The great host-pathogen war: UK cellular microbiology meeting 2020

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Abstract

In 2019 we started a new annual meeting, aimed at bringing together researchers from across the UK studying cellular microbiology and the cell biology of host-pathogen interactions. In contrast to large glamourous meetings, featuring the great and the good from across the world, we wanted to create a forum for early career researchers to present their work and enjoy lively discussion. In particular, we hope that focussing on making the meeting accessible, affordable, and informal would help integrate and build the UK community working on this exciting topic.

Main text

Following on from a highly successful inaugural meeting at the Francis Crick Institute last year (Rohn, Mostowy, King, Unnikrishnan, & Gutierrez, 2019), the second meeting of the UK Cellular Microbiology network was held at the University of Sheffield on the 10th of March 2020. A traditionally quiet time for conferences, we were fortunate to hold it just before the COVID-19 pandemic took hold. Despite extensive travel chaos the evening before due to a strong storm, about 100 participants made it to Sheffield from across the UK, as well as a few other European countries, to attend an excellent day of talks and posters followed by a thorough debrief over a buffet dinner and drinks. This was all made possible thanks to generous sponsorship from the British Society of Cell Biology, *Frontiers in Cellular and Infection Microbiology*, and this journal – *Cellular Microbiology*.

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This year, the meeting was marked by the notable absence of Prof. Stefania Spanò (University of Aberdeen), who suddenly and tragically passed away at the end of last year. Stefania was a major contributor to the UK cellular microbiology field, and both a friend and collaborator to many at the meeting. She will be greatly missed by the community, and the best talk prizes were named in her honour in recognition of her achievements, and commitment to mentoring and nurturing new talent.

The meeting was gracefully kicked off by the first of our keynote speakers, Annemarie Meijer from Leiden University, Netherlands. She demonstrated the power that zebrafish brings to the study of host-pathogen interactions and how she leverages that model to shed light on the mechanisms of host defence against intracellular bacterial pathogens responsible for infectious diseases, including tuberculosis and typhoid fever. One such defence is inflammasome activation, a key event that triggers inflammation whose role in tuberculosis disease progression is not well understood. Annemarie revealed new insights into the role of pyroptosis, a very specific cell death mechanism, in tuberculosis, with interesting links between nucleic acid sensing and Caspase 11-dependent pyroptosis.

Zebrafish are being increasingly used to model human infection, and have also helped reveal key roles for neutrophils and emergency granulopoiesis in host defence against *Shigella flexneri*. Vincenzo Torraca (Wellcome / LSHTM ISSF Research Fellow in the Mostowy lab), who did an excellent job stepping in as a last-minute invitation, described how he uses zebrafish to investigate the virulence of *Shigella sonnei*, an emerging pathogen that is replacing *S. flexneri* as the principal cause of shigellosis in industrialized regions. Vincenzo discovered that *S. sonnei* is more virulent than *S. flexneri in vivo* because of its distinctive O-antigen which allows tolerance against phagolysosome acidification and resistance to neutrophil-mediated killing.

Bacterial pathogens are known to interfere in a range of host immune responses. Charlotte Odendall (Kings College London) gave us an outstanding overview of the type I and III interferons and their signalling pathways, highlighting that these antiviral cytokines are also induced by many bacterial pathogens. Her research, which focuses on the enteric pathogen, *Shigella*, showed how OspC, a family of *Shigella* type III secretion system (T3SS) effectors, differentially blocks interferon I and III signalling. Interestingly, she discovered that interferon treatment protected epithelial barriers from *Shigella* invasion, indicating new antibacterial roles for these interferons at barrier sites. This is yet another example of how bacterial pathogens have evolved clever mechanisms to evade host defences.

Many Gram-negative bacterial pathogens are masters at tweaking the innate immune response, paving the way for a smoother ride in the host. *Salmonella enterica* serovar Typhimurium is a good example of a bacteria that flexes this handy adaptation, injecting the cell with proteins that disarm host defences. One such protein is SteE, a bacterial effector

that can activate a key transcriptional programme to establish an infection-permissive, anti-inflammatory environment. Although previously the Thurston lab (Imperial College) had established that the host kinase GSK3, along with host transcription factor STAT3, were both involved in this process, no one knew how it worked. PhD student Ioanna Panagi and colleagues therefore set out to explore the mechanism. What they discovered was surprising: although GSK3 has always been thought to be a serine-threonine kinase, they were able to show that SteE converts both the amino acid and substrate specificity of GSK3, directing the kinase to phosphorylate STAT3 (not its usual substrate) on a tyrosine residue: Y705. It is SteE that brings GSK3 and STAT3 together into a 3-way complex, thereby allowing the unusual phosphorylation event to take place. Such an unusual mechanism has never been reported before, and describes a novel trick by which bacteria can reprogramme host innate immune signalling.

The survival tactics of *Salmonella* Typhimurium were also the subject of an entertaining talk from Anthony Davidson from the University of Cambridge (Vassilis Koronakis lab). Anthony's talk focussed on how the bacteria gain entry into host cells by subversion of p21 activated kinase (PAK), which is best known for being a downstream target of the actin cytoskeletal regulators CDC42 and Rac1. Despite its importance, how PAK controls the cytoskeleton remains slightly enigmatic with the majority of PAK functions ascribed to its C-terminal kinase activity. Surprisingly, Anthony and colleagues discovered that this kinase activity is dispensable for infection by both *S.* Typhimurium and the extracellular pathogen enteropathogenic *Escherichia coli* (EPEC). Instead, the N-terminal scaffold domain of PAK appears to be key, via a series of four poly-proline motifs which directly interact with the Rac1/Cdc42 GEF β-PIX to facilitate bacterial invasion. This study puts the spotlight on a previously unappreciated role for the N-terminus of PAK in cytoskeletal control, beautifully demonstrating how pathogens remain powerful tools for unearthing new secrets in cell biology.

Salmonella species encompass a large and diverse group of bacteria with varying ability to invade different hosts. Whilst typhoidal serovars are exquisitely restricted to humans, non-typhoidal *Salmonella* (NTS) serovars are host generalists and cause zoonotic infections in humans. What are the factors that enable NTS serovar such as *S.* Typhimurium to infect a broad range of hosts? Natalia Cattelan from the University of Aberdeen showcased beautiful research from the Spanò lab, revealing how NTS disables the host protein Rab32 to bypass a novel bactericidal pathway in macrophages. This is achieved through the NTS-specific virulence effectors SopD2 (Rab32 GAP protein) and GtgE (Rab32 protease), which are absent in typhoidal serovars, making them susceptible to the killing activity of Rab32 in a mouse model of infection. Intriguingly, Natalia revealed that GtgE, like Rab32, has a CaaX motif at its C-terminus, a prenylation signal for anchoring proteins in the membrane of eukaryotic cells. She showed that this motif was essential for GtgE localisation, and key for both NTS survival inside murine macrophages and for infection *in vivo*.

Bacteria use a number of sophisticated mechanisms for antibiotic evasion, and in the era of increasing antibiotic resistance, more research is required to fully understand and tackle the problem. Kasia Mickiewicz (Newcastle University Faculty of Medical Sciences Research Fellow) won one of our best talk prizes for her excellent presentation on a little-known mechanism of antibiotic evasion referred to as 'L-form switching'. L-form switching involves a temporary loss of bacterial cell wall rendering bacteria resilient to all classes of cell wall-targeting antibiotics. A controversial concept when first proposed, Kasia demonstrated that L-form bacteria exist in patients with recurrent urinary tract infections, and used a zebrafish infection model to show that L-form switching can be adopted by pathogenic bacteria *in vivo* to evade cell wall-targeting antibiotics.

The rise of multi-drug resistance is becoming particularly worrisome in the case of tuberculosis infection. To counter this, Phil Elks (standing in for his PhD student Ffion Hammond, University of Sheffield) described how they took the approach of targetting the host response, rather than the pathogen itself, to improve the outcome of infection. Using a zebrafish tuberculosis model, Ffion found that trib1, a pseudokinase associated with immunity, was required for host protection against mycobacteria and overexpression of trib1 significantly reduced bacterial burden. Mechanistically, she demonstrated that trib1 overexpression protects the host by causing an increased pro-inflammatory innate immune response, increasing production of pro-inflammatory factors il1β and nitric oxide. The zebrafish model is a novel tool to study trib1 and adds to increasing evidence that trib1 is involved in immune cell function, highlighting its potential as a host-derived therapeutic target.

Whilst targeting the host immune response offers a promising way to counter infection, it is also frequently targeted by the pathogens themselves. For example, many pathogens enhance infection by modulating host cell survival through manipulation of cell death pathways, triggering pathways such as apoptosis to kill hostile macrophages or inhibiting death by pyroptosis to evade inflammatory responses. At the centre of cell death regulation are the host caspases. Intriguingly, it has become clear that low levels of caspase activation cause sub-lethal signaling of the mitochondrial apoptosis pathway, which promotes transformation and tumorigenesis. Remarkably, Dominik Brokatzky (PhD student in the Georg Häcker lab, Germany) described how sub-lethal apoptosis activation is hijacked by a range of pathogens during infection including viruses (Herpes simplex virus, Influenza A, modified vaccinia virus Ankara), bacteria (*S.* Typhimurium, *Chlamydia trachomatis*) and one parasite (*Toxoplasma gondii*). All these pathogens triggered this sub-lethal apoptosis activation through low-level activation of the mitochondrial apoptosis apparatus, inducing cytokine secretion from epithelial cells under control of the Bcl-2-protein family (Bax, Bak, Bcl-XL). Importantly, blocking Bax and Bak enhanced infection by both *Salmonella* and

Chlamydia. Thus, Dominik revealed that sub-lethal apoptotic signalling provides a conserved cell-autonomous defence pathway against a broad array of pathogens.

This year the meeting also embraced the growing field of fungal infections. Fungi are a major and growing cause of life threatening and debilitating infection in both the developed and the developing world. Candida species, and in particular *Candida albicans,* cause a high proportion of hospital-acquired infections each year in the UK and USA, causing significant mortality within the immunocompromised. Stella Christou (Ayscough lab, University of Sheffield) described how the fungal cell wall represents the major interaction surface between the pathogen and host cells, and altering its composition can both disrupt the physical properties of *Candida* and its ability to invade host cells. Previous work from the group showed that cell wall composition and structure is changed upon disruption of the AP-2 endocytic adaptor complex via deletion of the gene encoding APM4. In her talk, Stella described how, despite being able to grow at a rate similar to wild type and retaining their ability to switch to their hyphal form in vitro, APM4-deficient *Candida* behaved differently in a range of infection models. Intriguingly, whilst hyphae still formed on epithelial monolayers, this capacity was severely impaired after engulfment by macrophages and *in vivo* in zebrafish. They propose that cell wall chitin might underpin important physical properties of hyphae that allow them to lyse macrophages and invade epithelia and endothelial layers but that these responses are nuanced by its interactions with the host.

Host cells also deploy physical mechanisms to restrain pathogens. One example of this is via the septins, a poorly understood component of the host cytoskeleton, which entrap *S. flexneri* in cage-like structures to prevent actin-based motility. Although this role in suppressing infection is clear, the molecular determinants that underpin bacterial recognition and entrapment by septins are mostly unknown. To answer these questions, Dr. Damián Lobato-Márquez (Marie Curie Postdoc Fellow in the Mostowy lab, LSHTM, and winner of the Stefania Spanò best talk prize, See Figure 1) developed an *in vitro* reconstitution assay based on purified septin proteins and host cell extracts. This 'bottom-up' approach enabled him to discover bacterial factors recognized by septins, and directly visualise septin assemblies on bacterial membranes using high-resolution microscopy. This elegantly demonstrated how *in vitro* reconstitution illuminate new concepts in cell biology that are difficult (or impossible) to address using whole cells or animal models.

The scientific meeting came to an end with a very enlightening presentation from our second keynote speaker Jost Enninga from the Pasteur Institute in Paris, sponsored by *Cellular Microbiology*. Jost featured his recent *Shigella* research, both a technical *tour de force* and a testimony to the power of "seeing is believing". Using different strategies to visualise the process of infection at the subcellular level, Jost showed, among other things, that *Shigella* and *Salmonella* subvert intracellular pathways regulated by Rab GTPases and the cytoskeleton to drive interactions with macropinosomes and create a niche for

intracellular replication. The devil is in the details, and he nicely demonstrated how high-resolution imaging approaches such as FIB-SEM can provide critical information about the places where bacteria replicate and the host components that contribute to this process.

Thus concluded the second in this series of meetings, after which people moved on to a buffet dinner for some animated discussion and networking. The interactions and engagement from the audience during the talks and poster sessions were also excellent, providing a great forum for early career researchers to present their work. Whilst COVID-19 restrictions may prevent a repeat of the meeting in person next February, we hope to build on the enthusiasm of the UK cellular microbiology community and maintain this forum for many years to come.

Conflict of interest statement
The authors declare no conflict of interests regarding this work.

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Figure legend:
Figure 1: Damián Lobato-Márquez receiving his prize for the Stefania Spanò best talk award.