques regardless of whether antibodies derived from convalescent or vaccinated individuals. Such correlates can aid novel vaccines through clinical trials and subsequent licensing.

Assessing the effect of inter-isolate variation on the function of RVFV virulence determinants

Philie Hollinghurst[1], Kevin Maringer[1], Dan Horton[2], Victoria Sy[1], Simon Gubbins[1], Isabelle Dietrich[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Rift Valley fever (RVF) is a vector-transmitted disease with a widespread pattern of endemism across Africa and the Arabian Peninsula. Despite the significant threat posed to humans and ruminant animals, the impact of genomic sequence variation among Rift Valley fever virus (RVFV) isolates remains poorly understood. Putative increases in RVFV virulence and transmissibility were explored by comparing virulence associated genomic regions of two Kenyan RVFV isolates; one taken from a widespread outbreak in 2006 and a second ancestral isolate taken from a mosquito during at inter-epidemic period in 1983. Reverse genetics approaches were used to generate recombinant viruses by cloning the M segment ORFs or the NSs ORF of the desired isolates into a plasmid-based rescue system for the recovery of RVFV strain ZH-548. The NSs proteins to be functionally analogous, as the NSs recombinant viruses were found to comparably modulate the IFN-β response and exhibited similar replication kinetics in human, bovine and mosquito cells. By contrast, the 2006 M recombinant virus exhibited a 20% increase in viral growth rate relative to the 1983 virus. A mathematical model developed to examine the effect of within-host viral replication on the R_0 of RVFV did not find this to significantly impact transmission. However, peak titre disparities reported in lambs and calves challenged with comparable viruses were found to elicit large changes in viral transmission, indicating the need for further animal experiments. The modelling results additionally identified increases in peak in titre, particularly in lambs, as high risk for RVFV transmission.

Unleashing the aromatic arsenal: Exploring the antiviral potential of volatile compounds from *Nigella sativa* against Coronaviruses

Gemma Cooper[1], Hesham F. I. Khodeir[2], Karrar Kamoona[2], Ahmed Alalaqi[2], Parvez I. Haris[2], Maitreyi Shivkumar ORCID iD[1]

[1]Leicester School of Pharmacy, De Montfort University, Leicester, United Kingdom. [2]Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom

Abstract

The Covid-19 pandemic highlighted the need for pan-coronavirus antivirals that can be deployed in response to emerging viruses. Here, we investigated the antiviral activities of *Nigella sativa* oil extracts and bioactive compounds against seasonal human coronaviruses OC43 and 229E, and SARS-CoV-2 pseudoviruses.

Coronavirus-infected cells were directly treated with oil extracts and antiviral activity determined by quantifying viral titres. In diffusion assays, virus was pre-treated with oils by incubating in adjacent wells of microwell plates and infectivity subsequently determined.

Diffusion assays showed antiviral activity of *Nigella* extracts, with no cytotoxicity observed. After 24 hours a significant reduction of viral infectivity was seen; the most potent oils showing a ≥3 log10 reduction. Direct treatment with oils did not inhibit infection at non-cytotoxic concentrations, suggesting the vapor phase may provide higher concentrations of bioactive compounds without compromising cell viability.

We identified five key bioactive compounds present in the oil vapour phase by mass spectroscopy; pre-treatment of viruses with these compounds in diffusion assays showed reduction in infection. We hypothesised that the compounds impact viral entry by affecting the lipid bilayer, and preliminary TEM analysis indicates disruption of the viral lipid envelope upon treatment. This is also consistent with our observation that the volatiles have a limited impact on antiviral activity of the non-enveloped murine norovirus.

Evaluation of the antiviral activity of the key volatile compounds in isolation and in synergy are ongoing. This is a key step towards developing novel pan-coronavirus antivirals, delivered directly to the infection site through sprays or inhalers.

Characterisation of a neutralising (D9-like) epitope within the G-H loop of Foot-and-mouth disease virus serotypes O, A and SAT1

Abdelaziz Yassin[1,2], Santina Grazioli[3], Emiliana Brocchi[3], Daniel Horton[2], Anna Ludi[1], Donald King[1], Amin Asfor[2]

[1]The Pirbright Institute, Surrey, United Kingdom. [2]University of Surrey, Surrey, United Kingdom. [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy

Abstract

Foot-and-mouth disease virus (FMDV) possesses a hypervariable G-H loop region located within the VP1 capsid protein which is associated with virus neutralisation. The G-H loop contains the virus attachment motif (RGD) which binds to the cellular receptor (integrin) facilitating virus entry for all seven FMDV serotypes. A monoclonal antibody (mAb) D9 produced against a serotype O FMDV binds to an epitope in G-H loop that is neutralising. Its binding ability in ELISA and neutralising activity is affected by changes at critical amino acid positions on VP1. Previous studies have shown that these amino acids (L144, L148 and K154) affect serotype O neutralisation. In this study, we developed an indirect peptide ELISA using synthetic peptides representing the G-H loops from different FMDV serotypes O, A, SAT1 and SAT2. D9 was one of a panel of monoclonals tested. In addition, virus neutralisation test (VNT) was performed to assess the ability of D9 to neutralise other FMDV serotypes besides serotype O and to compare with the ELISA binding data. These preliminary ELISA results showed that D9 bound to serotype O, A and SAT1 peptides in a dose dependent manner but not to SAT2 and A22 peptides. Furthermore, there was agreement with the VNT results, confirming the presence of D9-like neutralising epitopes on some of the viruses used in this study. These findings help to map epitopes within the G-H loop and determine critical amino acids that affect the epitope orientation and thus binding affinity with antibodies.

African Swine Fever Virus Multigene Family Interferon Inhibitory Proteins: Functions and Application to Vaccine Development

Rodrigo Gonzalez Gomez ORCID iD[1,2], Anusyah Rathakrishnan ORCID iD[1], Samuel Connell ORCID iD[3], Linda Dixon ORCID iD[1], Teresa Lambe ORCID iD[2], Ana Luisa Reis ORCID iD[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]Zoetis Inc., Brussels, Belgium

Abstract

African swine fever virus (ASFV), for which there is no available vaccine, is a large DNA virus which encodes for 150 to 170 proteins, many of which are implicated in the evasion of host defences. Many ASFV proteins appear to inhibit interferon (IFN) induction and its response. Key among these are the genes from the ASFV Multigene Family (MGF), which flank the viral genome and are expressed shortly after cellular entry. The deletion of viral MGFs, particularly MGF360-12L and MGF505-1R, has been shown to attenuate the virulence of ASFV in pigs as well as viral replication in porcine macrophages, yet their molecular mechanism of action remains elusive. Part of the basic research required to develop an effective live-attenuated vaccine against ASFV involves achieving a comprehensive understanding of how the virus nullifies the induction of IFN signalling and downregulates its response in nearby cells. To facilitate the ongoing work for the development of a vaccine against ASFV, we used viral deletion mutants to assess the impact of MGF deletions on ASFV infection in porcine macrophages. We also worked to deconvolute the possible mechanisms of action of several MGF proteins by corroborating hypothetical interactions with host proteins and by assessing their impact on the IFN signalling cascade *in vitro*. The results of our study provide invaluable insight into how ASFV evades host innate immunity and contribute to the ongoing efforts to develop a successful live-attenuated vaccine for ASFV.

From ponds to pharmacies: cyanobacteria as a source of broad-spectrum antivirals

Sole Lancerin[1], Joseph Palmer[2], Nelly Mak[1], Nigel Temperton[3], Richard Sloan[1,4]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]ScotBio Naturally Pioneering, Livingston, United Kingdom. [3]University of Kent, Canterbury, United Kingdom. [4]Zhejiang University, Haining, China

Abstract

Viruses still pose a global threat, driving the need for innovative, broad-spectrum antivirals. One promising approach involves exploring bioactive compounds from overlooked sources. Cyanobacteria are of interest as there is established industry based on their use as dyes and nutraceuticals, but they may also produce pharmacologically active compounds.

In this study we sought to characterize compounds from the cyanobacterium *Arthrospira platensis* to verify its previously reported broad-spectrum antiviral activity and determine its mechanistic bases.

Systematic analysis of different biochemical fractions isolated from *A. platensis* revealed extracellular polysaccharides (EPS) to be active against a panel of enveloped viruses (human respiratory viruses), non-enveloped viruses (Coxsackievirus) and lentiviral pseudotypes (SARS-CoV-2, Nipah virus). We demonstrated that EPS not only blocks viral entry, but also that its mode of action may in part be virucidal, in that EPS directly inactivates viruses, thus preventing infection.

EPS effectively inhibits both enveloped and non-enveloped viruses, and both heparan-sulfate and sialic-acid dependent viruses, suggesting the target to be a shared mechanism. We hypothesize that EPS, with its intricate chemical structure, mimics the host glycocalyx, which all viruses interact with, trapping the viral particles. Experiments are ongoing to detail the molecular composition and structure of EPS and to define its interactions with virions at the molecular level.

EPS displays minimal cytotoxicity, broad-spectrum activity, and potential virucidal properties. Extraction involves scalable processes using industrial waste from *A. platensis* cultivation, ensuring efficiency, cost-effectiveness, and a minimal environmental footprint. These characteristics position EPS as a compelling candidate for globally impactful and effective antiviral therapeutics.

Murine models of SARS-CoV-2 Omicron variants recapitulate aspects of pathogenicity observed in humans

Jamie Barrett-Rodger[1], Katja Finsterbusch[1], David Greenwood[1], Emma Wall[1,2], Andreas Wack[1], David LV Bauer ORCID iD[1]

[1]The Francis Crick Institute, London, United Kingdom. [2]UCLH Biomedical Research Centre, London, United Kingdom

Abstract

Animal models remain essential for assessing pathogenicity and transmissibility of SARS-CoV-2 variants. Reports by multiple groups in early 2020 indicated that SARS-CoV-2 was unable to infect commonly used strains of laboratory mice as a result of differences between human and murine ACE2 receptor. As a result, transgenic mice overexpressing the human ACE2 receptor under the control of the K18 promoter (K18-hACE2) or the Syrian Golden Hamster, which was readily infected by SARS-CoV-2, were rapidly developed as models for SARS-CoV-2.

While pre-Omicron variants of SARS-CoV-2 were highly lethal to K18-hACE2 mice, we found that Omicron BA.1 readily infected these mice, but led to only mild disease with little to no weight loss — despite high levels of viral replication in the lungs, evidenced by RT-qPCR and histopathological examination and quantification of fixed lung sections.

Subsequent Omicron variants, including BA.5 and XBB, however, resulted in more severe disease in the K18-hACE2 model, with significant weight loss observed in mice. We observed deeper lung penetration and more markers of lung damage detected during RNAseq analysis of infected lung tissue as well. In contrast, the more recent BA.2.86 variant caused little to no disease in the K18-hACE2 model.

Remarkably, this increase in pathogenicity paralleled observations of disease symptoms within the Crick/UCLH Legacy Study cohort of healthy, vaccinated adults (NCT04750356): participants infected with BA.5 and XBB reported a significantly higher incidence of fever compared to those infected with BA.1.

Together, our results suggest murine models are useful for the assessment of Omicron-descended SARS-CoV-2 variants.

Virus Workshop: Virus Interaction with the Host Organism and Implications for Pathogenesis

Recombinant Infectious Bronchitis virus containing attenuating mutations in Nsp 10, 14 and in the Macrodomain of Nsp 3 provides complete protection against homologous challenge.

Sarah Keep, Katalin Foldes, Adam Rayment, Giulia Dowgier, Paul Britton, Erica Bickerton

The Pirbright Institute, Pirbright, United Kingdom

Abstract

Infectious Bronchitis Virus (IBV) is a *gammacoronavirus* that infects chickens. IBV results in acute respiratory disease typified by snicking, tracheal rales and loss of tracheal ciliary activity. Vaccination is commonly practiced using live attenuated vaccines (LAVs). Generation of an LAV involves the serial passage of a virulent field isolate through embryonated hens' eggs, typically 80 to 100 times. The molecular basis of attenuation is unknown, and a fine balance needs to be achieved as over-passaging can impact vaccine efficacy. A method of rational attenuation is required. We have previously identified mutations in non-structural proteins (Nsps) 10 and 14 that can attenuate IBV. A concern for LAVs is not only reversion but recombination that could potentially generate a pathogenic IBV. To address this, using reverse genetics we identified a second set of mutations, within the macrodomain of Nsp 3, that results in attenuation. The macrodomain mutant (rIBV-Mac) replicated comparable to the parental IBV *in vitro*. The mutations were stably maintained during passaging *in vitro* and *in ovo*. Chickens infected with rIBV-Mac exhibited reduced clinical disease and retained tracheal ciliary activity. Vaccination of chickens with either rIBV-Mac or a rIBV containing both the macrodomain mutations and previously identified attenuating Nsp 10 and 14 mutations offered complete protection from homologous challenge. The presence of multiple attenuating mutations did not negatively impact vaccine efficacy. The residues targeted in this study are conserved amongst IBV strains and the coronavirus family offering a potential method of rational attenuation that can be universally applied for vaccine development.

ZIKV sfRNA production: a tale of two hosts

Taissa Ricciardi-Jorge[1], Skye Storrie[2], Stephen Nuthalapati[1], Fanni Gergely[3], Trevor Sweeney[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]University of Oxford, Oxford, United Kingdom

Abstract

As a Flavivirus species, *Zika virus* (ZIKV) genomic RNA consists of a linear positive sense single-stranded RNA ~11kb in length. Its genome encodes structural and nonstructural proteins and has highly structured 5' and 3' untranslated regions (UTR). During ZIKV infection, short flaviviral RNAs (sfRNAs) are produced together with full-length genomic RNA. sfRNAs in flaviviruses have been reported to enhance virus replication by various mechanisms including the sequestration of host proteins and the inhibition of interferon (IFN) expression and IFN-simulated gene (ISG) translation. sfRNAs are generated by stalling the highly conserved 5'-3' exoribonuclease 1 (XrnI) at specific tertiary structures (xrRNA) in the viral 3'UTR. ZIKV has three such structures, resulting in the presence of three sfRNA species. Recent studies have reported that disruption of xrRNA2 structure disrupts all sfRNA production during ZIKV infection and, using an alternative mutation strategy, we confirm this finding. However, combining traditional *in vitro* RNA digestion assays and a newly developed in vitro RNA structure probing assay we show that, although the mutations efficiently disrupt xrRNA2, surprisingly all other elements of the 3'UTR remain intact. Furthermore, analysis of the stability of the mutation revealed the distinct selective pressure mammalian and insect cells impose on the virus evolution. Together these results show that the presence of RNA structure alone is not sufficient for sfRNA generation during infection and point to the existence of an additional host factor involved in sfRNA production in mammalian cells.

Neurovirulence of SARS-CoV-2 and associated differences between variants of concern (VOC)

Luke Jones ORCID iD, Edward Drydale, Katie Holden, Laurissa Havins, James Bancroft, Phalguni Rath, Lahiru Handunnetthi, Miles Carroll

Center for Human Genetics, University of Oxford, Oxford, United Kingdom

Abstract

SARS-CoV-2 is the aetiological agent of COVID-19, a disease responsible for a world-wide pandemic and considerable mortality and morbidity. Like other coronaviruses, it has been previously linked with a neurological tropism, although the exact virological and cellular mechanisms underpinning these infections remain under-studied. Here we show not only is SARS-CoV-2 neurovirulent, we identify that it can specifically infect and replicate within neurons and is largely non-infectious in microglial cells. We also observe differences between VOCs, with the ancestral strain (Vic01) displaying the highest levels of neurotropism. The predominant mode of spread appears to be cell-to-cell, with minimal levels of infectious virus detectable in the supernatant, regardless of VOC. We have also infected 3D neuronal models (human brain organoids) as a more physiologically relevant model system, and continue to see infection within defined mature neuronal populations. Together our results illustrate that relatively minor genetic changes can underpin major phenotypic differences in tropism and neurovirulence, as well as shedding light on how SARS-CoV-2 infection could lead to serious neurological sequelae such as encephalitis and long COVID.

Unravelling the structure of the interaction between the 5’ Chikungunya virus RNA and the cellular RNA-binding protein Musashi-2

Francesca Appadoo, Kaiwen Sun, Yuqian Liu, Juan Fontana, Andrew Tuplin

University of Leeds, Leeds, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a positive-single stranded RNA virus, belonging to the alphavirus family. CHIKV is linked to arthralgia symptoms, which can persist for years, making it a highly morbid pathogen. From ~2000, CHIKV has been the cause of epidemics in India, Southern Europe and the Americas. To date, no antivirals are available.

We have previously shown that the 5’ end of CHIKV RNA contains secondary structures essential for the survival of the virus via interactions with other RNA structures present within the viral genome. Here we present results showing that this highly dynamic region can be visualised by electron microscopy (EM) approaches. Initially, we confirmed by negative staining EM that the folded 5' end of CHIKV RNA appears in a small number of defined conformations, as assessed by 2D classification. We have also used cryo-EM together with an optimised data processing workflow to determine a higher resolution 3D model of dynamic RNA structures within the CHIKV genome.

Following optimisation of this approach, we are currently investigating by cryo-EM the interaction between RNA structures in the 5’end of the CHIKV genome and Musashi-2 RNA binding protein (MSI-2) – a host encoded protein that we demonstrated is essential to CHIKV genome replication. Initial biochemical and reverse genetics results suggest the formation of an RNA-protein complex. Overall, this work will allow us to further elucidate the role of this region within the infection cycle of CHIKV and better understand how essential these RNA structures are within the replication and translation of the genome.

Quantitative Temporal Viromic Analysis of HCMV Infected Dendritic Cells Reveals Virus Manipulation of Adaptive Immunity and Host Restriction of Viral Replication.

Lauren Kerr-Jones[1], Lior Soday[2], Nia Cwyfan Hughes[1], Kelly Miners[1], Edward Wang[1], Michael Weekes[2], Richard Stanton[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

Human cytomegalovirus (HCMV) is a herpesvirus that causes serious complications in immunocompromised individuals, including in transplant recipients and infants. *In vivo*, HCMV displays a wide tropism with myeloid cells a key site of both latency and reactivation. However passaged strains lose tropism for many of these cell types, thus a significant proportion of our understanding of HCMV arises from studies in fibroblasts. Yet due to the unique roles that myeloid cells play in the induction of protective immunity, the biology of infection in these cell types may be very different. Analysis of HCMV infection in these cells has previously been limited due to passaged HCMV strains accruing mutations that change the route of virus entry, hence passaged strains infect predominantly by the cell-free route, as opposed to the cell-cell route that dominates *in vivo*.

We therefore developed systems to infect dendritic cells (DCs) with wildtype HCMV, using direct cell-cell transfer, then to purify newly infected DCs in order to generate an unbiased proteome-wide view of how HCMV modulates primary DCs across an infectious time course. This revealed >400 proteins modulated by infection, the majority of which were unique to DCs, and had important roles in the induction of adaptive immunity. Furthermore, we found that in DCs, production of viral progeny was limited as a result of APOBEC proteins. Our data demonstrate the complex interactions between HCMV and primary immune cells, and provide a way to investigate how HCMV systematically subverts the induction of an adaptive immune response *in vivo*.

Dengue virus NS4A modulates NF-κB signalling and viral replication in Aedes aegypti mosquitoes

Mathilde Laureti

The Pirbright Institute, Woking, United Kingdom

Abstract

Dengue viruses (DENVs) are flaviviruses transmitted by mosquitoes and responsible for significant acute arthropod-borne viral (arboviral) diseases in humans. No antiviral treatments are available for DENV, and the licensed vaccine cannot be used in all settings. As such, the development of new vector control strategies is crucial. The immune system of DENV's main vector, *Aedes* aegypti, is a known barrier to transmission that could be manipulated to develop transmission-incompetent mosquitoes. As such, it is imperative to better understand virus-immune interaction in the vector. We demonstrated for the first time that viral RNA can be sensed by the NF-κB-regulated immune-deficiency (IMD) signalling pathway *in vitro*. Using RNA-Seq and qRT-PCR, we show that poly(I:C) induces an NF-κB-regulated immune response *in vivo* that is distinct from the repertoire of immune genes activated by other immune stimuli. We also demonstrated that DENV-2 infection and its viral protein NS4A can inhibit the exogenous induction of the IMD pathway. Transgenic mosquito lines were engineered to express the NS4A protein from DENV-2. Immune stimulation of these transgenic mosquito lines showed that DENV NS4A can significantly reduce the IMD pathway. The influence of NS4A was also studied during viral infection with Semliki Forest virus, an alphavirus known to induce NF-κB-regulated genes *in vivo*. Our findings give us a deeper understanding of how the mosquito immune system detects viral infections and how DENV's ability to escape the mosquito immune response facilitates viral transmission, which could potentially allow us to develop transmission-incompetent vectors to reduce the burden of dengue disease.

Human astrovirus causes region-specific changes in the intestinal epithelial barrier

Andres Rivera, Jonathan Golob, Christiane Wobus ORCID iD

University of Michigan, Ann Arbor, USA

Abstract

The intestinal epithelium balances selective permeability to ions and solutes with barrier functions towards pathogens, but its make-up differs along the cephalocaudal axis. Pathogen-induced disruptions of this barrier is one trigger for intestinal diseases, which can also be region specific. One pathogen that infects the intestine and breaches the intestinal epithelial barrier is human astrovirus (HAstV). HAstVs are positive-sense single-stranded RNA viruses responsible for causing millions of diarrhea cases all around the world, especially in children, the elderly, and immunocompromised people. Despite their significant burden, no effective antivirals or vaccine candidates against HAstV have been developed due to major knowledge gaps in astrovirus pathogenesis.

Previous work has shown that classical HAstV serotype 1, but not the non-classical HAstV VA1, can disrupt polarized Caco-2 cell monolayers by degrading tight junction proteins. Intriguingly, our data show that VA1 can alter the transepithelial electrical resistance of polarized colon-like T84 monolayers but not small intestinal organoids, suggesting region-specific alterations in the barrier. However, VA1 did not alter the paracellular flux of a 4kDa FITC-dextran tracer molecule in T84, nor did it change the differential release of virions into the apical vs basolateral compartment. These data are consistent with virus-induced changes in the pore but not leak pathway, two types of paracellular passages. Transcriptomic analysis of VA1-infected polarized monolayers from T84 and human intestinal organoids revealed dysregulation of gene expression of tight junction proteins and ion transporters, some of these in a colon-specific manner. Studies are ongoing to identify proteins mediating these region-specific barrier changes.

Complement-Mediated Enhancement of SARS-CoV-2 Neutralisation: Insights into Mechanism

Jack Mellors ORCID iD[1,2], Stephen Laidlaw[1,2], OCTAVE Consortium[1], OPTIC Consortium[1], Eleanor Barnes[1], Susanna J. Dunachie[1], Paul Klenerman[1], Miles Carroll[1,2]

[1]University of Oxford, Oxford, United Kingdom. [2]Pandemic Sciences Institute, Oxford, United Kingdom

Abstract

The complement system is part of the innate immune response to pathogens and has the potential to enhance antibody-mediated neutralisation potency. Despite its significance, the complement system is commonly excluded from neutralisation assays via the heat-inactivation, or processing methods, of plasma/sera samples. This can negatively impact our interpretations from conventional neutralisation assays, which are often used to assess vaccine efficacy, therapeutic options, and correlates of protection.

We have shown that the addition of exogenous plasma (as a source of complement) to SARS-CoV-2 neutralisation assays can enhance the neutralising antibody potency of vaccinee serum samples. This effect was lost following heat-inactivation of the complement source. The level of neutralisation enhancement also differed significantly between: serum from healthy or immunocompromised vaccine recipients, neutralisation of the VIC01 or BA.1 SARS-CoV-2 strains, and the use of Vero E6 or Calu-3 cells. These differences may offer some insight into the mechanism of enhancement. We are utilising purified proteins from the complement system, complement C3/C5-depleted plasma, and electron microscopy to determine the possible mechanisms of action. Our observations that a heat-labile component of plasma (indicative of complement) can enhance antibody neutralisation titres by up to a 32-fold increase has implications for vaccines, therapeutics, and correlates of protection. Our ongoing efforts to identify the mechanism of enhancement could be extrapolated to determine when the complement system is an asset to neutralisation, which could further our understanding of protection against new viral variants and provide considerations for vaccine design.

Dynamics of antigen-specific B cell response to porcine respiratory coronavirus infection

Emily Briggs[1,2], Basudev Paudyal[1], Joseph Newman[1], Brigid Veronica Carr[1], Sarah Keep[1], Erica Bickerton[1], Christine Rollier[2], Marie Di Placido[1], Elma Tchilian[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Coronavirus emergence in humans is associated with zoonotic transmission and has been a significant public health threat in recent years. Given the recent public health threat caused by coronaviruses, it is essential to dissect the antibody response and understand how these viruses are controlled by the immune system. We have established a robust pig model for porcine respiratory coronavirus (PRCV) infection and shown that the immune responses and lung pathology are similar to those induced by SARS-CoV-2 in humans.

Pigs were challenged with PRCV and culled at 1-, 5- and 12-days post infection (DPI). An extensive tissue panel including blood, spleen, nasal swabs, lymph nodes, bronchoalveolar lavage (BAL), lung, and tracheobronchial lymph nodes were collected to assess virological, clinicopathological and immunological parameters of disease. The antigen specificity and function of humoral responses were evaluated in serum and BAL, detecting antigen-specific and neutralizing antibodies from 7 DPI. Extensive flow cytometry staining panels were used to analyse the kinetic of the spike-specific B cell response in local and systemic tissues, and to isolate spike-specific B cells from the PRCV-infected animals to generate monoclonal antibodies. Single cell sequencing was performed on the isolated B cells, followed by cloning and expression of the resulting heavy and light chains. These were subsequently tested for their binding and functional properties.

This study provides comprehensive insights into the antibody response to coronaviruses in the highly relevant pig model. The findings will aid in identifying better control strategies for both human and animal health.

A novel pipeline for the identification of accessory genes in coronaviruses.

Federico De Angelis ORCID iD, Hou Wei Chook, Samantha Lycett ORCID iD, Paul Digard ORCID iD

The University of Edinburgh, Edinburgh, United Kingdom

Abstract

Many clinically important coronaviruses encode functionally-important accessory genes in the 2nd frame of their N gene, expressed through leaky ribosomal scanning. To ask whether this is a general feature of coronaviruses, we developed a pipeline to identify and score potential accessory genes. For 113 coronavirus species from all genera, we analysed open reading frame (ORF) lengths, genomic coordinates, start codon Kozak contexts and the presence of intervening AUG codons between N and putative accessory ORF starts. Results showed that β-coronaviruses had the highest number of strong candidate accessory genes, followed by δ- and then α-coronaviruses, while γ-coronaviruses had none. Our pipeline also identified many more gene candidates than a previously published method which only assesses ORF length. Transient expression of N genes from merbecoviruses not previously shown to produce poly-cistronic N mRNAs confirmed expression of accessory polypeptides with the expected molecular weights, supporting the sensitivity of our approach. Sequence analyses of sub-genera showed conservation of accessory gene start and stop codons and that introduction of premature termination codons is the cause of accessory gene variability among closely related viruses. Human-infecting MERS-CoV, OC43, HKU1 and NL63 have truncated ORFs compared to other members of their respective sub-genera. Temporal phylogenetic analyses of MERS-CoV indicated that that its N-encoded accessory gene may be reverting to an ancestral merbecovirus longer form. Overall, our pipeline shows that a functional overlapping ORF in the N gene is a widespread feature of β- and δ-coronaviruses that may have evolved after a split from an α-coronavirus ancestor.

Synergy between HA cleavage site sequence and NA-mediated plasminogen recruitment as a virulence mechanism for low pathogenic avian influenza

Hui Min Lee[1], Kate Sutton[1], Rute Maria Pinto[1], Lonneke Vervelde[1], Sjaak de Wit[2], Paul Digard[1]

[1]The Roslin Institute, Edinburgh, United Kingdom. [2]Royal GD, Deventer, Netherlands

Abstract

An outbreak of H3N1 low pathogenic avian influenza viruses in Belgium in 2019 caused unexpected levels of mortality and morbidity for a virus with an IVPI of 0.13. Schon and colleagues(a) proposed that HA cleavage mediated by NA-driven plasminogen recruitment was responsible for the systemic spread and pathogenicity of this virus. Aligning HA sequences of the outbreak viruses with other H3 strains also showed an unusual sequence around the HA cleavage site, as well as the previously noted plasminogen binding-associated NA polymorphisms. As part of the FluNuance ICRAD consortium, we established a reverse genetics system for A/chicken/Belgium/460/2019 (Ck/Belgium) and created single mutations in HA (K345R), and NA (S122N) that restored the viruses to normal consensus, as well as an HA/NA double mutant. Confirming previous work(a), trypsin-independent spread in cell culture and HA cleavage of wild type Ck/Belgium was observed in the presence of serum containing plasminogen. Dose-dependent HA cleavage and trypsin-independent spread was also observed in the presence of purified chicken plasminogen. Compared to the wild type, both HA cleavage and spread were lower in HA mutant and further blocked by NA mutation S122N. Similar observations were also found in chicken enteroids infected with the wild type or mutant viruses in the presence of plasminogen. Furthermore, *in ovo* tests showed that all the mutant viruses had significantly lower titres in embryos. We conclude that the unusual HA cleavage site and NA-mediated plasminogen recruitment work in concert to increase the virulence of Ck/Belgium.

(a)DOI: 10.1371/journal.ppat.1009490

A burning problem: Sunburn enhances host susceptibility to arbovirus infection

Ailish McCafferty ORCID iD[1], Andrew Durham[1], Joe Fryer[1], Lewis Kajtar[1], Emilie Pondeville[2], Kave Shams[1], Clive McKimmie[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

As climate change expands mosquito habitats, mosquito-borne virus infections such as Zika, Dengue, and Chikungunya are on the rise. These arbovirus infections are transmitted in warm climates with high UV radiation levels from the sun. A rapid, robust antiviral immune response, marked by Type I interferon induction in the skin, is crucial for curbing arbovirus replication and dissemination, which in turn limits severe disease. However, UV exposure has immunomodulatory effects on the skin. We hypothesised that UV interferes with antiviral responses, altering host susceptibility to arbovirus infections in UV-exposed skin. Here we show that prior erythemal UV exposure, equivalent to sunburn, increases host susceptibility to arbovirus infection *in vivo* by driving recruitment of cellular targets of infection whilst delaying antiviral interferon responses. Mice exposed to erythemal UV before infection had significantly higher virus levels at the inoculation site. The UV exposure drove a strong inflammatory response to the mosquito bite, including an influx of monocytes to the infection site. Rather than being beneficial to the host response, these cells were preferentially infected by the virus. Critically, the induction of antiviral type I interferons in UV-exposed skin occurred too late to influence the overall infection outcome. UV-mediated modulation of the antiviral immune response facilitated virus dissemination to other tissues, a pivotal step in severe disease development, resulting in reduced survival rates in UV-exposed mice. This work identifies UV as a crucial environmental factor influencing arboviral disease severity, which is particularly relevant given the climate in arbovirus transmission zones.

Emergence and spread of feline infectious peritonitis due to a highly pathogenic canine/feline recombinant coronavirus

Amanda Warr ORCID iD[1], Charalampos Attipa ORCID iD[2,3], Demetris Epaminondas ORCID iD[4], Marie O'Shea ORCID iD[1], Sarah Fletcher ORCID iD[1], Alexandra Malbon ORCID iD[2], Maria Lyraki ORCID iD[5], Rachael Hammond[2], Alexandros Hardas ORCID iD[6], Antria Zanti[3], Stavroula Loukaidou[3], Michaela Gentil[7], Danielle Gunn-Moore ORCID iD[2], Stella Mazeri ORCID iD[2,1], Christine Tait-Burkard ORCID iD[1]

[1]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, United Kingdom. [2]Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, United Kingdom. [3]Vet Dia Gnosis Ltd, Limassol, Cyprus. [4]Veterinary Services, Ministry of Agriculture, Natural Resources and Environment, Nicosia, Cyprus. [5]Vets4Life Referral Hospita, Athens, Greece. [6]Department of Pathobiology and Pouplation Sciences, Royal Veterinary College, Hatfield, United Kingdom. [7]Laboklin GmbH and Co KG, Bad Kissingen, Germany

Abstract

Cross-species transmission of coronaviruses (CoVs) poses a serious threat to both animal and human health. Whilst the large RNA genome of CoVs shows relatively low mutation rates, recombination within genera is frequently observed and demonstrated. Companion animals are often overlooked in the transmission cycle of viral diseases; however, the close relationship of feline (FCoV) and canine CoV (CCoV) to human hCoV-229E, as well as their susceptibility to SARS-CoV-2 highlight their importance in potential transmission cycles. Whilst recombination between CCoV and FCoV of a large fragment spanning orf1b to M has been previously described, here we report the emergence of a novel, highly pathogenic FCoV-CCoV recombinant responsible for a rapidly spreading outbreak of feline infectious peritonitis (FIP), originating in Cyprus. The recombination, spanning spike, shows 97% sequence identity to the pantropic canine coronavirus CB/05. Infection is spreading fast and infecting cats of all ages. Development of FIP appears rapid and likely non-reliant on biotype switch. High sequence identity of isolates from cats in different districts of the island is strongly supportive of direct transmission. A deletion and several amino acid changes in spike, particularly the receptor binding domain, compared to other FCoV-2s, indicate changes to receptor binding and likely cell tropism.

Insights on regulated proteolysis of host and viral proteins in a time course infection of Murine Norovirus and SARS-CoV-2

Leandro Neves ORCID iD, Philip Brownridge ORCID iD, Ed Emmott ORCID iD

University of Liverpool, Liverpool, United Kingdom

Abstract

Regulated proteolysis is pivotal for the replication of many single-stranded, positive-sense RNA (+ssRNA) viruses. In these viruses, replication starts with the translation of the 5' open reading frame encoding non-structural (NS) proteins that are co- and post-translationally cleaved by viral proteases. Although the proteolytic processing of polyproteins is conserved among +ssRNA viruses, the complexity of this cascade will vary depending on the number of proteases and non-structural proteins present in the virus genome. For instance, SARS-CoV-2 has 16 NS proteins of which two are papain-like (PLpro) and 3C-like proteases (3CLpro/Mpro) whilst Murine Norovirus (MNV) has a simpler genome with 7 NS proteins and one 3C-like protease (3CLpro). But markedly, upon infection and viral replication, cellular proteins are also prone to undergo proteolytic processing mediated by either viral proteases or host proteases, and in the absence of these cellular targets, both replication and production of virions are impaired. In this work, we applied mass spectrometry-based N-terminomics for the global characterisation of proteolysis using a time course infection model of MNV and SARS-CoV-2. Our results show the temporal dynamics of cleavage events during infection. These include proteolytic processing targeting viral and cellular proteins. We also identified cleavage sites within cellular proteins that match the MNV and SARS2 protease consensus sequences and show temporal regulation during infection. This work expanded the knowledge of novel substrates undergoing proteolytic processing during *in vitro* MNV and SARS-CoV-2 infection and presents quantitative information on how these events are regulated over time.

Investigating the differences in sgmRNA regulation between Alpha and Gamma coronaviruses.

Harry Francis[1,2], Graham Freimanis[1], Ian Brierley[2], Andrew Firth[2], Sarah Keep[1], Erica Bickerton[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]The University of Cambridge, Cambridge, United Kingdom

Abstract

Coronaviruses express their structural and accessory genes via the process of discontinuous transcription, that produces a nested set of subgenomic mRNAs (sgmRNAs) which relies on the use of two complementary transcription regulatory sequences (TRSs); one located at the 5' end of the genome (TRS-L) and one upstream of each gene (TRS-B) the TRS sequence and length differ between coronaviruses. work in the *alphacoronavirus* Transmissible gastroenteritis virus (TGEV) has identified a core sequence (CS) within the TRS, shared between the TRS-L and all TRS-Bs within the genome. Mutations within this CS and the adjacent flanking regions impact sgmRNA production. In th*e gammacoronavirus* infectious bronchitis virus (IBV) no CS has been identified with natural variations between the TRS-L and TRS-Bs observed. Moreover, IBV has low levels of conservation in the flanking regions of the TRS-Bs and sgmRNAs produced from noncanonical TRS-Bs have been demonstrated. Unlike IBV, using RNASeq and ribosomal profiling we have not identified sgmRNAs produced from noncanonical TRS-Bs during TGEV or the related PRCV *in vitro* infection suggesting less flexibility in TRS-B sequence. Using reverse genetics several IBV mutants have been produced containing mutations in or around a noncanonical TRS-B. The expression of the related sgmRNA, viral growth kinetics and genetic stability of the mutations have been assessed both *in vitro* and *ex vivo*. Mutation of a highly conserved downstream nucleotide causes a significant decrease in sgmRNA expression, whilst mutations within the TRS-B had less significant impact. Our work may suggest differences in regulation of sgmRNA expression between coronaviruses.

Comprehensive quantitative temporal proteomic, transcriptomic and effectome analysis of mpox

Martin Potts[1,2], Xinyue Wang[1,2], Yongxu Lu[3], Samuel Richardson[4], Robin Antrobus[1], Jenna Nichols[5], Quan Gu[5], Charlotte Cook[4], Dagmara Bialy[4], Pip Beard[4], Bryan Charleston[4], Ana Da Silva Filipe[5], Geoffrey Smith[3], Chris Boutell[5], Jonas Albarnaz[4], Michael Weekes[1,2]

[1]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [3]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. [4]Pirbright Institute, Woking, United Kingdom. [5]MRC-University of Glasgow Centre for Virus Research (CVR), Glasgow, United Kingdom

Abstract

Mpox is a zoonotic disease caused by monkeypox virus (MPXV). From May 2022, a multi-country outbreak has affected >80,000 individuals worldwide with sustained human-to-human transmission. Although therapeutic measures are available, including immunisation and antiviral chemotherapy with tecovirimat, these are not widely accessible in most countries. Resistance to tecovirimat has been detected, reinforcing the urgent need to study MPXV in human cells in order to develop novel therapies.

The outcome of infection is partly determined by the interplay between host antiviral restriction factors (ARFs) and their viral antagonists. Many viruses have evolved to subvert cellular protein degradation pathways to degrade ARFs. Here, we present quantitative temporal proteomic and transcriptomic analysis of MPXV infection in human fibroblasts utilising a currently circulating strain (CVR/MPXV/S1a), including the use of proteasome inhibitors to identify degraded ARFs. Notably, our data indicate that MPXV induces strong upregulation of the pro-inflammatory mediators IL-6 and IL-1β in contrast to other poxviruses such as vaccinia and initiates the proteasomal degradation of the cell-death regulators Caspase-1 and RIPK3.

We also present a novel mass spectrometry-based MPXV 'effectome' analysis, characterising the impact of expression of 96 individual MPXV proteins on the host proteome, enabling identification of viral proteins inducing degradation of individual host factors. This study will provide deeper insights into how MPXV interacts with human antiviral defences and may ultimately offer routes for generation of novel therapeutics against mpox.

Characterisation of Chikungunya virus (CHIKV) pathogenesis in the Tamarin (*Saguinus labiatus*) model

Claire Ham, Sarah Kempster, Deborah Ferguson, Daniel Yara, Jo Hall, Neil Berry, Neil Almond

MHRA, South Mimms, United Kingdom

Abstract

Chikungunya virus (CHIKV) is an arbovirus, within the family *Togaviridae*, genus *Alphavirus*. Over 2 million cases have been reported since 2005 and in 70% of cases, infected individuals suffer long-term arthralgia. As the range of the mosquito vector expands to include naïve populations, vaccine development is critical to control the economic burden of CHIKV disease.

To accelerate vaccine development, we have developed a new-world non-human primate model for CHIKV. The Red bellied Tamarin (*Saguinus labiatus*) model of CHIKV infection will support future studies designed to test the breadth and persistence of vaccine or innate protection to CHIKV infection. The smaller size of this model animal has advantages when investigating human vaccine response.

Here we present evidence of the susceptibility of Red bellied Tamarins to CHIKV infection with detectable viremia by RT-qPCR. Animals were challenged with a low dose of CHIKV East/Central/South African (ECSA) isolate OPY-1 and serum samples were taken during the first 14 days and tissues were taken at days 14 and 28 post-challenge. We also present innate transcription profiling and identify pathways that are differentially expressed after challenge and correlate these with blood cytokine levels. We compare this with similar profiles from other model species.

Understanding differentially regulated pathways, affected during CHIKV infection, will improve modelling of human disease states and support vaccine and therapeutic developments.

Optimising synergistic latency reversal approaches for host-mediated immune clearance of the latent HIV-1 viral reservoir

Dylan Postmus ORCID iD[1,2,3], Baxolele Mhlekude ORCID iD[2,3,4,5], Jenny Jansen[2,3], Bengisu Akbil ORCID iD[2,3], Christine Goffinet ORCID iD[1,2,3]

[1]Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]Institute of Virology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany. [3]Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Berlin, Germany. [4]Virology and Innate Immunity Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany. [5]Institute of Genetics, Technische Universität Braunschweig, Braunschweig, Germany

Abstract

A considerable barrier in the development of an effective and scalable HIV-1 cure is the longevous reservoir of latently infected cells that persist despite highly effective, combinatorial antiretroviral therapy. The “shock and kill” approach, however, proposes the pharmacological reactivation of proviral transcription with the goal of rendering latently infected cells, now producing *de novo* viral components, targets for killing by patrolling immune cells. We propose that the observed ineffectiveness of this approach in shrinking the viral reservoir, despite relatively efficient reactivation, is underpinned by counterproductive host responses to therapy and a frequently overlooked viral component. To explore this, we investigated the implications of treatment with latency reversal agents Bryostatin-1, a PKCa, and AZD5582, an iIAP, as activators of the canonical and non-canonical NF-κB signalling pathways respectively, alone or in combination with the iBET, JQ-1. In focus are the effects of these synergistically-acting treatment regimes on transcriptomic and epigenomic profiles, altered host-virus interaction and the susceptibility of latently infected cells to immune clearance. Using a novel reporter model of HIV-1 latency, we are able to detect dynamics of viral protein expression and presentation of antigens to humoral immune mechanisms. Furthermore, we show that treatment with Bryostatin-1 induces several blocks to immune-mediated killing of target cells. In contrast, the iAP-iBET combination appears to foster a cell state more conducive to immune clearance. However, we are also currently investigating how the action of the HIV-1 accessory protein Nef may be a major, underappreciated obstacle for “shock and kill” by inhibiting apoptosis.

Detection of insertions and deletions (indels) in high-throughput sequencing data of foot-and-mouth disease virus isolates circulating in the field.

Lidia Lasecka-Dykes ORCID iD, Valérie Mioulet, Jemma Wadsworth, Hayley M. Hicks, Nick J. Knowles, Donald P. King

The Pirbright Institute, Pirbright, Woking, United Kingdom

Abstract

RNA viruses exist as a swarm of closely related, but non-identical genomes. This relatively high frequency of mutations is due to error-prone viral polymerase and high replication turnover. While large number of studies focused on identification of single nucleotide variants (SNVs), the impact of insertions and deletions (jointly called indels) on diversity and evolution of RNA viruses remains less studied. This is due to more problematic detection of indels comparing to the SNVs.

Foot-and-mouth disease virus (FMDV) causes an economically important disease of livestock, and FMDV genomes carrying indels even up to 76 nucleotides long were previously identified in field samples. However, the process in which these indels occur remains unknown.

In here, we attempted detection of indels in high-throughput sequencing (HTS) data, obtained as a part of routine diagnostic of FMDV field samples. The sensitivity and specificity of the method was assessed by spiking HTS data with artificial reads containing known indels, allowing us to detect small and medium indels carried by individual reads. Using this approach to investigate FMDV field samples, most indels occurred at a low frequency, oscillating at the level of sequencing error, and were equally distributed along the genome. Contrastingly, sporadic genomic regions showed accumulation of indels exceeding the sequencing error threshold. Overall, our work confirms that indels are a constant feature of viral swamp structure, with occasional insertions or deletions being promoted by the positive selection.

SARS-COV2 and Influenza A virus co-infection in hACE2 mice model

Parul Sharma[1], Ellie Bentley[1], Samar Alosaimi[1], Adam Kirby[1], Anja Kipar[1,2], James Stewart[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Zurich, Zurich, Switzerland

Abstract

The coronavirus disease 19 (COVID-19) pandemic has devastating effects on human health worldwide. It is a respiratory disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV2) and studies reported that to date 6.9 million people lost their lives and infected over 699 million cases worldwide.

SARS-COV2 and influenza viruses are transmitted via similar mechanisms and co-infection of SARS-COV2 virus with seasonal respiratory viruses, predominantly influenza A virus (IAV) is a global health concern. In this study, we investigated the outcomes of disease when hACE2 mice were sequentially infected with influenza A virus (IAV) followed by SARS-COV2 virus. In this study, we utilized 6-8-week-old transgenic mice expressing the human K18-hACE2 receptor on epithelial cells. Mice were infected with 100 PFU of the X31 strain of IAV and after 3 days post-infection, mice were sequentially infected with 10000 PFU of SARS-COV-2 virus. Mice were regularly health monitored and sacrificed after 7 days post SARS-COV2 infection. The tissue samples such as lung, brain, and nasal turbinates were harvested for qPCR and histopathology analysis. The transcriptomic analysis was also performed to see differential gene expression and gene ontology pathways. Our data suggested that mice with co-infection of both viruses experienced rapid weight loss, more severe clinical symptoms, and lung damage as shown by histopathology. SARS-COV2 and IAV Co-infection also leads to cytokine storm and causes severe pneumonia and encephalitis. Transcriptomics analysis showed processes such as “cytokine production” and “innate immune response” are upregulated in co-infected mice.

The role of ORF10 in SARS-CoV-2 pathogenesis

Shichun Gu[1], Rachel Milligan[1], Isobel Webb[1], Abdulaziz Almuqrin[1], Andrew Davidson[1], James Stewart[2], David Matthews[1]

[1]University of Bristol, Bristol, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

ORF10 is predicted to be the last gene on the SARS-CoV-2 genome but it is still an open question as to whether it is a functional ORF – concrete evidence of its expression in infection is limited and a reliable antibody for detection of the encoded protein is not available. It has been reported that ORF10 over expression in cell culture has an effect on innate immune pathways – possibly through mitochondria - but cilia function has also been implicated. RNA structure analysis indicates that ORF10 is in a highly structured part of the genome which may explain it's apparent conservation.

We made an ORF10KO virus (by mutating the start codon and an internal methionine codon to stop codons) and a control virus in a Wuhan type background (with the D614G mutation in the spike protein). We found that the two viruses have distinct growth properties in different cell lines. Moreover, in the hamster model of infection the ORF10KO virus is highly attenuated in vivo and rapidly reverts to wild type. Further analysis of the ORF10KO virus and derivatives should help us determine if there is indeed a functionally relevant protein or if this striking phenotype is solely due to a highly specific RNA structure.

Phylodynamic analyses of A(H5N1) highly pathogenic avian influenza viruses reveal reassortment dynamics and diversification of host specificity

William Harvey ORCID iD, Lu Lu, Rute Pinto, Paul Digard, Samantha Lycett

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Since 2021, highly pathogenic avian influenza (HPAI) A(H5N1) viruses have caused a panzootic of unprecedented scale. This has affected both wild and domestic birds with high mortality outbreaks in atypical avian host species. Spill-overs in a diverse range of mammals have raised concerns over zoonotic potential. These viruses readily exchange gene segments with local low pathogenic avian influenza viruses via reassortment, a mechanism that can facilitate dramatic phenotypic change. Observational data suggest changes in the seasonality of recent HPAI A(H5N1) viruses and relaxation of host specificity, however virological or ecological explanations remain elusive.

Using phylodynamic approaches, we reconstructed spatio-temporally resolved phylogenetic trees and examined genomic trends relative to pre-panzootic A(H5N8) viruses. We detect several lineages emerging through reassortment of the internal gene segments and infer variation in the evolutionary trajectories of reassortant lineages. To explore changes in host specificity, we used tree-based metrics to estimate fitness in different host types, finding for example, a lineage adapted to spread in the avian order *Charadriiformes*. This demonstrates that phenotypic diversification accompanies the genotypic diversification. Machine learning approaches identify environmental factors and wild bird distributions that predict the areas in which different reassortment lineages spread highlighting ecological diversification.

We explain how genetic diversification of A(H5N1) through reassortment has consequences for spill-over opportunities at the wildlife-domestic poultry interface. Furthermore, a relaxation of host tropism increases the likelihood of endemicity in a range of host types raising the opportunities for further reassortment resulting in novel genotypes with potentially further changed characteristics.

Virus Workshop: Viruses: Molecular Machines to Understand Cellular Processes

Identification of influenza A virus segment 7 nucleotide 730 as a key residue that controls differential mRNA splicing

Shu Zhou[1], Josh D Jones[2,3], Hui Min Lee[1], Rute Maria Pinto[1], Eleanor R Gaunt[1], Helen Wise[1,4,5], Paul Digard[1]

[1]The Roslin Institute, University of Edinburgh, EH25 9RG, Edinburgh, United Kingdom. [2]Division of Virology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [3]Infection Medicine, Edinburgh Medical School: Biomedical Sciences, University of Edinburgh, Chancellor's Building, 49 Little France Crescent, EH16 4SB, Edinburgh, United Kingdom. [4]Division of Virology, Department of Pathology, University of Cambridge,, Cambridge, United Kingdom. [5]Royal Infirmary of Edinburgh, NHS Lothian, Edinburgh, United Kingdom

Abstract

Alternative splicing events allow production of up to 4 mRNA species from segment 7 of influenza A viruses (mRNA1-4). Unspliced mRNA1 encodes the M1 protein whilst the M2 ion channel is translated from mRNA2. mRNA4 was more recently identified in a minority of virus strains to encode M42 which can functionally replace M2. mRNA3 encodes a hypothetical 9-amino acid peptide with no attributed function. Sequence polymorphisms around the splice donor sites for mRNAs 2 and 4 determine relative expression of M2 and M42. However, bioinformatic analysis also shows marked RNA conservation in the "polypyrimidine tract" region upstream of the single splice acceptor site in the segment, although in IAV this sequence is purine-rich. The introduction of synonymous mutations into this region of the A/Puerto Rico/8/1934 virus drastically reduced virus fitness and led to selection of reversion or adjacent pseudo-reversion mutations. Examination of segment 7 expression in a minireplicon system showed that the original mutations led to loss of mRNA2 and hence M2 expression, which were restored by the pseudo-reversion mutations. Further analysis identified mutation A730G as the key disruptor of mRNA2 production. Introduction of this mutation into segment 7s from other strains of IAV also blocked mRNA2 production. In addition, mutating residue 730 in an H5N2 segment 7 that naturally produces mRNA4 led to loss of this spliced mRNA. However, A730G did not inhibit mRNA3 splicing in the A/Udorn/72 segment, but increased it. We conclude that nucleotide 730 is a key modulator of segment 7 splice acceptor function.

Large scale screening of rotavirus genome segments identified a conserved upstream start codon in the NSP3 segment

Hou Wei Chook, Blanka Tesla, Paul Digard, Lu Lu, Eleanor Gaunt

The Roslin Institute, The University of Edinburgh, Midlothian, United Kingdom

Abstract

Due to constraints in genome size, RNA viral genomes encode polycistronic genes through non-canonical gene expression mechanisms, such as alternative translation initiation sites, programmed ribosomal frameshifting and the utilisation of non-AUG start codons. However, there is a lack of available bioinformatic tools that identify putative open reading frames (ORFs). To address this, we developed R pipelines that identify two definitions of ORFs in all reading frames in inputted sequences. These definitions are: (1) nucleotide sequences that do not contain an in-frame stop codon; and (2) a nucleotide sequence flanked immediately by in-frame start and stop codons, where overlaps are permitted. Importantly, these pipelines do not require prior knowledge of the reading frame containing the canonical ORF in the inputted sequences. Through analyses of rotavirus genome segments, we have identified a conserved in-frame start codon (uAUG) in the NSP3 segment in a weak Kozak context. Translation initiation at the uAUG would result in a 3 amino acid N-terminally extended form of NSP3. Mutagenesis and reverse genetics experiments have demonstrated that the uAUG is dispensable for successful rescue of rotavirus strain SA11, but that uAUG can replace the canonical NSP3 start codon for successful virus rescue. The significance of the NSP3 uAUG during infection is currently being investigated.

Investigating compositional bias in influenza A virus using a synonymously recoded GFP library

Colin Sharp[1], Blanka Tesla[1], Laura Cano Aroca[2], Thomas Hofmeister[3], Andrew Mehle[4], Michael Liss[3], Paul Digard[1], Grzegorz Kudla[2], Finn Grey[1], Eleanor Gaunt[1]

[1]The Roslin Institute/University of Edinburgh, Edinburgh, United Kingdom. [2]University of Edinburgh, Edinburgh, United Kingdom. [3]Thermo Fisher Scientific, Regensburg, Germany. [4]University of Wisconsin, Madison, USA

Abstract

The genomes of many viruses mimic their hosts' compositional biases in terms of nucleotide, dinucleotide, codon and codon pair usage. We have previously highlighted the importance of the underrepresentation of CpG and UpA dinucleotides in the influenza A virus (IAV) genome by demonstrating that reversal of the natural suppression of these dinucleotides by synonymous recoding results in significant viral attenuation. In order to assess the influence of other compositional biases in a tractable and unbiased system, we are utilising a library of 195 synonymous permutations of the GFP coding sequence that exhibit wide variation in compositional features. To achieve expression of GFP sequence by the IAV polymerase, GFP was cloned in place of the majority of the HA coding sequence in genome segment 4 reverse genetics plasmids. Using this approach, we have generated GFP-sequence-containing transcripts that mimic IAV genomic RNAs in their ability to act as reporter templates for viral polymerase, both in minireplicon assays and in the production of GFP-encoding viruses in HA-expressing cell lines. Experiments using a subset of eight of the GFP mutants show significant differences in fluorescent signal in both systems, indicating that gene composition has a profound impact on protein production in a manner directed by multifactorial influences in the context of IAV infection. By expanding these studies to include the whole library and testing how these influence polymerase efficiency, transcript turnover, translational efficiency or host response we have begun to identify the most important compositional features of transcripts for protein production during IAV infection.

Dissecting the molecular mechanisms of coronavirus genome packaging.

Harriet Mears ORCID iD[1], Ziyi Yang[1], Kristijonas Raibuzis[2], Ciaran Gilbride[1], Flora Lee[3], Anob Chakrabarti[4], David Bauer[1]

[1]The Francis Crick Institute, London, United Kingdom. [2]University of Worcester, Worcester, United Kingdom. [3]Kings College London, London, United Kingdom. [4]UCL, London, United Kingdom

Abstract

For all viruses, specifically packaging their genome into progeny virions is crucially important for efficient transmission between hosts. This process must be tightly regulated to prevent spurious incorporation of defective viral genomes, subgenomic RNAs or host nucleic acids, resulting in non-infectious particles. Many viruses encode specific packaging signals (PS) which interact with viral structural proteins during particle assembly. While PSs have been identified in model coronaviruses from mouse and swine, genome packaging in human coronaviruses is still poorly understood.

We focussed our initial analyses on Mouse Hepatitis Virus (MHV), for which there is a characterised PS and mutant, and its close relative, human coronavirus OC43. Purified virions were subjected to high throughput RNA structure mapping, to establish the first high quality full-genome MHV and OC43 RNA structures. Next, we performed cross-linking and immunoprecipitation of the viral structural proteins Membrane and Nucleocapsid. This allowed us to examine structural protein binding sites around the PS, as well as to identify other regions of the genome which are specifically bound. Concurrently, we developed both reverse genetics and transfection-based virus-like particle systems for OC43; these allowed us to mutationally characterise the interactions between the structural proteins and the PS, to unpick the mechanism of genome packaging.

Together, our work provides an initial framework to understand the essential features of two coronavirus PSs. In the future, this will allow us to analyse more divergent coronaviruses to establish the fundamental principles of genome packaging family-wide.

Norovirus non-structural proteins contribute towards virion assembly and stability.

Jake Mills ORCID iD, David Rowlands ORCID iD, Morgan Herod ORCID iD

University of Leeds, Leeds, United Kingdom

Abstract

Human noroviruses are estimated to cause >200,000 deaths due to gastroenteritis yearly, and there is currently no efficacious vaccine or anti-viral therapy to treat norovirus infections. During the viral life-cycle, nascent genomes must assemble with two capsid proteins, VP1 and VP2, to generate infectious virions. However, how this process is coordinated is not understood.

Using the murine norovirus (MNV) model system, we previously generated heat-stable viruses by *in vitro* evolution. Next generation sequencing revealed mutations across the MNV genome, including synonymous and non-synonymous changes to the non-structural protein coding region (e.g. NS3), in combination with mutations to VP1. We hypothesised that these nucleotide substitutions in the non-structural coding region were required in conjunction with changes in the capsid for virion stability and infectivity. Reintroduction of these substitutions in the non-structural coding region individually into an infectious clone reduced the recovered viral titre. However, the individual non-structural mutations conferred greater particle stability in both infectivity assays following exposure to high temperatures and particle stability thermal release assays. Intriguingly, a synonymous change in NS3 induced the greatest increase in stability, suggesting RNA structure may be crucial to particle formation and stability. Work is underway to investigate multiple substitutions in combination, together with alternative synonymous substitutions and RNA SHAPE mapping, before the structural implications and RNA-protein interactions are investigated. These studies will illuminate the roles of non-structural proteins in virion assembly and help to inform the design of stable and structurally relevant virus-like particles (VLPs) for use in future vaccine trials.

Investigating the role of a recently identified antigenic domain (AD-6) of glycoprotein B in herpes virus entry

Alexander Hargreaves ORCID iD, Anastasia Lankina, Paul Griffiths, Matthew Reeves

Institute of Immunity and Transplantation, Division of Infection and Immunity, University College London, London, United Kingdom

Abstract

Ongoing studies of the humoral immune response to a recombinant vaccine based on human cytomegalovirus (HCMV) glycoprotein B (gB) identified the novel antigenic domain 6 (AD-6) as an important correlate of protection. Recently, we have also shown that AD-6 is structurally and immunogenically conserved across gBs of the herpesvirus family. The precise role of AD-6 in gB function remains unclear, although we have previously shown that a recombinant HCMV AD-6 peptide is able to inhibit HCMV infection of fibroblasts suggesting a role for AD-6 in the entry process of HCMV and thus, potentially, other herpes viruses.

Here we confirm that HCMV AD-6 inhibits entry into fibroblasts but, interestingly, not into epithelial cells. In contrast, we observe that the HSV-1 AD-6 analogue peptide was able to neutralise HSV-1 infection in both fibroblasts and epithelial cells. Intriguingly, both AD-6 analogues were able to cross-inhibit infection of fibroblasts by HSV-1 and HCMV suggesting a shared mechanism. However, the HSV-1 AD-6 analogue also demonstrated partial inhibition of HCMV infection in epithelial cells, but not vice versa. We used alphafold-generated structures to predict differential binding of the AD-6 peptides to virion glycoproteins of HCMV and HSV and hypothesise that AD-6 has general conserved functions between HHVs but also herpes virus-specific functions and interactions that may explain important differences to their routes of viral entry. Our findings help to further understand the role of AD-6 as an important mechanism for viral entry and gB function to consider when developing AD-6 based HHV immunotherapies.

Unravelling the components of the *Bacillus subtilis* SPβ bacteriophage viral particle

Abida Bano[1,2], Aisling Brady[3,4], Laura Miguel-Romero[5], Cora Chmielowska[1], José R Penadés[1], Nuria Quiles-Puchalt[6]

[1]MRC Centre for Bacterial Resistance Biology, Imperial College London, London, United Kingdom. [2]Institute of Microbiology and Molecular genetics, University of the Punjab, Lahore, Pakistan. [3]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom. [4]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [5]Instituto de Biomedicina de Valencia, Valencia, Spain. [6]Department of Biomedical Sciences, Faculty of Health Sciences, Universidad CEU Cardenal Herrera, CEU Universities, Valencia, Spain

Abstract

Bacteriophages, or phages, are naturally occurring viruses that infect a diverse range of bacterial hosts, showcasing variations in lifestyles, genetic content, and viral particle components. The SPBeta family of bacteriophages specifically targets *Bacillus subtilis*. Classified as temperate phages within the Siphoviridae family of double-stranded DNA viruses, phage SPβ serves as a model for this family and was initially identified in the *Bacillus subtilis* strain 168. Its genome comprises 138,418 base pairs, encoding 188 open reading frames (ORFs). Recently, the SPBeta phages have garnered attention due they encode a quorum sensing system that regulates the decision-making process in the phage life cycle. In this study, we conducted a comprehensive examination of this intriguing phage, focusing on a systematic search for proteins involved in the production of infective viral particles. Utilising mass spectrometry analysis, we performed a proteomic characterisation of the viral particle, identifying 7 proteins within its proteinaceous structure. Upon identifying the genetic locus responsible for packaging-related genes, we further characterised 6 additional proteins crucial for the packaging process of the phage. Additionally, we successfully pinpointed the receptor protein that the phage recognises during infection of *Bacillus* cells. This research represents the first-time characterisation of novel proteins involved in the packaging process of the SPBeta family of phages.

Improved Hepatitis E virus sub-genomic replicons permit novel functional studies

Frazer Buchanan, Mark Harris, Morgan Herod

University of Leeds, Leeds, United Kingdom

Abstract

Hepatitis E virus (HEV) is a leading global cause of acute viral hepatitis. HEV is classified into 8 genotypes (G1-G8), G1 is transmitted between humans faecal-orally, while G3 can be transmitted to humans through consuming contaminated pork products. There is a relatively poor understanding of the virus replication cycle, in part due to limitations in the cell culture systems and reagents available. It is therefore important to develop improved HEV culture systems that will permit tractable study of fundamental virus biology and the pathogen-host interactions. In our previous work, we tackled these limitations by employing transposon mutagenesis on a G1 HEV sub-genomic replicon (SGR; self-replicating mini-genomes that permit study of viral replication in isolation) to successfully HA-tag the viral putative cysteine protease (PCP) domain. Here, we have been able to incorporate this HA-tag into a G3 SGR that also contained a V5-tag within a separate viral domain, the hypervariable region (HVR), to generate unique dual-tagged SGRs. We were able to improve replication of this dual-tagged SGR 100-fold in culture by modifying the HVR through sequence insertions that originated from a patient strain of HEV. This next generation of SGRs is allowing simultaneous visualisation of PCP and HVR domains with host cell factors. Our preliminary results suggest no significant co-localisation of these proteins by immunofluorescence and multiple products by western blotting implicating processing of the viral polyprotein is possible. This next-generation of SGRs will be important for understanding the molecular basis of host-pathogen interactions.

Applying 3D correlative structured illumination microscopy and X-ray tomography to characterise herpes simplex virus-1 morphogenesis.

Kamal Nahas[1,2], Maria Harkiolaki[2], Stephen Graham[1], Colin Crump[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Diamond Light Source, Didcot, United Kingdom

Abstract

Herpes simplex virus-1 (HSV-1) is a large DNA virus composed of a genome-containing capsid, a complex tegument layer, and an envelope. Numerous viral genes products are involved in virion assembly, but the relative importance and function of many remains poorly characterised. We have conducted a comparative analysis of nine HSV-1 mutants, each lacking a different viral protein, using correlative structured illumination microscopy and soft-X-ray tomography under cryogenic conditions (cryoSIM and cryoSXT). We have studied the relative importance of different HSV-1 proteins during nuclear egress and cytoplasmic envelopment by observing defects or delays in virus assembly, such as nuclear retention of capsids and the crowding of unenveloped capsids in the cytoplasm. We also observed a process not previously seen using other imaging strategies: capsids interacting with a viral glycoprotein-enriched pole of cytoplasmic vesicles, supporting the hypothesis of envelopment at specific vesicle microdomains. Lastly, we studied virion morphogenesis in 3D using this imaging strategy. Transmission electron microscopy has previously suggested that cytoplasmic HSV-1 capsids become wrapped by tubular vesicles. However, this 2D depiction is compatible with more than one 3D model (e.g., wrapping by tubules or cisternae, or budding into spherical vesicles). By capturing the entire depth of the cell using cryoSIM and cryoSXT, we confirmed that capsids bud into spherical vesicles in every observed instance, clarifying the mechanism behind virus assembly. 3D correlative cryo-imaging of cell ultrastructure is an emerging technology for virus research that we have employed to shed new light on viral protein functions and virion morphogenesis.

Turning up the heat; mechanistic insights from thermal inactivation of influenza A virus

Jenna Schafers ORCID iD[1,2], Aidan Brown ORCID iD[2], Wilson Poon ORCID iD[2], Paul Digard ORCID iD[1]

[1]Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [2]School of Physics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Even prior to recent pandemics, viral inactivation has been crucial in the food industry for centuries[1]. Surprisingly, we lack a mechanistic understanding for even simple thermal inactivation. Using influenza A virus (IAV), we explore the limits of virus survival following heat exposure, gleaning important clues as to the inactivation mechanism from thermodynamic analysis.

IAV was exposed to a range of temperatures over time in bulk solution. Remaining viral infectivity was assayed by plaque assay. Infectivity decayed faster over time at higher temperatures as intuitively expected. The first-order exponential decay allowed determination of inactivation rates for each temperature, and revealed 'Arrhenius behaviour' between 40-50°C. Such behaviour is consistent with a single, dominant inactivation mechanism, requiring an activation enthalpy of 248(2) kJ/mol (100(1) k_BT). This is remarkably close to the energy barrier predicted by simulations of haemagglutinin$_2$ (HA) conformational change[2]. The activation entropy we obtained for inactivation (0.56(08) kJ/mol/K (67(1) k_B)) is consistent with a partially-unfolded (rather than, say, aggregated) state.

Studying the combined effect of pH and temperature revealed pH catalyses thermal inactivation; suggesting electrostatic forces are integral to the mechanism. Another temperature-independent inactivation process appears present initially at low pH, implying heterogeneity in the underlying population.

Taken together, this strongly suggests that premature HA-triggering, required for membrane fusion during infection, is key to thermal inactivation.

Considering the ubiquity of fusion proteins such as HA across enveloped viral species[3], mechanistic insights in this area will have broader implications for understanding viral stability and disinfection.

Length variation in Hepatitis C Virus E2 – a novel regulator of viral entry and antibody sensitivity.

Stephen Devlin ORCID iD, Michael Oliver, Joe Grove ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Currently 58 million people globally are living with Hepatitis C Virus (HCV) and whilst direct-acting antivirals offer a cure for those treated, there is no vaccine for HCV and infection rates remain high. One major barrier to the development of an effective HCV vaccine is the lack of mechanistic understanding of E1E2, the HCV viral entry proteins and major targets of the immune response. Of particular importance for vaccine design and entry mechanism is the Hypervariable Region One (HVR-1) of the E2 glycoprotein. HVR-1 participates in host receptor interactions and HCV immune evasion; HVR-1 is an intrinsically disordered tail which modulates entry and immune evasion through a currently unknown mechanism. Using bioinformatics, we systematically analysed all known HVR-1 sequences (> 20,000) to compare distribution of length polymorphisms across all HCV genotypes. To investigate whether length polymorphism affects virus phenotype, we cloned HVR-1s of varying length into the prototypical J6 E1E2. We evaluated the infectivity, receptor dependency, and neutralisation of this panel using pseudotyped viruses. In addition, AlphaFold was used to model E1E2 complexes with HVR-1s of differing lengths. These models were then used in molecular dynamics simulations to investigate the disorder and dynamics of these HVR-1 regions. This study demonstrates that HVR-1 length polymorphism is a common feature across all HCV genotypes and can modulate the infectivity, receptor dependency, and neutralisation of pseudotyped HCV viruses. Altogether, this suggests a novel regulatory mechanism in which length polymorphism of HVR-1 alters the function of the E1E2 HCV viral glycoproteins.

Rab27 GTPases and Myosin Va are host factors required for efficient OROV cell egress.

Juan Oswaldo Concha Casaverde ORCID iD[1,2], Kristel Irma Gutierrez Manchay[3], Colin Crump[1], Luis Lamberti Pinto da Silva[2]

[1]University of Cambridge, Cambridge, United Kingdom. [2]University of Sao Paulo, Sao Paulo, Brazil. [3]Sao Paulo, Sao Paulo, Brazil

Abstract

Oropouche fever, a debilitating illness common in South America, is caused by Oropouche virus (OROV), an arbovirus. OROV belongs to the Peribunyaviridae family, a large group of RNA viruses. Little is known about the biology of Peribunyaviridae in host cells, especially assembly and egress processes. Our research reveals that Rab27 GTPases mediate intracellular transport of OROV induced compartments and viral release from infected cells. We show that Rab27a interacts with OROV proteins and is recruited to OROV compartments during assembly. Moreover, Rab27a expression is required for OROV trafficking to the cell periphery and efficient release of infectious particles. Consistently, depleting Rab27a's downstream effector, Myosin Va, or inhibiting actin polymerization also hinders OROV targeting to the cell periphery and infectious viral particle egress. These data indicate that OROV hijacks Rab27 activity for successful intracellular trafficking and egress of virions from infected cells. Understanding these crucial mechanisms of OROV's replication cycle may offer potential targets for therapeutic interventions and aid in controlling the spread of Oropouche fever.

Infectious bursal disease virus (IBDV) VP3 contains a predicted C-terminal intrinsically disordered region (IDR) that modulates the physical properties of virus factories (VFs)

Andrew Brodrick ORCID iD, Andrew Broadbent ORCID iD

University of Maryland, College Park, USA

Abstract

Infectious bursal disease virus (IBDV), a double-stranded RNA virus of the family Birnaviridae, produces cytoplasmic replicative bodies, termed "virus factories" (VFs), that form by liquid-liquid phase separation (LLPS). Little is understood regarding IBDV VF formation, or how the virus drives LLPS, although viral protein (VP)3 is known to be an essential factory component. In this study, we generated a predicted structure of full-length VP3 using AlphaFold2, revealing a 36 residue carboxy (C-) terminal intrinsically disordered region (IDR). As IDRs have been implicated in driving LLPS, we hypothesized this region to be essential for VF formation. To test this, we generated mNeonGreen (mNG)-tagged VP3, and an mNG-VP3 lacking the C-terminal IDR (mNG-VP3ΔC). We then assayed for the presence of biomolecular condensates in avian DF-1 cells expressing mNG-VP3 or mNG-VP3ΔC either individually, in the presence of infection, or simultaneously with other viral proteins. Additionally, we performed live cell imaging and fluorescence recovery after photobleaching (FRAP) experiments to detect LLPS. Interestingly, we found that the VP3 C-terminal IDR was neither required for the formation of puncta, nor the recruitment of VP3 to VFs. However, we found that puncta in cells expressing VP3ΔC had significantly reduced molecular mobility as measured by FRAP, where the mobile fraction was reduced from 0.95 to 0.40 ($p<0.01$), demonstrating that the IDR modulates the physical properties of the puncta. Taken together, our data suggest that IBDV VP3 contains a C-terminal IDR that, while not required for the formation of VFs, nevertheless modulates their physical properties, driving LLPS.

Investigating the basis of RNA selectivity and dynamics inside viral replication factories

Cyril Haller ORCID iD, Alexander Borodavka ORCID iD, Julia Acker ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

Many viral replication factories are biomolecular condensates, with distinct physicochemical properties and molecular compositions from their surroundings that promote virus assembly. One such example is in rotaviruses, which selectively accumulate viral RNA and proteins into phase separating replication factories and assemble them into viral particles. However, it remains to be determined how the presence of RNA in rotaviral replication factories affects their properties and function. The factors determining selectivity and specificity of these condensates for viral RNA are similarly unclear. Here we use *in vitro* partitioning assays of diverse RNAs to reveal the properties that permit entry into replication factories. Additionally, fluorescence recovery after photobleaching (FRAP) and Förster resonance energy transfer (FRET) experiments reveal how the fluidity of these liquid-like condensates, which may be critical for their function, is affected by localisation and structural changes of such RNA. This work sheds light on how RNA-RNA and RNA-protein interactions shape the material properties of rotavirus replication factories, and how these properties impact condensate behaviour through RNA recruitment or exclusion.

The Art of Viral Construction: Exploring Phase Transitions in Assembly Pathways

Julia Acker, Alexander Borodavka

University of Cambridge, Cambridge, United Kingdom

Abstract

Viral replication factories are specialised organelles that sequester viral proteins and cognate RNAs and thus support both, viral replication and genome packaging. We have shown that Rotavirus viral replication factories form via condensation of the viral RNA chaperone NSP2 and a an intrinsically disordered scaffold protein NSP5 that undergoes multiple post-translational modifications, including hyperphosphorylation, during infection. Despite our understanding of how viroplasms are nucleated, it remains a mystery how viroplasms gradually mature, and what triggers the transition from viral replication to genome assembly.

By employing an array of biophysical techniques, such as microscopy, microfluidics, mass photometry and small angle x-ray scattering (SAXS), we investigated the impact of hyperphosphorylation on the condensate-forming properties of recombinantly expressed NSP5. Additionally, we explored its altered propensity to form interactions with NSP2 and viral RNAs. Our findings suggest that phosphorylation may regulate the spatiotemporal dynamics of rotavirus replication and assembly by modulating protein-protein and protein-RNA interactions within viral replication factories.

Lifestyle switching in pathogenic RNA viruses.

Stephen Nuthalapati, Trevor Sweeney

The Pirbright Institute, Pirbright, Woking, United Kingdom

Abstract

From paralytic poliomyelitis caused by polio virus to respiratory illness inflicted by the novel SARS-CoV-2, positive-strand RNA viruses remain a global threat to human health. Because these pathogenic viruses use the same single-strand positive sense RNA (+ssRNA) as template for viral genome replication and protein synthesis, temporal regulation of the viral genome usage is needed to avoid abortive translation or replication. The 'lifestyle switch' model suggests that viral translation is inhibited following initial rounds, thus freeing the RNA of ribosomes allowing it to efficiently serve as a replication template. This central and critical role of temporal regulation of genome usage presents an attractive therapeutic target, however, how such a lifestyle switch is regulated is poorly understood.

Using *in vitro* reconstitution systems, we show that binding of viral polymerase at the 5'-end of flaviviral genome triggers a transition from the translation-competent linear form to replication-competent circular form, in a process that is likely regulated by other viral and host proteins. Furthermore, we show that flaviviral polymerase bears striking similarity to the poliovirus system, suggesting a convergence of viral replicase control of gene expression in two +ssRNA viruses using very different translation mechanisms. Currently, we address how viral and host proteins regulate genome usage and, by SHAPE (Selective Hydroxyl Acylation analysed by Primer Extension) mapping, additionally examine the role of RNA structural changes affecting lifestyle switching .

A deep mechanistic understanding +ssRNA virus-replication in the host cell will help identify virus-specific processes that can be effectively targeted to help contain new outbreaks.

Dynamics of SARS-CoV-2 RNA-dependent RNA polymerase association with the viral genome

Nuno Brito Santos ORCID iD[1], Anob Chakrabarti[2,1], David LV Bauer[1]

[1]The Francis Crick Institute, London, United Kingdom. [2]University College London, London, United Kingdom

Abstract

Since establishing in humans, novel SARS-CoV-2 variants have been recurrently generating, through accumulation of mutations and/or recombination. From circulating sequences, particular genomic regions have been proposed to be more prone to mutation/recombination. However, the exact mechanism and dynamics of the association of nsp12, the viral RNA-dependent RNA polymerase (RdRp), with the viral genome remain undefined. Particularly, it is critical to address those before the action of natural selection.

To tackle this, we developed a CrossLinking and ImmunoPrecipitation (CLIP)-seq assay. Briefly, infected cells were crosslinked, and then probed for the genomic position of nsp12 molecules at that moment. Importantly, CLIP-seq allow us to study these interactions at single nucleotide resolution. Furthermore, we explored the impact of anti-RdRp drugs remdesivir (chain terminator) and molnupiravir (mutagen) in the association with the viral genome, and therefore their potential consequences for viral evolution. Integration of these results with available data on viral genome secondary structure and conservation reveals candidate regions with direct impact in CoV evolution, before selective pressure.

This work will advance the understanding of fundamental and conserved mechanisms driving viral evolution. Ultimately, the generated knowledge may improve the preventive monitoring of viruses with zoonotic potential and refine public health measures to limit viral evolution during an outbreak.

The post-transcriptional basis of T2 phage development in *Escherichia coli*

Alice Collins ORCID iD, Sivaramesh Wigneshweraraj ORCID iD

Imperial College London, London, United Kingdom

Abstract

Post-transcriptional regulation of RNA is a pivotal regulatory step in gene-expression, in which the fate of RNA is determined by non-coding RNA (also called small RNA; sRNA). sRNAs can influence whether the targeted mRNAs are destined for translation, translational repression, degradation, or protection from degradation. Some sRNAs engage in 'sponging,' an unclear process thought to regulate sRNAs by sequestering them in inactive complexes or causing their degradation. In many bacteria, a core component of post-transcriptional regulation is a protein called Hfq, which facilitates the interaction between sRNAs and their cognate mRNA or sRNA targets. Therefore, Hfq is the major post-transcriptional regulator of bacterial gene-expression. Phages are viruses that infect bacteria. Individual sRNAs of bacterial and phage origin that regulate phage gene expression or bacterial processes to benefit phage replication have been described. However, our total lack of understanding of the Hfq-licenced post-transcriptional regulatory basis of bacteria-phage interactions at the molecular and system level represents a major gap in our fundamental understanding of bacteria-phage interaction biology. Here, we have screened a library of 11 common lytic *Escherichia coli* phages in bacteria devoid of Hfq and describe the molecular basis underpinning optimal T2 phage development in *E. coli*.

Using Protein Structure Prediction to Map Glycoprotein Diversity Reveals Defining Events in the Evolutionary History of the Flaviviridae.

Jonathon C.O. Mifsud[1], Spyros Lytras[2], Michael Oliver[2], Vincenzo A. Costa[1], Edward C. Holmes[1], Joe Grove ORCID iD[2]

[1]The University of Sydney, Sydney, Australia. [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Enveloped viruses encode specialised glycoproteins that mediate fusion of viral and host membranes. These proteins are important determinants of viral tropism and pathogenesis, and are targets for host immunity. Discovery and understanding of the molecular mechanisms of viral membrane fusion has, thus far, been achieved through experimental determination of glycoprotein structure. Nonetheless, there is an incomplete understanding of membrane fusion mechanisms across the Flaviviridae. The E glycoprotein of the Orthoflaviviruses, e.g. Dengue virus, is a prototypical class II fusion protein. However, the glycoproteins from the Jingmen Viruses, Large Genome Flaviviruses, Pesti-, Pegi- and Hepaciviruses remain poorly characterised and unclassified. This knowledge gap is a barrier to mechanistic understanding and vaccinology for important animal and human pathogens.

We employed state-of-the-art protein structure prediction to explore glycoprotein diversity in >450 species of viruses distributed across the Flaviviridae. To overcome challenges of ambiguous genome annotation we chose to systematically predict structures for entire genomes for each of the viral species (resulting in >33,000 structural models). Structural similarity searches allowed us to unambiguously identify fusion mechanisms for the majority of species, including those from the poorly understood Jingmen Viruses and Large Genome Flaviviruses. This revealed a complex pattern of protein loss and gain, with fusion mechanism correlating with commitment to specific replication strategies and/or ecological niches.

In conclusion this study provides insights on the ancient evolutionary events that define the diversity of viral lifestyles found across the Flaviviridae. Moreover, it provides a blueprint for mapping protein structure and function across the virosphere.

Looking inside a complex biological condensate - the structure of avian reovirus replication factories.

Eva Durinova[1,2], Tomas Bily[2,1], Frantisek Kitzberger[2,1], Zdenek Franta[2], Peter Mojzes[3], Petr Chlanda[4], Roman Tuma[2,5]

[1]Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic. [2]Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic. [3]Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic. [4]Department of Infectious Diseases, Virology, Heidelberg University Hospital, Heidelberg, Germany. [5]University of Leeds, Leeds, United Kingdom

Abstract

Avian reovirus (ARV) belongs to *Reoviridae* family with segmented dsRNA genomes. ARV is responsible for significant economic losses to the poultry industry by causing tenosynovitis and chronic respiratory disease in chicken[H1] . ARV virion exhibits an outer protein shell enclosing an inner core that contains [H2] 10 unique dsRNA segments and multiple copies of viral polymerase. ARV assembly takes place in so-called viroplasms or viral factories (VF). They are cytoplasmic membrane-less inclusions which accumulate viral proteins and RNA, and assist capsid assembly, genome packaging, and replication. VFs also provide protection from the host cell defense mechanisms by sequestering viral RNA. Given the dense nature of VFs, little is known about ARV assembly and packaging intermediates. Using previously developed host cell lines and imaging techniques [1-2] we demonstrate that ARV VFs are inhomogeneous biological condensates in which the inner, fluid phase is surrounded by densely packed virion arrays. The inner phase concentrates viral non-structural proteins and assists in the assembly of viral cores. The larger, RNA-filled and double-layered particles are excluded and emanate from the inner fluid phase at the periphery. This suggests that ARV VFs are functionally compartmentalized: the inner fluid phase assists RNA assortment and core particle assembly while the peripheral phase is the site of RNA replication inside viral cores and virion completion.

[1] Durinova et al. (2023) Shedding light on reovirus assembly-Multimodal imaging of viral factories. Adv Virus Res 116, 173-213.

[2] Zimmermann & Chlanda (2023) Cryo-electron tomography of viral infection-from applications to biosafety. Curr Op Virology 61, 101338.

Influenza A Virus M2 Biogenesis Highways and Byways

Christian Diwo[1], Colin Adrain[2], Maria João Amorim[3]

[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal. [2]Queen's University Belfast, Belfast, United Kingdom. [3]Católica Medical School, Lisboa, Portugal

Abstract

Compared to the production of soluble proteins, synthesising and secreting membrane proteins is a slow and error-prone process. Viruses rely entirely on the cellular protein synthesis machinery to produce their protein components which makes membrane protein biogenesis a likely bottleneck for virus particle production and in turn limits viral transmission. Influenza A virus (IAV) expresses three essential membrane proteins that are incorporated into the viral envelope and play essential roles during viral entry and egress. IAVs small ion channel M2 is heavily studied due to its contribution to pathogenesis and its promise as a therapeutic target, however M2's specific biogenesis route remains undiscovered. We find that the only recently characterised, but highly conserved endoplasmic reticulum membrane protein complex (EMC) facilitates efficient membrane insertion of M2 at the endoplasmic reticulum (ER). Interestingly, the genetic ablation of EMC has a significant impact on titers at early stage replication of IAV, which in turn limits cell to cell transmission measured by plaque size. However M2 is able to enter the membrane and is able to function in replication without EMC assistance, hinting at a yet undiscovered pathway for membrane protein biogenesis. We are able to identify features in the M2 sequence that make M2 EMC dependent as well as features which allow M2 to enter a membrane in absence of EMC.

The Microbiology Society is a membership charity for scientists interested in microbes, their effects and their practical uses. It has a worldwide membership based in universities, industry, hospitals, research institutes, schools, and other organisations. Our members have a unique depth and breadth of knowledge about the discipline. The Society's role is to help unlock and harness the potential of that knowledge.

Microbiology Society @microbiosoc @microbiosoc @MicrobiologySocietyOrg1945 Microbiology Society

Microbiology Society 14–16 Meredith Street, London EC1R 0AB, UK +44 (0)20 3034 4870 microbiologysociety.org

Registered as a Charity in England and Wales 264017. A Charity registered in Scotland SC039250.
Company Limited by Guarantee. Registered in England 1039582.