Florey Institute PGR Symposium 2016

Abstract Book

Sponsors

Programme

Friday 24th June 2016, Firth Hall

09:15-09:45	Registration (presenters to put up posters)
09:45-10:10	Welcome and Introduction to the Florey Institute
10:10-11:10	PGR talks 10:10-10:30: Magdalena Widziolek (Jagiellonian University) 10:30-10:50: Jason Wilson (University of Sheffield) 10:50-11:10: Isabel Johnston (University of East Anglia)
11:10-11:50	Keynote: Dr Suzan Rooijakkers (University of Utrecht)
11:50-13:15	Lunch and Poster Viewing
13:15-14:15	PGR talks 13:15-13:35: Joby Cole (University of Sheffield) 13:35-13:55: Sayali Haldipurkar (University of Sheffield) 13:55-14:15: Friederike Uhlig (University of Sheffield)
14:15-14:55	Keynote: Dr Neil Stokes (RedX Pharma)
14:55-15:25	Break
15:25-16:05	PGR talks 15:25-15:45: Oishik Banerji (University of Sheffield) 15:45-16:05: Catherine Buckley (University of Sheffield)
16:05-16:45	Keynote: Dr Hasan Yesilkaya (University of Leicester)
16:45-17:00	Prize Giving and Close

Oral 1: ***Porphyromonas gingivalis*** **Type IX Secretion System Components and its gingipain substrates are crucial for the bacterial pathogenic potential in vitro and in vivo**

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Porphyromonas gingivalis (Pg) is the main pathogen involved in the development and progression of chronic periodontitis. Recently this pathogen has also been strongly associated with systemic disorders such as cardiovascular diseases. The main virulence factors of *P. gingivalis* that contribute to its pathogenicity are cysteine proteases referred to as gingipains. These proteases are secreted via an unique, still poorly understood, secretion system known as T9SS (PorSS). The aim of this study was to determine the involvement of gingipains and T9SS components in the pathogenesis of *Pg*.

To investigate the pathogenic potential of *P.gingivalis*, we first analysed the subcellular localisation of the RgpB gingipain in the wild type strain, W83, and mutants of the T9SS components. The cellular localization of RgpB was determined using immunofluorescent staining, protein fractionation and western blot techniques. We showed that RgpB is located in the outer membrane of bacteria and is exposed to the extracellular environment in the wild type strain. Whereas in the secretion system mutant, ΔPorU, the full length, inactive form of the enzyme accumulates in the periplasm.

Next, we investigated the virulence of the wild type and mutant bacteria using *in vitro* invasion and killing assays. In addition, *in vivo* analysis was performed using the zebrafish embryo infection model. These results indicate a crucial role of gingipains as well as PorSS system components in bacterial invasion and survival in macrophages and endothelial cells. This observation was confirmed for the first time using the zebrafish model. Neither gingipain nor PorSS system component mutants affected zebrafish embryo survival. In contrast, infection with the wild type strain caused the development of severe oedemas and led to fish mortality. KYT – gingipain inhibitors also demonstrated the importance of this protealytically active enzymes in bacterial pathogenesis.

Pg infections have been studied in many animal models. However, none of these allows visualization of *Pg* dissemination and pathological outcomes of the infection in different tissues. One major advantage of zebrafish embryos is their transparency allowing real-time imaging of the progression of bacterial infection and associated systemic outcomes. To image *Pg* dissemination in real-time we infected embryos with fluorescently stained bacteria. Within 2h *Pg* localized to tissues distant to the inoculation site with many bacterial cells already ingested by phagocytes. In the case of the gigipain null mutant, we observed much less free

bacteria, and the majority already phagocytosed in infected fish by 24 hpi. This suggests that the mutant bacteria are more effectively eradicated by the host in comparison to the wild type *Pg*. Furthermore, using a transgenic zebrafish line expressing a red fluorescent protein in endothelial cells we found that the wild type *Pg* dissemination occurred directly through the vascular system. However, the gingipain null mutant appears to be less efficient at this process.

In summary, we showed that gingipains and the components of the T9SS secretion system are crucial for bacterial virulence. Our results also clearly suggest that the zebrafish embryos are a suitable model to study the mechanisms of *Pg* pathogenicity and its link to systemic diseases.

Oral 2: **Structural studies into a family of tripartite pore-forming toxins from *Aeromonas hydrophila***

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The ClyA superfamily of alpha helical pore forming toxins (PFTs) is found in a number of pathogenic bacteria, where the action of the toxin is to lyse host cells. The prototypical member, ClyA, from pathogenic *E. coli,* is a single protein that forms a 12-mer pore embedded in the membrane[1]. In contrast, the superfamily member (NheA, NheB, NheC) from the foodborne pathogen, *Bacillus cereus* is a tripartite PFT, with the active toxin formed from the three proteins, which form an operon[2]. We have recently identified another tripartite PFT family member (AhlA, AhlB, AhlC) from the fish pathogen, *Aeromonas hydrophila*. To help understand the pathogenicity of these organisms, we are studying the structure and function of these PFTs to understand the role of each of the tripartite protomers.

Rationale & Hypothesis: Each of the components of the tripartite pore forming toxin system has a different specific role in pore formation, possibly to allow pore formation in specialised cells.

Objectives: To elucidate the function of each component of the Ahl tripartite pore forming toxin system through structural and functional studies

Methodology: X-ray crystallography, electron microscopy, cell-based in vitro assays, mutational studies, biochemical analysis

Findings:

- AhlC X-ray structure suggests a novel receptor forming function for AhlC, which agrees with electron microscopy data and hemolytic assays.
- AhlB is able to form pre-pores in lipid membranes, and preliminary X-ray data suggests AhlB forms a pre-pore with 10-fold symmetry

Oral 3: **Contribution of the NsrR regulon to nitric oxide detoxification: linking the nitric oxide and tellurite response of *Salmonella* Typhimurium**

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Rationale & Hypothesis: The intracellular pathogen *Salmonella* Typhimurium is a facultative anaerobe, with O_2 as the preferred electron acceptor. During periods of anoxia *Salmonella* can switch to using nitrate in a respiratory process called denitrification. Unlike many soil bacteria, *Salmonella* undergo truncated denitrification, where nitrate is converted into nitrous oxide via sequential reduction of nitrite and nitric oxide (NO). During infection, *Salmonella* also encounters NO as part of the host response when residing inside macrophages. It is therefore important that *Salmonella* has the ability to efficiently detoxify this potent NO. There are three characterised NO detoxification systems in *Salmonella,* HmpA, NrfA, and NorVW, however deletion of these three genes does not eliminate *Salmonella*'s survival under these conditions. It is therefore expected that there are further, yet unknown NO detoxification genes. NsrR, an NO sensing transcriptional repressor, is known to regulate, three putative, tellurite resistance genes in *Salmonella*, *yeaR*, *tehB* and *STM1808*, yet the physiological relevance of tellurite is questionable; we therefore hypothesise that they are involved in detoxifying NO. We also aim to determine the role of NsrR regulated genes in resistance against tellurite.

Objectives: Using a range of NsrR regulated gene *Salmonella* knock-out strains we will:
1: Determine the contribution of NsrR regulated genes to nitric oxide sensitivity.
2: Determine the contribution of NsrR regulated genes to tellurite sensitivity.
3: Monitor NO production of novel NO-sensitive strains.
4: Assess the survival of the strains in macrophages.

Methodology:
1 + 2: Subject *Salmonella* knock out mutant strains to tellurite and the NO donor, deta NONOate both aerobically and anaerobically to assess the importance of each gene to survival in these conditions.
3: Grow the different strains anerobically under denitrifying conditions and monitor the amount of NO released – higher levels would suggest less ability to detoxify.
4: Monitor the survival of the strains in macrophages, which use NO as a defence mechanism; including IFN-γ (more NO) and L-NAME (less NO) treated cells.

Findings: The three tested 'tellurite resistance' mutants are sensitive not just to tellurite but also to nitric oxide; furthermore a quadruple mutant strain lacking *yeaR*, *tehB*, *STM18*08 and the well charactised NO reductase, *hmpA*, is severely attenuated to both compounds and also in macrophages. This strain's virulence will therefore be further investigated *in vivo*. A clear link has also been shown

been strains that are sensitive to nitric oxide and those that are sensitive to tellurite – suggesting a similar mode of action.

In addition we have discovered differences in the MIC of tellurite in LB and M9 minimal media – it is counter intuitively more toxic in LB. We have also shown the protective effect that selenite affords *Salmonella* during growth on tellurite, which has recently been shown in *E. coli*. These results in combination suggest that in contrast to the current thinking, the toxic effect of tellurite to *Salmonella* is not due to reactive oxygen species.

Oral 4: **Global changes in histone post-translational modifications arising during infection of primary macrophages with *Streptococcus pneumoniae***

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Rationale & Hypothesis: Histone post-translational modifications are increasingly recognised as playing a pivotal role in the pathogenesis of infectious diseases. Recently bacterial virulence factors have been identified as stimuli for acute epigenetic changes in innate immune cells. We hypothesised that the key virulence factor, pneumolysin, expressed by S. pneumoniae could induce transcriptional change through epigenetic modification.

Methodology: Peripheral blood mononuclear cells were obtained from healthy volunteers following ficoll gradient separation. Macrophages were differentiated by plastic adherence and matured for 14 days. These were then exposed to opsonised mid log phase D39 serotype 2 S. pneumoniae or an isogenic mutant lacking pneumolysin at a multiplicity of infection of 10. Three hours after infection histones were extracted and analysed using mass spectrometry to identify and quantify histone post-translational modifications. In addition RNA was extracted for transcriptomic analysis.

Findings: Our preliminary data shows a number of histone PTMs are altered following challenge with S. pneumoniae decreased dimethylation at lysine 9, acetylation of lysine 23, and in monomethylation of lysine 79. In addition, transcriptomic analysis also reveals pneumolysin dependent differential gene expression.

Conclusions: We conclude that there is evidence of acute changes to the histone tails in response to pneumococcal infection which appear to represent the removal of poised mark on histone H3 (lysine 9 dimethylation) to allow gene expression. Future work will explore the interaction between these marks, gene expression and protein production.

Oral 5: **Transcriptional regulation by OrbS in the opportunistic pathogen *Burkholderia cenocepacia* in iron-stress response**

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Rationale & Hypothesis: *Burkholderia cenocepacia,* a Gram negative bacterium is involved in causing serious opportunistic infections in patients with cystic fibrosis and chronic granulomatous disease. These infections are difficult to treat since *B. cenocepacia* is intrinsically resistant to most antibiotics.

In order to infect and survive in host tissues bacteria require effective signalling mechanisms that allow the bacteria to respond appropriately to the prevailing conditions. One of these is through the use of extra-cytoplasmic function (ECF) sigma factors that are alternative subunits of RNA polymerase. When activated under required conditions they associate with core RNAP to recognise specific promoter elements and drive transcription of genes to efficiently respond to the respective environmental stress. Conventionally, ECF sigma factors are associated with a membrane anchored anti-sigma factor that senses the stress condition and regulates the activity of the ECf sigma factor. However, alternative regulatory mechanisms do exist.

The ECF sigma factor OrbS of *B. cenocepacia*, is expressed under iron depleted conditions. Iron is an extremely important nutrient for colonisation that bacteria need to acquire even though it is largely absent in the free form in host tissues. Many pathogens are capable of synthesizing iron chelating compounds called 'siderophores'. As these compounds are able to acquire iron from an unusable form and make it available in a biologically usable form, thereby promoting bacterial growth, they are considered as virulence determinants. OrbS is involved in transcriptionally regulating genes required for the biosynthesis and utilisation of the siderophore 'ornibactin'. Unusually, OrbS does not have an anti-sigma factor for regulating its activity and its only known regulator is the master transcription regulator, Fur.

Objectives: (1) To elucidate DNA sequence requirements for promoter utilisation by OrbS and (2) To investigate presence of an alternative OrbS regulatory mechanism

Methodology: A systematic mutagenesis study was carried out to identify important bases for OrbS-dependent promoter activity by introducing single or multiple basepair substitutions in the OrbS-dependent promoter and using reporter-fusion assays to measure the promoter activity. To address whether the activity of OrbS is directly regulated by iron availability, the activity of the OrbS-dependent promoter was measured in response to iron in the absence of Fur

using a reporter plasmid. As Fur is highly conserved, the experiment was carried out in *E. coli*.

Findings: Results demonstrate that OrbS recognises the promoter through two nucleotide sequence motifs: CGTC (-10 region) and TAAA (-35 region). These regions are separated by a conformationally important GC rich spacer whose length appears to be crucial for promoter recognition. Promoter activity measurements in an *E. coli fur* mutant suggest the presence of an alternative mechanism for regulating OrbS activity other than transcriptional regulation by Fur but this needs to be confirmed in a *B. cenocepacia fur* mutant.

Oral 6: **Modulation of visceral sensitivity by supernatants of *Staphylococcus aureus***

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Rationale & Hypothesis: The mammalian host is home to huge numbers of bacteria that colonise the skin as well as mucosal surfaces in the airways and in particular the gastrointestinal tract. Dysbiosis of the gastrointestinal microbiota has been linked to numerous pathologies such as diabetes, Alzheimer's and chronic bowel diseases. In addition, gastrointestinal infection or antibiotic treatment frequently co-occur with neurological symptoms such as pain, anorexia and vomiting. These are thought result from an immune response, secondary to immune cells recognising intruders. Recent findings show that soluble factors from bacteria activate neurons and this suggests that alternative mechanisms may underlie the occurrence of neurological symptoms (Chiu et al., 2013). As disease conditions weaken the gastrointestinal barrier, we hypothesise that bacterial substances might be involved in pain arising from the gut.

Objectives: To test the above hypothesis, we investigated the effect of supernatants of the well-known opportunistic pathogen *Staphylococcus aureus*. The aim of the present study is to understand whether *S. aureus* produces substances that can directly interact with sensory neurons in the small intestine.

Methodology: The distal small intestine was removed from adult C57BL/6 mice and sensory nerves attached to a suction electrode to record extracellular electrical activity. Supernatants from *Staphylococcus aureus* JE2 (SSA) were prepared in brain heart infusion (BHI) broth and applied as a 20 % v/v solution to the serosal side of the intestine for 90 min. Tissue was distended every 15 min (0 – 30 mmHg) and baseline activity as well as the response to distension were quantified.

In-vitro cytotoxicity assays using Trypan Blue were performed on primary dorsal root ganglia neurons, bone marrow-derived mast cells and HEK293 cells.

Findings: Baseline firing was decreased from 49.06 ± 9.3 spikes/s to 1.92 ± 1.5 spikes/s 90 min after application of SSAs from overnight cultures (p = 0.024, N = 7). The distension-induced increase of nerve activity to 85.47 ± 17.46 spikes/s (1.7 fold compared to baseline) was also significantly reduced to 2.13 ± 1.35 spikes/s (p = 0.011, N = 7). We also investigated supernatants taken at different time points after inoculation of BHI with bacteria and found that there was a moderate, but significant, negative correlation between the optical density (range OD_{600} 5.46 – 23.36) of SSAs and the fold change of nerve activity after one hour SSA application, i. e. four distensions (r = -0.425, p = 0.043, n = 23, N = 15).

As experimental outcomes showed a degree of variability, we compared the effects of SSAs in *in-vitro* cell viability assays using primary dorsal root ganglia neurons (DRG), bone marrow-derived mast cells (BMMC) and HEK293 cells. Thirty minutes after SSA application, the number of DRG neurons was markedly reduced and quantification of cell death indicated that 50.85 ± 5.38 % of HEK cells and 25.00 ± 8.87 % of BMMC were sensitive to SSA. Variation of SSA's cytoxicity was related to their inhibitory actions in electrophysiology experiments.

Conclusions: These results suggest that intestinal sensory neurons may be potential target structures for soluble substances produced by *S. aureus*. Further studies will investigate whether this interaction is specific to neurons and aim to identify the substance(s) that mediate SSA's inhibitory effect on sensory nerve activity.

Oral 7: **Structure of the crystalline native *Clostridium difficile* S-layer**

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Rationale & Objectives: The important nosocomial pathogen, *Clostridium difficile*, causes antibiotic-associated diarrhoea and life-threatening pseudomembraneous colitis. The mechanism of gut colonisation of the bacterium is poorly understood, but the surface layer or S-layer is thought to be involved. The bulk of the *C. difficile* S-layer consists of the essential protein SlpA, which is the most abundant protein in the cell. Following expression, SlpA undergoes post-translational cleavage to form low molecular weight (LMW) and high molecular weight (HMW) S-layer proteins or SLPs. These form a complex, which self-assembles into a paracrystalline S-layer. Despite its importance very little is known about the structure of this layer.

Methodology & Findings: Here we present a 20 Å three-dimensional structure of the native S-layer of the ribotype 027 strain R20291, isolated from intact cells and resolved using electron crystallography. We also report a 10 Å projection structure of the S-layer in a frozen-hydrated state. Using a novel SlpA negative mutant, we have created a strain natively expressing a truncated SlpA lacking part of the LMW SLP. We have a generated a three-dimensional model of this mutant S-layer, which shows significant differences from the wild type, and has allowed us to propose the likely location of the LMW SLP within this layer. This putative region is hypothesised to be involved in the host-pathogen interaction and subsequent colonisation.

Oral 8: **What is the perfect cup size: how do cells shape their phagocytic cups?**

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Capturing invading pathogens is critical during the immune response in order to defend the body against infection. To do this, immune cells such as macrophages need to be able to correctly form and shape phagocytic cups. Dictyostelium are predatory soil-dwelling amoeba that hunt and feed on bacteria in the wild. In the lab, we can harness this property to study formation of phagocytic cups. We have discovered a protein, BAR8, which is crucial for phagocytic cup formation. BAR8 is a unique and currently unstudied protein. It contains multiple small GTPase regulatory domains (RCC1, RasGAP and RhoGEF) as well as a BAR domain. These domains provide a mechanism to connect membrane curvature to regulation of small GTPases. Here we show that BAR8 localises to both phagocytic and macropinocytic cups and is essential for normal formation of both structures. Loss of BAR8 affects the ability of cells to phagocytose particles and bacteria of different sizes and geometries demonstrating the importance of phagocytic cup shape in engulfment.

Poster 1: **Alteration in mitochondrial function influencing antimicrobial responses to bacteria in macrophages during COPD**

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Rationale & Hypothesis: Infectious stimuli alter the metabolic profile of macrophages and this shift to glycolytic metabolism is essential for innate immunity. Down-regulation of mitochondrial oxidative phosphorylation (OXPHOS) is associated with an increase in production of mitochondrial ROS, a microbicidal used by tissue macrophages. Chronic Obstructive Pulmonary Disease Macrophages (COPD-Mϕs) have altered microbicidal responses to bacteria and show signs of oxidative stress. I hypothesized that COPD-Mϕs might have altered capacity for this metabolic shift with altered mitochondrial function and that upregulation of Mcl-1, a feature of COPD-Mϕs.

Objectives: To investigating how the mitochondrial metabolic profile in response to bacterial challenge is altered in COPD-Mϕs and in macrophages over-expressing Mcl-1.

Methodology: Bone marrow cells from wild type and Mcl-1 transgenic C57BL/6 mice were cultured in 10% L929 conditional media and differentiated into bone marrow derived macrophages (BMDMs). COPD and healthy volunteer's monocytes were differentiated into monocyte derived macrophages. Macrophages were embedded into XF/cell culture microplates followed by challenge with opsonised *Streptococcus pneumoniae* (D39, MOI=10) for 4 hours. Subsequently, the glycolytic rate, OXPHOS and loss of inner mitochondrial transmembrane potential ($\Delta\Psi m$) were measured using a XF24 Flux analyser and flow cytometry respectively.

Findings: *S. pneumoniae* significantly increased basal and glycolytic capacity of both BMDMs ($P<0.04$, $n=4$), whereas its noticeably decreased OXPHOS and increased proton leak (PL). However, the baseline basal mitochondrial respiration showed a higher trends and increased PL in Transgenic macrophages. On the other hand, mitochondrial respiration capacity (MRC) and PL showed an opposite trends in COPD-Mϕs compared to healthy macrophages after infection. Although comparable trends noticed for the baseline MRC, PL was significantly elevated in COPD-Mϕs ($P=0.009$, $n=3$) which could observably disrupted $\Delta\Psi m$.

Conclusion: My preliminary data suggests COPD and enhanced Mcl-1 production may modulate mitochondrial function resulting in enhanced PL which may compromise the ability to produce mtROS as a microbicidal Radical.

Poster 2: **The role of the tetraspanin, CD151, in neutrophil phagocytosis**

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Rationale & Hypothesis: Inflammatory lung diseases are characterized by persistent or chronic inflammation, and represent a major economic burden worldwide. Neutrophils are innate immune cells that play a major role in the pathogenesis of inflammatory lung disease. Their prolonged survival in the tissue results in tissue damage and prolonged tissue inflammation. Bacterial clearance by neutrophils at site of inflammation was also shown to be decreased compared to healthy individuals. Tetraspanins are a family of four membrane spanning proteins that have key roles in immune functions including cell survival, bacterial adhesion and phagocytosis, cell motility and proliferation although little is known in the neutrophil. We hypothesize that tetraspanins play a role in neutrophil phagocytosis.

Objectives: Investigate the roles of the tetraspanins CD151 in neutrophil phagocytosis in *in vitro* studies.

Methodology: Primary human neutrophils were isolated from the blood of healthy volunteers by Percoll gradient centrifugation. Anti-CD151 antibodies were used to probe tetraspanin function. Phagocytosis of *Staphylococcus aureus* and *Streptococcus pneumonia* was assessed by bright field, fluorescent microscopy and bacterial viability assays.

Findings: The use of anti-CD151 antibodies showed inhibition in phagocytosis of heat killed and live *S. aureus* but not *S. pneumoniae*. Bacterial viability assays confirmed that in the presence of anti-CD151 antibodies there is less viable *S. aureus* present in the early stages of phagocytosis. However, the later time point showed similar viable *S. aureus* levels inside the neutrophils incubated with anti-CD151 antibodies as the earlier time point. Fluorescent microscopy data from single experiment showed less heat killed *S. aureus* present in the acidified phagosome, as indicated by the pHrodo stain, when neutrophils are incubated with anti-CD151 antibody. This data indicated a potential role of the CD151 tetraspanin in targeting phagocytosis of *S. aureus* to the phagolysosome.

The data presents novel and potential roles for tetraspanins in pathways underpinning neutrophil phagocytosis. Further research must be done to elucidate mechanisms by which they act. These finding may reveal potential therapeutic strategies for the treatment of infection and inflammatory diseases.

Poster 3: **Characterisation of proteins potentially involved in haem uptake and utilization in pathogenic *Neisseria***

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Neisseria meningitidis (NM) is the most common cause of bacterial meningitis and septicaemia in the UK. It affects over 2000 people per year with a mortality rate of up to 10%, and many survivors are left with permanent disabilities. However, the bacterium harmlessly colonises the nasopharynx of around 10% of the population. There are still significant gaps in our understanding of the mechanisms by which *NM causes invasive disease,* particularly surrounding the survival of the pathogen after invading the bloodstream. A significant obstacle to bacterial survival in the bloodstream is the scarcity of key nutrients such as iron. A large proportion of iron is sequestered by haem, which is bound to a range of carrier proteins, making it very difficult to access.

Two neighbouring genes potentially involved in haem uptake and metabolism in NM are being studied in the Sayers group. Previous work suggested that one of them – a small secreted protein – may bind to red blood cells, a rich source of iron in the form of intracellular haem. The aim of this project is to identify the function of these proteins and their potential role in host-pathogen interactions.

Relevant genes have been cloned from NM MC58 genomic DNA and expressed in *E. coli* overproducing strains. Affinity and ion exchange purification methods have been used to purify the proteins with yields of up to 3.6 mg per gram of cell paste and this has been used to begin crystallisation trials. A variety of techniques will be applied to characterise their roles in haem uptake or metabolism and identify any interacting proteins. Potential interactions with human cells will be studied using microscopy techniques and flow cytometry. A bank of mutants is available to compare with wild type proteins to further characterise the proteins. The findings from this project could improve understanding of how *Neisseria meningitidis* survive in the human host and potentially pave the way to improved treatment and prevention.

Poster 4: **Investigating the role of tetraspanin proteins in *Salmonella* infection**

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Tetraspanins are a family of transmembrane proteins that have a key roles in basic cellular functions, cancer malignancy and infectious diseases. In bacterial infections, tetraspanin proteins have been shown to be involved in adhesion and invasion of different human epithelial and macrophage cells (Green et al.(2011) Infection and Immunity 79 2241).

Rationale & Hypothesis: Treatments that target tetraspanins could interrupt bacterial-cell association and eliminate bacterial invasion, offering an alternative to antibiotics, in infections where resistance is an increasing problem. *Salmonella* is an infectious pathogen linked with human and animal diseases world-wide. These bacteria are characterized by the ability to infect a variety of phagocytic or non-phagocytic human cells either

Objectives: The aim of this research is to investigate the mechanism whereby tetraspanins are involved in *Salmonella* infection using different cell types.

Methodology: The well-characterised mouse macrophage cell line J774 and mouse macrophage cell lines established from CD9 KO and wild type mice were pre-treated with anti-tetraspanin antibodies or recombinant proteins representing the large extracellular domain (EC2) prior to infection with *Salmonella* bacteria. The human epithelial cell line HEC-1-B was similarly investigated. Assays were developed to monitor infection by flow cytometry (FACS) analysis in addition to conventional microscopy.

Findings: Pre-treatment of mouse macrophage cells with anti-CD9, anti-CD81 and a lesser extent anti-CD63 antibodies significantly decreased *Salmonella* infection. Differences in the infectivity of the CD9KO and wild type mouse macrophage cell lines were noted, although this varied depending on the virulence of the *Salmonella* strain. In this study, no effect of anti-tetraspanin antibodies on the HEC-1-B cell line was noted. Taken together, this study suggests a prominent role for tetraspanin CD9 and other tetraspanins in *Salmonella* infection of mouse macrophages. These findings, therefore, could have a promising future in treatment of bacterial infections instead of using antibiotics.

Poster 5: **TMEM203 is a putative coreceptor of STING in antiviral and antibacterial immunity**

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The STING-mediated signalling is of great importance in antiviral and antibacterial immunity, while its functions and mechanism of interactions with other inflammatory signalling receptors is only partially understood. From a previous functional screen, we discovered a novel proinflammatory regulator, transmembrane protein 203 (TMEM203). In a recent unpublished study, we showed that TMEM203 is colocalised with STING in the endoplasmic reticulum and that it facilitates STING-mediated expression of type I interferons and cxcl2.

Rationale & Hypothesis: TMEM203 is a coreceptor of STING and influence its functions during viral and bacterial infections.

Objectives: To characterise the role of TMEM203 in STING-mediated expression of type I interferons and chemokines during bacterial and viral infections.

Methodology: Building on the previous results which suggest TMEM203 colocalise with STING and that its overexpression significantly enhances the STING-mediated release of cxcl2 in RAW264.7 cells (mouse macrophages), here we applied siRNA-based gene interfering technique to knockdown TMEM203 in THP-1 cells (human monocytes) and primary macrophages. The transfected cells were then treated with STING ligand 3'-3' cGAMP to stimulate cellular expression of IFN-β and IL-8. Cytokine expression levels were assessed by qPCR and compared to control groups.

Findings: In the current study, we demonstrate that TMEM203 knockdown in THP-1 cells and primary macrophages reduces the cells ability to produce interferon-β and IL-8 via STING signalling, and may consequently weaken the innate immune defence against viral and bacterial infections. Therefore, the current studies support that TMEM203 is an essential signalling partner of STING.

Poster 6

Fisk E

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Rationale & Hypothesis: *Streptococcus pneumoniae* is a gram-positive bacterium, commonly found in the upper respiratory tract of humans, and is the most common cause of community acquired pneumonia, observed mainly in children, the elderly and immune-compromised. Alveolar macrophages present the first line of defence during respiratory infection and so their recognition and response towards bacterial infection are critical for an appropriate immune response. Literature shows that immune cells can mediate their metabolism in response to infectious stimuli such as LPS, but few studies have used whole and/or live bacteria to assess this, or how it impacts infection outcomes. By better understanding the interaction between host and pathogen at a cellular level, we hope to reveal ways in which the bacterium, or host, could be targeted to promote better patient outcomes in the future.

I hypothesise that an increase in glycolysis, and associated metabolic pathways such as the pentose phosphate, are required during infection, for macrophage activation and effector functions such as bacterial killing.

Methodology: Murine bone marrow-derived macrophages (BMDMs) were used to model *S. pneumoniae* infection. Metabolism was assessed via Seahorse Extracellular Flux (XF), to evaluate changes in glycolytic and respiratory metabolism, and ^{1}H-NMR Spectroscopy, to assess levels of metabolites both in the extracellular supernatant and the intracellular extracts. To consider the influences of metabolism on effector functions, metabolic inhibitors and altered media conditions have been used during infection. Internalisation and killing of bacteria is measured by gentamycin-protection assay and cell viability by flow cytometry.

Findings: A protocol has been optimised for using Seahorse XF assays and preliminary work demonstrates an increase in metabolic capacity of BMDMs following infection with *S. pneumoniae*. NMR Spectroscopy has also shown changes in levels of metabolites such as lactate, pyruvate and acetate over an infection time course. Findings thus far can be summarised as an increase in glycolytic metabolism during infection.

Poster 7: **Detection of subtle immune defects in individuals at risk of pneumococcal disease**

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Rationale & Hypothesis: Several risk groups have been identified in the literature, to be at greater risk of developing invasive pneumococcal disease (IPD.) This includes imunocompromised individuals, such as HIV-infected individuals, and myeloma patients. Our group has previously uncovered a subtle defect in B-cell responses of unvaccinated IPD sufferers, *in vitro*, to a T-independent antigen mimic (αδdex.) The hypothesis for this study is that this same B-cell defect would be found in HIV-infected and myeloma patients, *in vitro*.

Objectives: This study used a validated immunologic assay previously used in our group to study B- cell responses to a T-independent antigen mimic (αδdex) and T-cell help thereafter (after stimulation of T-cells by T-dependent anti-CD3.) Our objectives were to assess B- and T-cell activation and proliferation to these stimulants, and measure these responses using two optimised flow cytometry panels.

Methodology: Lymphocytes enriched from whole blood of consenting adults, were cultured with αδdex alone and combination with anti-CD3, to assess both direct B and T-cell effects, and T-cell help to B-cells. B and T cell activation and proliferation were assessed using standardised flow cytometry and appropriate markers. Total B-cells and B-cells subsets stratified by CD19, CD10, CD21, and CD27 into naïve, activated memory cells, tissue-like memory cells and resting memory cells were evaluated.

Findings: Results from eight HIV infected individuals [median CD4 count (IQR): 612 (494.75 – 828.25) and undetectable viral load] showed no change in activation or proliferation of CD19+ B-cells after stimulation with αδdex, compared to age-, sex- and ethnicity-matched controls. However, there was significantly higher ($p \leq 0.01$) activation in the resting memory B cell compartment in the HIV patient group after αδdex stimulation. Furthermore, in the entire study cohort there was significantly higher ($p \leq 0.05$) CD4+ T cell activation in white individuals, compared to black individuals.

Poster 8: **Response of intracellular *Staphylococcus aureus* to the redox environment of host cells**

Christmas B

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Staphylococcus aureus is a successful human pathogen with many mechanisms for surviving host immune responses and resisting antibiotic stress. The ability to survive intracellularly, inside host cells, is a key characteristic of S. aurues that enables it to evade host immune responses and be sheltered from antibiotics. This ability of S. aureus to survive intracellularly is facilitated by a high resistance to host derived reactive oxygen species (ROS), encountered in phagolysosome, but also by its ability to escape from such compartments and successfully proliferate in the low redox potential environment of the eukaryotic cytosol.

In order to better understand the ability of S. aureus to adapt and thrive following a sudden change in redox environment, we have investigated the transcriptomic and physiological response of S. aureus to a rapid change in redox environment using continuous culture, microarrays and NMR. This has enabled a redox response regulatory network to be mapped that has confirmed the role of some well-established regulatory genes such as agr (accessory gene regulator) and rot (repressor of toxins), and also started to highlight the involvement of others.

Furthermore, H2S is an important gasotransmitter in eukaryotic cells with recently proposed ROS and antibiotic resistance roles in bacteria. Our investigations into the role of H2S as a mediator of ROS resistance has shown that increased production of H2S increases resistance to ROS in S. aurues but that suggested links with antibiotic resistance are less clear. Increased understanding of these key defensive processes will inform future development of antibacterial treatments targeted at intracellular S. aureus infections.

Poster 9: **Identifying novel immune modulating factors in a genome-wide *S. aureus* screen in human neutrophils**

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Rationale: Staphylococcus aureus is a highly adaptive and widespread bacterial pathogen causing numerous clinical problems due to antibiotic resistance. Recent human-pathogen interaction studies reveal S. aureus uses multiple evasion mechanisms to survive and replicate within neutrophils, the primary cellular defence against this pathogen. In addition, S. aureus induces rapid and profound neutrophil necrosis, which further disables the immune response.

Objectives: Our study aims to identify novel immune modulators through the screening of a genome-wide S. aureus mutant library in neutrophil cell death assay.

Methodology: The 1,952 strain mutant library was constructed by transposon insertion in the clinically relevant community acquired methicillin-resistant S. aureus (USA300) background. Individual S. aureus strains were co-incubated with primary human neutrophils isolated from healthy volunteers, at an MOI of 10 for 3 hours before ToPro-3 staining and assessment of cell loss via flow cytometry.

Findings: Wild type USA300 caused profound neutrophil cell loss by 3 hours (70.4%±2.9%). Assessment of ToPro-3 negativity and viable cell counts identified 118 S. aureus mutants that showed attenuated neutrophil cell death. Following the first round of screening, this was reduced to 34 mutants after further validation by focused secondary screens (n=3). A number of internal controls with known pro-death functions including lukAB, lukGH, agrA and saeS were among the identified attenuated strains. Four gene mutations not previously associated with cell death: purB, lspA, clpP and pfo were also among the most highly-attenuated strains. These genes have been further validated by transduction strategies, and current studies to verify them by gene complementation are underway. Chemical complementation of adenine and inosine was required to recover the attenuation of neutrophil killing by purB mutant. These findings may identify novel mechanisms of S. aureus induced neutrophil cell death, which may aid in the design of future antibiotic-independent therapeutic strategies to restore failures of innate immunity.

Poster 10: **Biochemical and structural studies on the streptococcal DNA polymerase I flap endonuclease domain**

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Drug-resistant and multidrug-resistant strains of *Streptococcus pneumoniae* are among some of the most concerning pathogens to confront modern medicine. Today, the pressing need for new drugs demands the innovation of new strategies. One such strategy is the identification of essential proteins as drug targets in highly conserved components of bacterial machinery, in the hope that drugs developed against these will be more resilient to antibiotic resistance. The streptococcal flap endonuclease (*Sp*FEN) represents a potential target since it plays a crucial role in DNA replication, removing unwanted flap intermediates during lagging-strand synthesis. The FEN or 5'-3' exonuclease forms the first part of DNA polymerase I as a ~300 a.a. domain; the second domain being a polymerase highly homologous with the *E. coli* Klenow fragment. Previous studies have shown that the FEN domain is essential for cell viability but not the Klenow domain.

Objectives: Characterize *Sp*FEN in terms of its biochemical and structural properties so enabling the development of specific inhibitors via rational drug design.

Findings:

- Comparison of biochemical data between the WT nuclease with four active-site mutants reveal conserved aspartate residues that are required for both exo- and endonuclease activities. Quantitative results of kinetic analyses will be presented.
- We have also determined the 3D structure of the *Sp*FEN protein:DNA complex in two slightly different conformations at 2.2 Å and 1.65 Å resolution by X-ray crystallography.

Structural data for FENs from pathogenic organisms have yet to be reported in the literature. Hence, this work provides an exciting first step towards facilitating long-term possibilities for future structure-based drug design.

Poster 11

Buchan K

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Current research into *Staphyloccocus aureus* depends on *in vivo* models to discover how infection is established, however the field is reliant on the mouse model for dissecting these processes. A number of recent studies have shown that several important virulence factors are uniquely tailored to a human host, undermining any infection model that does not contain these proteins. Currently, no established infection model for studying human-adapted virulence of *S. aureus* exists, so my project proposes to use the zebrafish, an excellent model for studying the innate immune system, as an alternative.

I aim to develop a number of transgenic zebrafish lines that express the human immune components that are targeted by *S. aureus*. Combining this with fluorescent labelling will allow visualisation of the infection process *in vivo*, in real time and with high resolution. In addition I will characterise a newly discovered virulence factor that inhibits the enzyme myeloperoxidase, which is important for generating reactive oxygen species within neutrophils. My primary aim is to establish the humanised zebrafish as an alternative model for studying the human-specific interactions of *S. aureus*.

Constructs containing human proteins fused to fluorescent reporters and driven by a neutrophil-specific promoter were assembled using Gateway cloning and injected into zebrafish at the single-cell stage to introduce the construct into the genome. *In vivo* imaging of transgenic embryos infected with *S. aureus* was carried out using numerous microscopy techniques, including wide-field fluorescence, confocal, and light-sheet microscopy. Survival of zebrafish against Staphylococcal infection was assessed by injection into the circulation. Preliminary experiments demonstrate a functional humanised construct that labels neutrophil subcellular components. Results show that humanised virulence factors do not contribute to infection in the zebrafish, supporting published data. Additionally, experiments demonstrate a clear role for myeloperoxidase in survival against *S. aureus* infection.

Poster 12: **Interactions of BPIFA1 and BPIFB1 with *Staphylococcus aureus***

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As part of a robust innate immune system, the cells of the airway epithelium secrete fluid and proteins to create the highly proteinaceous periciliary liquid (PCL). Many proteins present in the PCL have putative antimicrobial functions, including two of the most abundant proteins, BPIFA1 (SPLUNC1) and BPIFB1 (LPLUNC1). The function of these two proteins in host defence is unresolved and we hypothesize that they interact with the respiratory pathogen, *S. aureus*, to reduce the establishment of infection.

Air-liquid interface (ALI) cultures of primary bronchial epithelial cells secrete many proteins present in the PCL, including BPIFA1 and BPIFB1. Pull down assays interacting cell secretions with *S. aureus* were used to visualise protein-bacterial interactions. Both BPIFA1 and BPIFB1 were shown to interact strongly with *S. aureus*. Recombinant proteins generated in CHO cells exhibited similar binding to the endogenous proteins. Deglycosylation using PNGase F treatment prior to pull down assays suggested that these interactions were not dependent on the glycosylation state of BPIFA1 or BPIFB1.

We next used a selection of adhesion molecule and/or surface protein mutants of *S. aureus* from the Nebraska Transposon Mutant library in pull down assays to identify possible *S. aureus* proteins responsible for interactions with BPIFA1 and BPIFB1. Of the 10 mutants chosen for study, none were identified as mediating these interactions.

Our study has shown that BPIFA1 and BPIFB1 form strong interactions with the surface of *S. aureus*, in a potentially non-specific manner. The functional consequences of which remain unresolved but support the notion that BPIFA1 and BPIFB1 play a role in protecting airway epithelial cells from infection. To further investigate this continuing studies on *in vitro* ALI cultures of primary bronchial epithelial cells are being undertaken to analyse bacterial localisation with the epithelium and to study protein secretion during *S. aureus* infection.

Poster 13: **Functional characterisation of (p)ppGpp synthetases: enzymes required for bacterial stress adaptation and survival**

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Staphylococcus aureus is a Gram positive bacteria responsible for around 12,500 cases of bacteraemia a year in the UK. *S. aureus* can colonise the nose and skin asymptomatically but can also invade the host as a pathogen. In the host the bacteria encounters various nutrient stresses which trigger the activation of the stringent response. The stringent response is a conserved mechanism by which bacteria adapt to environmental changes such as amino acid starvation and cell wall stress. The response is mediated by two nucleotides, ppGpp and pppGpp, collectively known as (p)ppGpp. These small alarmones have many binding targets in the cell and cause the cells to enter a slow growing state through altering the transcriptional profile and inhibiting ribosome formation. In *S. aureus* (p)ppGpp is synthesised by three members of the RSH superfamily: RSH, RelP and RelQ. RSH is a bifunctional enzyme with both synthetase and hydrolase activity and a C-terminal regulatory region. RelP and RelQ are both small monofunctional synthetases with no regulatory region. The aim of this project is to learn more about how these synthetases are regulated transcriptionally, translationally and post-translationally and their function in the cell. The conditions that trigger the transcription of the synthetases will be determined using a lacZ reporter system and any transcription factor or two-component regulators of this will be identified using a transposon mutant library. Any binding partners of the sythetases will be assessed using a bacterial two hybrid system library. Altogether, this project aims to provide insights into the synthesis of (p)ppGpp by these enzymes and thus contribute to the mechanistic understanding of the pathogenesis of *S. aureus.*