UNIVERSITY OF LEICESTER

Department of Infection, Immunity & Inflammation

9th Annual Postgraduate Student Conference

27–29 March 2017

Programme and Abstracts

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For more information

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Preface

The Department of Infection, Immunity and Inflammation would like to welcome you to the Ninth Annual Postgraduate Departmental Conference. Within the department there is a diverse range of research areas, and in this conference PhD students representing research groups in Infection and Immunity, Renal Medicine and Immunology, TB and Respiratory disease, will be showcasing their research.

This experience is designed to prepare students across the department for their Viva and give them the opportunity to network with other students and staff members from different fields.

We would like to thank Dr Shaun Heaphy for opening the conference, and Professor Nigel Brunskill for kindly giving the keynote lectures.

We would also like to thank Starlab for sponsoring this event and providing a prize for the best paper.

Feedback from the presentations plays a vital part in the student experience of the conference and we would encourage you all to provide constructive criticism through comments and questions.

We hope you enjoy the presentations from both students and invited speakers, and we thank you for attending this conference.

Abstract Booklet Organisers:
Ananthi Ramachandran, Giannis Koukkidis, Felicity Easton

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Day 1, 27th March

Hasanain Alaridhee

Characterisation of the inflammatory response in Murine IgA nephropathy.
Supervisor(s): Dr Cordula Stover and Prof. Jonathan Barratt

IgA nephropathy (IgAN.) constitutes 45% of all cases of primary glomerulonephritides, and is defined by the presence of IgA associated IC deposition within glomeruli, associated with hematuria, proteinuria, fibrosis and necrosis. The disease progresses to renal failure in 25–30% of cases during a period of 20 years. So, IgA nephropathy a serious medical problem. However, there is no animal model for this disease, therefore a mouse model was developed by Dr Cheung and Professor Barratt at University of Leicester.

The aim of my work is to analyse the kidneys of experimental and control mice by qPCR, IF, IHC and histological staining. Complement activation was measured in serum samples of mice by ELISA. The results will be presented.

Ziyad Aldosari

The effects of mechanical stretch on the immunological and anti-inflammatory properties of skeletal muscle cells: An *in vitro* study of potential mechanisms that affect response to exercise therapy.
Supervisor(s): Dr Alan Bevington, Dr Emma Watson and Dr Alice Smith

A significant increase in output of potentially anti-inflammatory myokine IL-6 has been found from contraction of skeletal muscle that occurs during exercise in absence of muscle damage. This IL-6 output may be beneficial in inflammatory states such as chronic kidney disease (CKD). However, release of IL-6 from exercising muscle may be abnormally weak in CKD patients, possibly because of acidosis.

Evidence from cultured L6 rat skeletal muscle cells suggests that exertion stimulates protein synthesis through the amino acid-sensitive protein kinase mTORC1. In CKD exercise depletes the amino acids present in muscles *in vivo*; possibly because exercise-induced acidosis in muscle impairs the normal activation of MAPKs (e.g. p38)

and the amino acid transporter SNAT2 which should occur in exercising muscle, thus inhibiting mTORC1, protein synthesis and IL-6 production.
This study tests the following hypothesis: (1) higher levels of global protein synthesis and IL-6 secretion occur in mechanically stressed skeletal muscle during physical exercise or *in vitro* mechanical stretch as a result of activation of MAPKs, then SNAT2 up-regulation, followed by mTORC1 activation; and (2) this process is blocked by low pH.

Initial experiments on mechanically stretched L6 cells have shown that drug inhibition of MAPKs inhibitsSNAT2, suggesting that MAPKs regulate SNAT2 and hence protein synthesis.

Intriguingly, there was a significant increase in SNAT2 activity following physiological stretch lasting one hour, but not after 17h possibly because prolonged stretching leads to cell damage. Future work will optimise this model using lower maximal stretch for a shorter period of time, allowing effects of stretching at different pH's on IL-6 output to be studied.

Abdullah Alruwaili

Understanding C-peptide signalling in the kidney.
Supervisor(s): Prof. Nigel Brunskill and Dr Alan Bevington

Diabetes mellitus can result in very serious complications such as retinopathy, neuropathy, and nephropathy, especially when not treated. In the UK in the last decade, more than 2.5 million people were diagnosed with diabetes and this number is increasing continuously. C-peptide is made and secreted from the Beta cells in the pancreas and then released to the circulation in equimolar amounts with Insulin and it is deficient in type I diabetes. Human C-peptide is composed of 31 amino acid residues and may exert beneficial biological effects on kidney which are lost in type I diabetes, thus contributing to nephropathy.

The hypothesis to be tested in this project is that C-peptide signalling in cultured human renal tubular models (hTERT1 and HEK-293A cells) is mediated by the candidate C-peptide receptor GPR146, and that this changes in a diabetic milieu, especially in C-peptide depletion or excess, high glucose, and Insulin depletion or excess.

It was demonstrated here that C-peptide stimulates the activation of ERK in a dose-dependent manner. It was also shown that the response of ERK to C-peptide was

time-dependent. Q-PCR results show that C-peptide may down-regulate GPR146 mRNA.

It will now be interesting to investigate (by expressing eGFP-tagged GPR146 in TERT and cells and applying GFP-trapping), whether other signalling proteins associate with GPR146 in TERT cells.

Fateh Kadhim

The role of CARD9 in the pathogenesis of IgA nephropathy.

Supervisor(s): Dr Karen Molyneux and Prof. Jonathan Barratt

IgA nephropathy (IgAN) is a strong risk factor for chronic kidney disease (CKD) and end stage renal disease (ESRD), 20-40% of patients develop ESRD within 20 to 30 years of diagnosis. IgAN has a complex aetiology which is thought to include changes in the immune response. An increase in serum levels of aberrantly glycosylated IgA1 is a key element of initiating kidney injury in IgAN patients, as a result of mesangial IgA deposition. Subsequent to IgA deposition, several kinds of inflammatory and anti-inflammatory mediators are secreted, leading to recruitment of inflammatory cells from the circulation, which are associated with the histopathological changes in IgAN. CARD9 is an intracellular protein with a critical role in innate and adaptive immunity. Considerable evidence about the role of CARD9 in regulating pro-inflammatory signalling pathways in myeloid cells has been documented. Recently, reports have proposed a role for CARD9 in sterile inflammation in immune complex mediated disease such as IgAN and IBD. However, whether or not CARD9 participates in pathogenesis of IgAN remains unknown. We hypothesise that CARD9 signalling has a key role in the progression of IgAN in the development of monocyte and macrophage cell dysfunction, particularly when exposed to mesangial cell matrix during IgA1complex deposition. In this study, PBMCs were isolated from 50 individuals and then genomic DNA was extracted to determine CARD9 haplotype status. IgA1 was purified from human serum and used to stimulate an adherent immortalised monocyte cell line (U937) cells. The results of the CARD9 sequencing revealed individuals homozygous and heterozygous for the CARD9 rs4077515 haplotype. Interestingly results from stimulated U937 cells showed that IgA1 up regulated CARD9 mRNA expression and protein synthesis.

Heather MacKinnon

Intervention Updates and Design of Feasibility testing for the SPARK intervention to increase Physical Activity for people with Chronic Kidney Disease (CKD).

Supervisor(s): Dr Alice Smith, Prof. Sally Singh and Dr James Burton

The SPARK project aims to develop a self-directed intervention to promote physical activity for people with CKD, not requiring renal replacement, to improve the symptom burden of CKD, reduce cardio-vascular risk and improve Quality of Life. The intervention aims to promote walking and strength training and is being iteratively developed as recommended by the MRC Framework for the Development of Complex Interventions.

Initial testing of the SPARK intervention was conducted (n=8) yielding positive recruitment and retention data however engagement data showed the strength training element of the intervention was less successful. Reviewing the intervention design, several potentially contributory factors were identified. Starting the strength training at week 3 may have reduced engagement. Motivational Interviewing (MI) sessions focussed on initial goal-setting for the first 2weeks of walking and hence strength training was not robustly covered. Secondary outcome measurements included surrogate measures of strength however no direct strength measurement was included.

Changes made to the intervention for the next round of testing included:

- Increasing emphasis on strength training during the MI session
- Starting walking and strength training in week 1
- Including strength measurement to increase participants' awareness of this intervention element.

A focus group with the previous participants reviewed these adaptations to ensure patient acceptability. Recruitment centres have also been diversified to sample a wider population. Recruitment has started at LGH and plans to include Kettering General Hospital and Primary Care. Recruitment targets are pragmatic as this is feasibility testing however the aim is to recruit 30-50 participants before February 2018.

Dina Nilasari

KCa 3.1: A POTENTIAL ANTI FIBROTIC TARGET IN IgA NEPHROPATHY

Supervisor(s): Prof. Jonathan Barratt, Prof. Peter Bradding and Dr Karen Molyneux

Introduction IgA nephropathy (IgAN) is a primary glomerulonephritis characterised by the deposition of IgA1 containing immune complexes in the mesangium leading to glomerular and tubulointerstitial fibrosis. A direct effect of IgA1 on mesangial cells (MC), podocytes and proximal tubular epithelial cells (PTEC) is believed to be crucial for the development of renal fibrosis in IgAN. The intermediate-conductance Ca2+-activated potassium channel, KCa3.1, has emerged as an important regulator of fibroblast proliferation in renal and other diseases.

Aims The aim of this study was to investigate the effect of blocking KCa3.1 on the in vitro response of human renal cells to IgA1.

Methods Western blotting was used to determine whether human MC, podocytes and PTECs constitutively express KCa3. To evaluate the role of KCa3.1 in the pro-inflammatory and pro-fibrotic response of human renal cells to IgA1, the cells were incubated with increasing concentrations of IgA1, with or without the KCa3.1 blockers, TRAM-34 or ICA-17043. KCa3.1 expression was measured using western blotting and qPCR.

Results IgA1 induced an up-regulation of KCa3.1 synthesis in human MCs and PTECs which could be inhibited by TRAM-34 or ICA-17043. Administration of TRAM-34 inhibited the IgA1-mediated release of IL-6 by human MC.

Discussion The increased synthesis of KCa3.1 in human MCs and PTECs in the presence of IgA1 and the inhibition of IgA1 induced human MC activation suggests a role for this Ca2+-activated potassium channel in the glomerular and tubulointerstitial fibrosis characteristic of progressive IgAN and may offer a novel therapeutic target for IgAN

Conclusion KCa 3.1may offer a novel therapeutic target in IgAN.

David Wimbury

Common variation in C1GALT1 associated with galactosylation of IgA1 and altered C1GALT1 mRNA expression in IgA+ cells.

Supervisor(s): Dr Karen Molyneux and Prof. Jonathan Barratt

IgA nephropathy (IgAN), the commonest primary glomerulonephritis worldwide, is characterised by the deposition of IgA1 and IgA1-containing immune complexes in the renal mesangium leading to renal impairment. IgA1 from IgAN patients, in serum and isolated from mesangial deposits, has been found to contain lower levels of O-linked galactose. Serum levels of undergalactosylated IgA1 have also been linked to IgAN progression.

The first part of this work identified a single haplotype across the gene C1GALT1 strongly correlating with raised levels of undergalactosylated IgA1 in serum. C1GALT1 encodes the enzyme T-synthase which catalyses the addition of galactose to glycosylated proteins, including IgA1. The second part of this work involved assessing the effect the presence of this C1GALT1 haplotype has on the expression of T-synthase in IgA producing cells. Healthy volunteers carrying different combinations of the C1GALT1 haplotype were identified. B lymphocytes were isolated from whole blood from these volunteers and stained to identify IgA+/- and IgD+/- cells. The stained cells were sorted and expression of C1GALT1 mRNA was determined.
C1GALT1 was found to have reduced mRNA expression in individuals homozygous for the haplotype linked to IgA1 undergalactosylation; specifically in IgA+ cells.

These results show that the levels of undergalactosylated IgA1 in circulation are influenced by a variation of the C1GALT1 gene. This is reinforced by showing the expression of C1GALT1 mRNA is reduced in people with the haplotype associated with higher levels of Gd-IgA1. Further work to measure levels of T-synthase protein in the sorted cell populations is now needed for confirmation.

Yan Song

Exercise behaviour in unit-based maintenance Haemodialysis patients in the UK and China.

Supervisor(s): Dr Alice Smith and Dr James Burton

***Introduction*:** Patients on maintenance haemodialysis (HD) frequently suffer from reduced muscle mass and poor physical function and strength, associated with increased risk of morbidity and mortality. In order to design realistic, effective and person-centred interventions to promote exercise behaviour, a thorough

understanding of exercise levels and habits is required. Perceptions, attitudes and habits relating to lifestyle may be influenced by ethnicity and culture.

Aims : to explore self-reported exercise behaviour in HD patients from the UK and China using the Godin Leisure Time Exercise Questionnaire (LTEQ) which asks about strenuous, moderate and mild exercise activity, and allows calculation of total exercise activity and a Health Contribution Score (HCS) to categorise respondents as "Active", "Moderately Active" or "Insufficiently Active".

Methods: In this cross-sectional study, the LTEQ was administered to 768 patients at UK and Chinese HD units (UK: n=266; M: F 2.9:1; median (IQR) age 65 (21) years. China: n=502; M: F 1.4:1; age 52(19) years.

Results: UK HD patients reported participating in less strenuous (Z=-2.127, P=0.033), moderate (Z=-2.933, P=0.003), mild (Z=-3.094, P=0.002) and total (Z=-3.031, P=0.002) activity than the Chinese HD cohort. There were no differences between the groups in HCS (Z=-0.768, P=0.442). However, HCS analysis revealed that a higher proportion of UK HD patients than were "Insufficiently Active" (Z=-18.424, P<0.001).

Conclusion: UK HD patients reported lower levels of exercise than the Chinese HD cohort. Reasons for this are unclear, but may be related to the different age and gender profiles, diverse cultural perceptions of exercise, or other lifestyle factors. Further qualitative and longitudinal studies will explore the reasons for the discrepancy between UK and Chinese HD patients, and how exercise behaviour relates to disease severity, comorbidity and quality of life.

Yuanyuan Wu

Properdin Deficiency Increased Tissue Protective Receptor Expression for Regeneration in 72-hour Renal Ischemia Reperfusion Injury Mice.

Supervisor(s): Dr Bin Yang and Prof. Nigel Brunskill

Properdin is an only positive regulator in the alternative pathway of complement activation. As expected, ischemia-reperfusion (IR) induced acute kidney injury may be ameliorated in the properdin knockout (P^{KO}) mice. However, our previous work has demonstrated more severe damage in P^{KO} mice than its wild type (WT) in a 72 h renal IR injury model. It has also been noticed that proliferative factors PCNA and mitosis figure were elevated in P^{KO} mice post 72 h IR injury. Therefore, the aim of this study is to disclose its underlying mechanism with focus on erythropoietin (EPO) tissue protective receptor composed of EPO receptor (EPOR) and β common receptor

(βcR). Here, we found that EPOR protein level in WT kidneys was significantly increased by IR injury and further by properdin deficiency. EPOR accumulation was found enhanced on cellular plasma and membrane in both WT and P^{KO} IR kidneys compared with sham ones. Furthermore, βcR mRNA level in kidneys was significantly increased by IR injury about 9 folds in WT and 17 folds in P^{KO} mice. Both EPOR protein and βcR mRNA levels were significantly correlated with tubulointerstitial damage score, apoptosis, HMGB-1 and caspase-3 protein, as well as PCNA protein and cellular mitosis. The expression of EPOR protein and βcR mRNA was increased by IR insult and further enhanced by properdin deficiency, which might be associated with not only tissue damage, but also repair. The impact of properdin on these tissue protective related receptors and its biological involvement are worthy of further investigation.

Amy Clarke

Associations between physical function and survival in patients with chronic kidney disease.

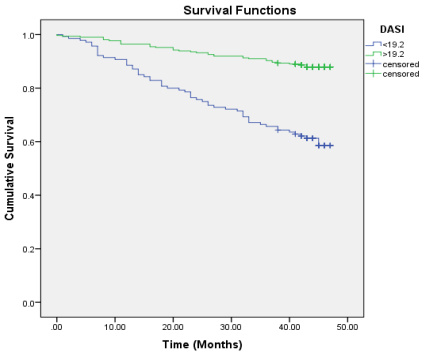
Supervisor(s): Dr Alice Smith and Dr Thomas Yates

Introduction: Reduced physical function is associated with an increased risk of mortality in CKD. Physical performance assessments can help to identify those at risk for adverse events. However, objective measures are not always feasible, and self-reported measures may provide a suitable surrogate.

Methods: Associations between self-reported physical function and survival were examined in 450 non-dialysis CKD patients (median follow up 44 months). Upon enrolment participants completed the Duke Activity Status Index (DASI) (measures physical capacity). Mortality was recorded from electronic records in September 2016. A receiver operating curve (ROC) was constructed to plot DASI scores as a binary classifier for survival. Sensitivity and specificity analyses were performed to identify a cut off score to predict mortality. Kaplan-Meier survival curves and log-rank tests were used to estimate survival proportions. Cox proportional hazard models were constructed to examine associations between DASI and mortality.

Results: 93 (20.4%) deaths occurred. The ROC showed that a DASI score of 19.2 predicted mortality, (60% sensitivity and 77% specificity) (area under curve 0.76; CI 95% 0.71-0.81; p <0.001). Kaplan-Meier showed a separation between survival curves (P<0.001, see figure). Adjusted hazard ratios (HR) for those scoring >19.2 on the DASI for mortality was 0.56 (95% confidence interval [95% CI], 0.35- 0.92, p<0.001). The DASI also indicated an overall association with mortality (HR=0.975,

95% CI 0.957-0.992, P=0.004).



Conclusion: The DASI was independently associated with survival in CKD patients, and may help to identify vulnerable patients. Physical function is modifiable and may be an amenable target for intervention.

Kawther AlQaseer

The role of Serine/Threonine protein phosphorylation in function of Lsr2.

Supervisor(s): Dr Galina Mukamolova and Dr Helen O'Hare

One third of the world population is estimated to have latent tuberculosis infection (LTI) associated with dormant *Mycobacterium tuberculosis* bacilli which are more resistant to antimicrobial treatment and can resuscitate resulting in development of active tuberculosis. Lsr2 is a global transcriptional regulator which has over 1000 DNA binding sites and controls gene expression in mycobacteria during growth, dormancy and resuscitation. A deletion of *lsr2* in *M. tuberculosis* resulted in a dramatic growth defect on solid medium, the inability to persist in hypoxia and impaired persistence in animals. However, *lsr2* inactivation in fast-growing *Mycobacterium smegmatis* did not affect growth and was important for production of specific lipids and biofilm formation. Our phosphoproteomic analysis of *M. tuberculosis* producing an essential serine/threonine protein kinase B (PknB) revealed phosphorylation of Lsr2 on threonine 112 (T112). Furthermore a recombinant PknB was able to phosphorylate a recombinant Lsr2 protein *in vitro.* Complementation studies using *M. tuberculosis* and *M. smegmatis lsr2* deletion mutants showed that T112A mutation led to a complete loss of complementation of the mutant phenotypes, hence suggesting an essential role of phosphorylation for function of Lsr2 in mycobacteria. Future experiments will be focused on influence of phosphorylation on Lsr2 DNA binding and protein-protein interactions.

Mutlaq Alshammri

Investigate the physiological state of Mycobacterium tuberculosis during aerosol transmission.

Supervisor(s): Prof. Mike Barer and Dr Primrose Freestone

Background: Although the aetiological agent of tuberculosis (TB) in humans, *Mycobacterium tuberculosis*, (*Mtb*) was first discovered more than 100 years ago, it remains a successful pathogen in terms of morbidity and mortality. The mechanisms used by *Mtb* to adapt itself against surrounding stresses within host tissues and aerosols to survive, transmit and infect new individuals are not fully understood. Identifying the features of the bacilli in aerosols and how these properties influence the survival of the pathogen within the aerosols to infect a new host is a key challenge that is largely unexplored.

Method: *M. bovis* BCG Glaxo strain was used as a model to begin an investigation into the relationships between cell lipids and propensities for aerosolisation. *M. bovis* BCG was grown to stationary phase then washed and re-suspended with PUM buffer. A comparison between the suspension before and after nebulisation was investigated.

Result: There was a significant reduction in the cell wall hydrophobicity level and percentage of cell imaged with lipid body positive of *M. bovis* BCG suspension after nebulisation.

Conclusion: Preliminary evidence has been obtained relating cell wall hydrophobicity and buoyancy of the pathogen to propensity for aerosolisation.

Malikah A. Sadik

The Interaction between *Mycobacterium tuberculosis* and Vitamin D.

Supervisor(s): Prof. Mike Barer and Dr Natalie Garton

Vitamin D deficiency has long been associated with the progress of tuberculosis (TB). TB, an infectious disease caused by bacterial pathogen *Mycobacterium tuberculosis* (M.tb) responsible for the loss of 4000 lives daily. In the pre-antibiotic era, cod-liver oil and sun exposure were reported as the only effective treatment to cure TB and both of these treatment would increase physiological vitamin D levels. However, recent randomized controlled trials have not proven the adjunct role of vitamin D3 in accelerating the recovery of TB patients.

In *vitro* the supplement of vitamin D3 metabolites dihydroxyvitamin D3, 5-logs higher than in the blood, showed to increase the doubling time of virulent M.tb within macrophages from 1 to 3 days with no overt bactericidal effect. This metabolite modifies the immune response by stimulating the macrophages production of nitric oxide, antibacterial peptides Calthicidin, promote phagolysosome fusion, and reduces the infection-induced lipid droplets accumulation.

Two studies reported a direct effect of vitamin D on M.tb. The earlier one reported a bactericidal effect of 2.5 mg of vitamin D2 on solid medium, while the most recent one reported a dose dependant inhibition of 64 mg of vitamin D3 in liquid medium broth. Therefore, the direct response of M.tb to vitamin D remains to be explored.

Methods are being developed to measure the effect of vitamin D3 on Mycobacterial growth. Dose dependent effects were seen on *Mycobacterium smegmatis* growth measured with optical density. These effects were eliminated in the presence of the detergent Tween80. *Mycobacterium bovis* BCG optical density measurements in the absence of Tween80 are not reliable, although the effect of vitamin D3 are observed. Alternative methods of measuring the growth rate or the cell activity are being explored, before investigating with M.tb.

Mariam Mohamed Nur

Elucidating the function of Resuscitation-promoting factors in secretion of major virulence factors and metal acquisition in mycobacteria.

Supervisor(s): Dr Galina Mukamolova and Dr Primrose Freestone

Tuberculosis remains a global health threat. An estimated one-third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb). Mtb is an exceptionally successful human pathogen due to its ability to enter a dormant state. Resuscitation promoting factors (Rpfs) are lytic transglycosylases (LTG) involved in the reactivation of dormant bacteria via peptidoglycan remodeling; the precise molecular mechanism has yet to be elucidated. LTG have previously been associated with bacterial secretion systems in gram-negative pathogens. Notably, Mycobacteria have 5 type VII secretion systems (T7SS), namely ESX1-ESX5. While ESX1 is critical for mycobacterial virulence, ESX-3 is the T7SS linked to iron and zinc acquisition. Our labs recent proteomics secretomics data have indicated significant impairment of ESX1 and ESX3 secretion systems in Mtb *rpf* null strains. We have therefore hypothesized that Rpfs are involved in the assembly of ESX3 and that the deletion of *rpf*s results in the improper assembly of ESX3, resulting in a metal deficient phenotype. Our results indicated that the addition of haemin as an iron source to *Mycobacterium marinum*

ΔrpfAB strain with a defective growth phenotype in Sauton's medium, moderately improves the growth of this strain. Full "chemical complementation" of *ΔrpfAB* to WT growth has been achieved with both magnesium and zinc. This work includes the generation of the first ever *rpf* triple mutant in *M. marinum*, a class two pathogen which has intact ESX1 and ESX3, and the characterization of this mutant may well provide a specific role to the currently limited knowledge of Rpf function.
The mutant could only be generated using haemin and zinc, confirming our hypothesis on a special role of Rpf in metal acquisition.

Moreover, the mutant did not grow on agar, it has a growth defect in minimal medium and the phenotypes in liquid can be complemented by addition of supernatant from WT or with the addition of haemin and zinc.

Valeria Quimper

Do Neutral Lipids Enhance Environmental Survival and Transmission of *Mycobacterium tuberculosis*?

Supervisor(s): Prof. Mike Barer and Dr Natalie Garton

Background: Understanding the way *M. tuberculosis* (Mtb) can tolerate environmental stresses can aid in the comprehension of how this pathogen is able to prevail and transmit to other hosts. *Mycobacterium tuberculosis* utilizes lipid metabolism for survival during nutrient availability restrictions induced by the immune system. Previous studies have shown that bacterial lipid bodies (LBs) increase the environmental survival of rhodococci (relatives of Mtb) and the aim of this work is to test whether these structures have similar effects in Mtb. Initial work has focussed on survival during desiccation and the use of luciferase reporters to assess viability.

Methods: The desiccation assay was done by sterilizing coverslips, inoculating with an exponential phase culture then leaving it to desiccate for different times at which samples were taken by washing and assessments by colony (CFU) counts and luciferase assay. For the luciferase assay, the luciferin substrate was dispensed onto samples and emitted light (RLU) was measured.

Results: Current results have shown a strong correlation between CFU counts and RLUs in serially diluted cultures. The desiccation assay did not show any significant difference between WT and Ffluc strains; luciferase and CFU assay showed different recovery percentages. Ratios of luciferase assay and cell lysis ATP assay showed similar ratios across 2 strains.

Conclusion: The results obtained have provided the basic methods needed to carry out this study.

Baleegh A. Kadhim

The Role of Muralytic Enzymes in Growth, Persistence and Virulence of *Mycobacterium Tuberculosis.*

Supervisor(s): Dr Galina Mukamolova and Prof. Russell Wallis

The problem of tuberculosis is further complicated by the emergence of multiple drug resistant strains of *M. tuberculosis.* Moreover, it has a remarkable cell wall structure that can assist as a significant barrier against multi-drug treatments. This leads to the death of about 1.5 million people annually (WHO, 2015). Peptidoglycan hydrolyzing enzymes such as N-acetylmuramyl-L-alanine amidase (CwlM) have several biological functions and can be considered as excellent drug targets. Amidases play a key role in controlling mycobacterial growth, antimicrobial resistance and cell wall biosynthesis. However, the precise mechanisms regulating these dynamic processes remain unknown. A recombinant *M. tuberculosis* CwlM was purified as a soluble protein. Protein purification will enable the study of CwlM activity in controlling mycobacterial growth. Firstly, phosphorylation of CwlM by a recombinant serine/threonine protein kinase in vitro has been confirmed by application of western blot and mass-spectrometry. Furthermore, the recombinant CwlM was sent for generation of polyclonal antibodies. The sensitivity and specificity of anti-ClwM antibodies were characterised using dot blot and western blot assays. The results have shown the anti-CwlM antibodies recognize the recombinant protein at a dilution of up to 1:200,000. The antibodies will be used for detection of CwlM in mycobacteria, pull-down assays and for purification of native CwlM from mycobacteria.

Finally, the muralytic activity of CwlM was investigated using zymography. The results have indicated that the full-length CwlM did not have muralytic activity in zymogram assays. The PknB phosphorylated full-length CwlM also failed to produce a clearance band on zymogram. Preliminary results indicate that a shorter form of CwlM is active in zymogram, thus suggesting that PknB phosphorylation may control cleavage and activation of CwlM. Further studies are underway to investigate the biological structure of truncated form of CwlM using crystallography.

Iswahyudi Iswahyudi

Serine/threonine protein phosphatase (PstP) of *Mycobacterium tuberculosis*
Supervisor(s): Dr Helen O'Hare and Dr Galina Mukamolova

Regarded at this time being to be the only serine/threonine phosphatase acting to oppose the activity of eleven serine/threonine protein kinases, PstP could potentially dephosphorylate more than 500 serine/threonine protein phosphorylation sites identified so far in the *M. tuberculosis* proteome.

Located in the operon region with genes encoding serine/threonine protein kinases PknA and PknB, *pstP* is probably involved in controlling mycobacterial cell growth.

Mutant strains with disrupted in *pstP* gene is likely to have a higher level of phosphorylated proteins because of the lack of de-phosphorylation activity. Using *pstP* conditional gene replacement mutant in *Mycobacterium smegmatis* (CM MSMEG_0033), the aim of this project is to investigate the effect of depletion and overexpression of PstP on the growth and morphology of the cells. Depletion of PstP has shown a decreasing phase in un-inducer cultures which might indicate the effect of the lack of *pstP* on the cells growth. The cells became unable to transcripted *pstP* in the absence of inducer. This evidence might have proved that *pstP* is essential to *M. smegmatis*. On the other hand, the growth of *pstP*-overexpressing of CM MSMEG_0033 growing without inducer was similar as the growth of the same strain in media with inducer. PstP depletion results in damaging the cell envelope integrity and septum formation, suggesting the role for PstP in regulating the cell division events. To examine whether *pstP* depletion and overexpression influences the global phosphoproteome, cell lysates from the strains will be compared by Western blotting using anti-phosphothreonine antibody.

Homam Helal Ageel

How does inhibition of either the lectin pathway or the alternative pathway or the terminal pathway of complement activation effect the resistance to *Neisseria meningitidis* infection in vaccinated and non-vaccinated mice?
Supervisor(s): Prof. Wilhelm Schwaeble and Prof. Peter Andrew

The complement system constitutes an important part of both the innate and adaptive immune system equipping host with defences against many pathogens including *N. meningitidis*. The Alternative pathway (AP) of complement and its roles in the protection from different infections, including meningococcal infections, has been well understood. Testing different serotypes of Neisseria meningitidis in serum

bactericidal assays (SBAs) have shown that MASP-3 is a key factor required for driving the AP and essential to mediate killing of *N. meningitidis*. Since the pathophysiology of various autoimmune diseases involves the AP as a driving force of chronic inflammation, my supervisor established therapeutic antibodies that target MASP-3 in order to control AP activation with his commercial partner OMEROS Corporation. A currently established treatment to target complement dependent inflammatory disease is to use an inhibitory anti-C5 antibody which inhibits the activation of the terminal complement component C5 and with that the release of the complement anaphylatoxin C5a and with that the formation of membrane attack complex (MAC). Since MAC formation is essential for the complement-mediated killing of Neisseria, this treatment predisposes to meningococcal infections, a complication that is supposedly handled by vaccinating patients that against Neisseria. However, even in presence of specific antibodies, no SBA is detectable in C5 inhibited serum. My present study aims to assess and compare the efficacy of vaccination against *Neisseria meningitidis* in mice with and without systemic pre-treatment with therapeutic inhibitors for MASP-2, MASP-3 and C5.

Mohenned Alsaadawi

Humanised mouse properdin could augment the alternative pathway of wild type mouse and normal human serua on zymosan and *Plasmodium falciparum* merozoites coated plate.

Supervisor(s): Dr Nicholas Lynch and Prof. Wilhelm Schwaeble

The complement system has a major role in the immune system and enhances the killing of infectious organisms. There are three pathways of complement activation, the classical, the alternative and the lectin pathways. They converge with the formation of a C3 convertase. The complement pathways are regulated by many proteins such as properdin, factor H, CR1 and factor H related proteins (CFHR1). All the known and probable complement evasion mechanisms used by *P. falciparum,* the causative agent of malaria, act on the alternative pathway C3 convertase. C3bBb, inhibited during *Plasmodium* infections, can be stabilised by properdin thus producing more C3b. My project aims are to produce chimeric properdin which can be used to augment the alternative pathway activity of mouse and human sera on *P. falciparum* surfaces. Three different forms of humanised mouse properdin were produced in mammalian cell culture and their activity in normal, factor P depleted human and wild type mouse serua was tested on zymosan.

Neda Nezam Abadi

Identification of novel prophage-like elements in the genomes of *Legionella* spp.
Supervisor(s): Dr Edouard Galyov and Prof. Martha Clokie

Legionella spp. are waterborne pathogens that can causes Legionnaires' disease, a potentially fatal acute pneumonia. These pathogens have been shown to be resistant to many disinfectants, and beta-lactam antibiotics. Furthermore, detection of *Legionella* spp. is often difficult, thus infections with these organisms can be difficult to both treat and diagnose. As such there is a great need to identify more accurate diagnostic tools and more effective therapeutic agents against *Legionella* spp. Bacteriophages (phages) are a potential tool to be exploited for this purpose with bacteriophage based diagnostics already successfully developed for Bacillus anthracis and bacteriophage based therapeutics in active development against numerous other bacteria. However, little is known about the bacteriophages with the capability of infecting *Legionella* spp. To date, no viable bacteriophages have been isolated, and only 1 potentially viable prophage has been described in the literature. Thus with little knowledge available in the literature, the aim of this study was to obtain more information on *Legionella* phage using a bioinformatics approach. To do this, the genomes of 612 *Legionella* belonging to 50 species were screened for putative prophages using PHASTER (PHAge Search Tool Enhanced Release). From this analysis 1495 putative prophage-like elements were identified, which across all *Legionella* spp. could be grouped into four major clusters.

Detection of these new prophages can serve as valuable source for future studies aiming to isolate bacteriophage infecting *Legionella* spp., e.g. using quantitative PCR (qPCR) to measure changes in bacteriophage densities in a culture independent manner, negating one major drawback of *Legionella* spp. bacteriophage research to date, the inability to observe single plaques.

Wafaa AlRashidi

Bacteriophage-Bacteria-Amoeba Interaction
Supervisor(s): Prof. Martha Clokie and Dr Edouard Galyov

In natural environments, bacteriophage have a major influence on controlling bacterial population, as they would directly kill their bacterial host in their lytic life cycle, or may alter the bacterial phenotypic characteristics while integrating to their

genomes through the lysogenic life cycle. However, and as it is apparent that in many environments, pathogenic bacteria can survive despite the abundance of bacteriophages and were able to transmit into the environment and into susceptible hosts. Some studies suggested that bacterial survival in nature is due to their endosymbiotic relationship with some protozoan species such as Acanthamoeba, which assist them to escape environmental challenges or chemical treatments, and multiply within the protozoan cell, producing enough numbers for their transmission through the environment and establishing infections. The ubiquitous protozoan *Acanthamoeba castellanii* will be used in this study, for its significance in acting as a Trojan horse and a reservoir for bacterial pathogens, hence, assisting pathogens persistence in various environments. Here, we have developed a novel study to investigate whether intracellular survival of bacteria into an amoebal host would protect them from bacteriophage infection. Thus, infection assays have been acquired and will be used to quantify intracellular bacteria on the basis of eliminating extracellular bacteria from a co-culture system.

Thekra Sideeq Al-Tayawi

Differential Expression of *Clostridium difficile* and phage genes during a one-step growth curve

Supervisor(s): Dr Shaun Heaphy and Prof. Martha Clokie

Clostridium difficile is a pathogenic bacterium causing antibiotic-induced diarrhoea in the immuno-compromised. Genomes of the majority of *C. difficile* strains encode inducible prophages that could access the lytic pathway in a susceptible host. Although *C. difficile* phages with lytic ability have been characterised in the literature, knowledge of the impact they exert on their hosts is limited. Previous studies revealed that the phages could transduce infected cells, influence toxin production and regulate phase variable genes during lysogeny. However, no studies currently exist to reveal the global transcriptional response of *C. difficile* during a phage lytic infection cycle. Here, we determined the lytic growth kinetics of phiCDHS1, a siphovirus infecting the R20291 strain and analysed the genes expressed during the phage cycle. Analysis of the one-step growth curve revealed that the phage completes its replication in 40 min with a burst size of 1000. The early-log, mid-log and stationary phases of the phage replication were observed at 20, 30 and 40 min respectively. Total RNA extracted at these time points was rRNA-depleted and sequenced using Illumina HiSeq^{TM}4000 technology. Analysis of the mapped transcripts against the reference bacterial genome suggests that 1713/3507 (~30%) genes at p value 0.001 were differentially expressed during the phage infection cycle. Phased gene expression was observed in the phage. The phage genes expressed

(44/51) at post-infection correspond to early transcripts associated with DNA replication and late transcripts for structural proteins. Analysis is currently ongoing to determine how expressions of these phage genes affect gene expression in the host bacterium. These findings will help to elucidate the transcriptional response of *C. difficile* to phage infection.

Ananthi Ramachandran

Development of robust *ex situ* models to investigate the therapeutic potential of *C. difficile* phages

Supervisor(s): Prof. Martha Clokie and Dr Shaun Heaphy

C. difficile, a Gram positive, spore forming, toxin producing, anaerobic bacteria is responsible for causing *C. difficile* associated diarrhoea (CDAD). Treatment of CDAD, particularly by antibiotics is problematic due to the organism's ability to develop resistance to such treatment and therefore alternative methods of treatment are being sought after.

Phage therapy (use of bacteriophages to treat bacterial infection) has shown promise due to its advantages over standard antibiotics including high efficiency, minimal disruption of the natural intestinal microbiota and ability to infect antibiotic resistant bacteria.

To date, no work has been carried out on understanding phage, *C. difficile* and human gut epithelial cell interactions. A cell culture model using mucus producing HT29 cells (HT29-MTX) was developed to mimic a natural setting where *C. difficile*, phage and cell interactions can be observed.

It has been previously revealed that mucus levels of HT29-MTX cells gradually increase over a 21 day period and remain stable thereafter. In this same time period, results have shown that varied levels of mucus also alter phage interactions with the epithelial cells as well as the phage activity displayed against *C. difficile*.

It was observed that when mucus levels of HT29-MTX cells increased, not only was the phage able to adsorb to the mucus but once adsorbed, it was evident that the phage could only kill *C. difficile* that was yet to attach to the epithelial cell mucus layer.

Ahmed Dowah

Identification of Receptors Binding proteins for *Clostridium difficile* Bacteriophage
Supervisor(s): Prof. Martha Clokie and Prof. Russel Wallis

Clostridium difficile is responsible for a range of gastrointestinal diseases referred to as C*. difficile* infection (CDI) and due to the emergence of antibiotic resistance, alternative treatments for CDI are urgently required. Phages could provide an alternative source of antimicrobials for this pathogen due to their specificity, minimal disruption of microbiota and ability to self-amplify at the site of infection. However, before phages can be used therapeutically, the *C. difficile* phage infection process needs to be extensively studied. To date no studies have identified to which receptors on the bacterial surface the phages bind or adsorb, to establish infection. In other words, how does the first physical contact between phage receptor binding proteins located in the distal part of the phage tail and the surface of the bacterium occur. The PhD project has aimed to identify the receptor binding proteins for phage phiCDHS1 (Siphovirus), which infects clinically relevant ribotypes. The approach employed to identify the receptor binding proteins was by over-expressing four predicted phage tail fiber proteins (Gp18, Gp19, Gp21 and Gp22), as presumably one or two of them was involved in a phage host binding. The expressed proteins were purified, polyclonal antibody were generated against them and used to neutralize phage infection. Only antibodies against Gp22 were able to block phiCDHS1 infection indicating that, this protein is the one responsible for recognition of *C. difficile* and subsequent adsorption. Work is currently underway to solve the structure of Gp22. These findings will lead to greater understanding of bacteriophage biology, the mechanisms behind the predator-prey interaction between the phages and their hosts.

Guillermo Rangel-Pineros

Exploration of the diversity and ecology of *Streptococcus pneumoniae* bacteriophages
Supervisor(s): Prof. Martha Clokie, Dr S Heaphy and Dr Brenda Kwambana

Streptococcus pneumoniae bacteriophages (pneumophages) have been studied since the early 1970s but whilst many reports have revealed a high prevalence of prophages in clinical isolates, only two lytic pneumophages have been reported to date. Our research has focused on the exploration of pneumophages in The Gambia and the role they play in the physiology of the pneumococcus.

123 nasopharyngeal swabs (NPS), collected from healthy children < 5 years old, were enriched for pneumophages. Phage characterization was conducted by transmission electron microscopy, host range, genome sequencing and isolation of the corresponding lysogen. Prophages were detected in the genomes of a set of Gambian pneumococcal isolates. These were compared with previously reported pneumophages and their phylogenetic relationships were inferred.

A temperate siphovirus SpGS-1 was isolated from an NPS of a 4 year old child. It has a 37631 bp genome with 53 putative ORFs organized in five modules. Genome comparisons revealed that SpGS-1 is related to pneumophage MM1 (a PNEM1 prophage), albeit major differences were detected in the lysogeny and morphogenesis modules. All Gambian isolates tested had significantly lower MitC-MIC in comparison with a set of non-prophage-containing pneumococcal strains. 11 complete prophages were detected in the genomes of the Gambian strains analysed, although two of them were found in genomic locations different from those identified in previous reports. The characterization of the identified pneumophages will shed light on the role these phages play in the ecology of the pneumococcus.

Aisha Salah Amer

Factors contributing to the control of A*rthrospira fusiformis* in East African Soda Lakes

Supervisor(s): Prof. Martha Clokie, Dr Shaun Heaphy and Prof. David Harper

East Africa's Central Rift Valley has the largest population of lesser flamingo (*Phoeniconaias minor)* in the world. The diet of lesser flamingos consists almost entirely of *Arthrospira fusiformis*. Unfortunately, in recent years, an irregular and unpredictable crash in the density of the *Arthrospira* population has been reported. The aim of this study is to investigate the role of cyanobacteriophages in regulating the *Arthrospira* biomass in some alkaline-saline lakes by metagenomic, microscopy and cultures techniques.

The result of electrical conductivity and chlorophyll content of Lake Bogoria showed that the chlorophyll content (μg L^{-1}) gradually increased from May to October 2015; this was associated with the increase in the salinity of lake water. The abundance of this cyanobacterium crashed significantly in July 2016 when the EC of the lake increased. The H-type *Arthrospira* morphotype was the less abundance of the three in the Central Basin; in the time of the *Arthrospira* breakdown (July 2016) the number of trichomes was zero. A microtome assay of four *Arthrospira* samples and a negative control, showed that one sample (from July 2016) had strange particles within

Arthrospira sections; their shape and size seem to indicate that they are phages. These results were in agreement with the NanoSight results, in which the concentrations of selected particles for the samples from June and July 2016 were significantly different from the control sample. These results could support the idea of the presence of cyanophages of *Arthrospira* in Lake Bogoria,

Faizal Patel

Developing a novel diagnostic test to detect bacteria, with the use of bacteriophages, microfluidics & spectroscopy

Supervisor(s): Prof. Martha Clokie, Dr E Galyov and Dr Andrew Hudson

Bacteriophages are well known for their therapeutic anti-bacterial properties. However, there is also potential to use phages in a diagnostic manner to identify bacteria. Phages are currently an underexploited resource within the biotechnology and diagnostic field. Increasing the accuracy of pathogen detection will allow for more efficient antibiotic therapy regimens thus minimising contribution to antibiotic resistance.

Here we are investigating the use of phages, which will directly confirm the presence of pathogens from poultry samples. This will be done through the use of quantum dots and microfluidics (lab on a chip based technology). Quantum dots (QDs) are semi-conductive nano-crystals which can act as reporter probes amongst other applications. QDs have many advantages over traditional reporters such as having broad excitation spectra, narrow emission spectra, tunable emission peaks and long fluorescence lifetimes.

Currently, locally isolated *Salmonella* phages are being investigated for properties such as both broad and narrow range specificity and high burst sizes. Initially a single chosen phage will be physically labelled with biotin, allowing for the coating of the phage with streptavidin coated QDs. These labelled reporter phages will be investigated within microfluidic devices with the use of spectroscopy for their ability to act as reporters, to confirm or deny the presence of *Salmonella* spp. We will then move on to quantifying bacterial presence using droplet microfluidics, and multiplexing to allow the detection of multiple bacterial cells within one assay by using multiple reporter phages.

Mohammed Imam

Studies of Host-Specific Non-culturable Phages

Supervisor(s): Dr Ed Galyov and Prof. Martha Clokie

Bacteriophages are the most abundant biological entities in the environment. They kill almost half of earth's bacterial population every two days. Bacteria exposed to phages can be infected in an either lytic or lysogenic way depending on the nature of phages and environmental factors. Phages can be used as a therapeutic agent or a prevention tool for bacterial infections.

Phages are a vast understudied and underexplored source of biodiversity; however, current methods of isolating host-specific phages from the environment are dependent on the culturability of the host, which is not always possible, and the ability of phage to form plaques. These methods are not suitable for phages that do not form plaques under laboratory conditions and for hosts that are resistant to phages due to the presence of anti-phage mechanisms. Therefore, the demand for developing new approaches to isolate host-specific non-culturable phages is increasing, given the need to expand understanding of biodiversity and bacteriophage applications.

In this project, a new method for the isolation of phage DNA using bacterial outer membrane vesicles (OMVs) has been designed and evaluated. The principle was proven using a previously isolated *Pseudomonas aeruginosa* phage and OMVs extracted from *P. aeruginosa* strain. Incubation of the phage with OMVs resulted in the release of phage DNA specifically. Further confirmations are required to assess the applicability of the developed method for environmental samples. To choose the ideal sample, water samples from different sources have been collected and screened for the presence of phages prior to isolation of non-culturable phages using the developed method.

Lamiaa Fingan Al-Maliki

Isolation and characterisation of bacteriophages infecting lyme *Borrelia* species

Supervisor(s): Prof. Martha Clokie and Dr Ed Galyov

Lyme borreliosis (LB) is caused by *Borrelia* spp. It is the most common vector-borne disease in the Northern Hemisphere. The disease is transmitted to humans by ticks and previous record showed that as many as 3,000 cases are diagnosed each year in the UK. LB is treated with amoxicillin and doxycycline and there are currently no vaccines available. In many cases patients may suffer from persistent long-term

symptoms, so called 'chronic Lyme disease', which could be due to antibiotic resistant bacteria. Therefore, alternative LB therapies are needed. Phages may complement or substitute the current treatments and may provide a diagnostic tool for the disease. The aim of this project is to isolate and characterise phages associated with *Borrelia* spp for therapeutic or diagnostic purposes. To do this, seven *Borrelia* strains were cultured in complete BSKII medium and treated with different inducing agents such as Mitomycin C, Norfloxacin to mediate prophage release. Transmission electron Microscopy analysis of the induced lysate revealed the presence of different morphologies of phages. Analysis of phage activity using spot test and SYBR GreenI /Propidium Iodine (PI) live and dead cell assay is underway to determine their potential application. This work will lead to the first systemic understanding of phages infecting *Borrelia.*

Iman Tajer Abdullah

Mechanistic and Phenotypic Characterisation of the Rgg/SHP Quorum Sensing System in *Streptococcus pneumoniae*

Supervisor(s): Dr Hasan Yesilkaya, Prof. Peter Andrew and Prof. Russell Wallis

The Rgg regulators with their short signaling peptides called SHP (short hydrophobic peptides) form part of a quorum sensing system in Gram positive bacteria. They play an important role in stress response, sugar metabolism, and virulence, but their function and mechanism of action remain unclear in the important human pathogen *Streptococcus pneumoniae*. The pneumococcal type 2 D39 strain has five homologues of Rggs, and two of them (SPD_0144 and SPD_0939) are predicted to be associated with putative *shp* genes encoding for SHP peptides, which regulate their own expressions, and are predicted to be required for Rgg activation. Therefore, this study was designed to identify the active form of SHP144 to stimulate Rgg144-mediated transcription, and quantify the functional importance of each selected SHP144 residue in Rgg144 activation and binding, and then establish their importance in Rgg144's phenotypic manifestation.

To achieve these aims, several synthetic peptides representing the C-terminal end of SHP144 were synthesised to identify active SHP144 using reporter strains P*shp144*: *lacZ*-wt and P*shp144*:: *lacZ*-Δ*shp144* ('P'-promoter). Site directed mutagenesis was used for substitution of selected individual amino acids residues of SHP144 with alanine, and the effect of each amino acid replacement was studied using a transcriptional reporter assay. The phenotypic impacts of mutations were determined by oxidative stress induced by paraquat, and by growth assays in chemically defined medium supplemented with different sugars.

The results showed that 12 and 13-aa long synthetic peptides representing the C-terminal end of SHP144 (C12 and C13, respectively) were sufficient to stimulate P*shp144*::*lacZ* both in wild type and *shp144* mutant background. Furthermore, replacement of the majority of selected residues with alanine abolished P*shp144* transcription, indicating the importance of selected residues in transcriptional activation. In addition, mutations of selected SHP144 residues decreased pneumococcal resistance against paraquat, and diminished its growth in CDM supplemented with mannose. Further studies are underway to define the importance of individual SHP144 amino acids in Rgg144 binding, and their impacts on pneumococcal Rgg144 conferred phenotype.

Rashed Alghamdi

Studies on the functional significance of the asparaginase and glutaminases of *Klebsiella pneumoniae*.

Supervisor(s): Dr Hasan Yesilkaya and Dr Primrose Freestone

Asparaginase and glutaminase are sets of enzymes that assist *K. pneumoniae* in acquiring necessary nitrogen sources when ammonia (NH_3), the preferred N source, is low or absent. My work has found that *K. pneumoniae* contains four putative asparaginase and glutaminase genes (*yneH*, *ybiK*, *ansA* and *KPN_01165*). The aim of my project is to investigate the contribution of these enzymes to *K. pneumoniae* N metabolism and virulence by creating isogenic unmarked mutations in these genes. Investigations showed that among all the 4 asparaginase and glutaminase genes, *KPN_01165* seem to be the most important metabolically, as the strain carrying this mutation suffered reduced growth in minimal culture media, low asparaginase and glutaminase activities, and reduced biofilm formation. I found that mutating the genes for the asparaginase and glutaminase enzymes had little effect in rich medium, but had more of a role in host like media such as bovine serum, or in defined minimal medium that was NH_3 deprived. Use of quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) showed that in minimal M9 medium containing asparagine or glutamine (without glucose), there was compensatory effect of the asparaginase/glutaminase gene mutations relative to wild type grown in the same conditions. Also, it has been found that *KPN_01165* was up-regulated in *ΔyneH*, and *ΔybiK* backgrounds by 2.53, and 2.37 fold, respectively indicating that in *K. pneumoniae* cross-talk exists between the asparaginase and glutaminase genes.

Ohoud Alhumaidan

The role of peptidoglycan remodelling in *Listeria monocytogenes* persistence
Supervisor(s): Dr Galina Mukamolova and Prof. Peter Andrew

Listeria monocytogenes is a food-borne bacterial pathogen, the causative agent of human listeriosis which affects mainly immune-compromised people and pregnant women. *L. monocytogenes* has many virulence factors that enable its replication in macrophages and the escape from lysosome to cytoplasm. One of these virulence factors is the actin-assembly inducing protein ActA that is essential for *L. monocytogenes* intra- and intercellular motility. Recently, ActA protein has been shown to regulate peptidoglycan (PG) biosynthesis during *L. monocytogenes* replication in macrophages. However the exact mechanism for this phenomenon is unknown. The central hypothesis of the present study is that ActA possesses peptidoglycan hydrolyzing activity and belongs to the family of lytic transglycosylases (LTGs) which are known to control PG biosynthesis and remodelling in other bacteria.

To address this hypothesis three His-tagged forms of ActA have been expressed in *Escherichia coli*. These forms included a full-length ActA protein and two versions of the N-terminal domain ($ActA_{A30\text{-}S157}$, ActAN1) and ($ActA_{S30\text{-}N233}$, ActAN2). Both ActAN1 and ActAN2 have been successfully purified and their identity has been confirmed by mass-spectrometry. Moreover both proteins showed significant peptidoglycan-hydrolysing activity in zymogram and FITC-labelled PG assays. Bioinformatics analysis has predicted Glutamate 98 (E98) to be essential for ActA catalytic activity. Site direct mutagenesis will be employed to generate E98A mutants of ActAN1 and ActAN2 and assessment of their PG-hydrolysing activity. Future experiments such as analysis of muropeptides released from PG by ActA, complementation studies and pull-down assays will shed light on the function of this protein in peptidoglycan remodelling.

Shamim Al-Husseini

Investigating the role of the *Pseudomonas aeruginosa* porin proteins as transferrin and lactoferrin binding proteins
Supervisor(s): Dr Primrose Freestone and Dr Neeta Kulkarni

Cystic fibrosis (CF) is an autosomal recessive disorder affecting mucosal surfaces within the lung and other organs. Due to this defect an imbalance in electrolytes and water secretions causes accumulation of a thick mucus layer in the CF airways. CF patients are exposed to variety of bacterial and fungal infections which can cause high morbidity and mortality. A particularly severe CF pathogen is *Pseudomonas*

aeruginosa. For *P. aeruginosa* to be able to colonise or grow within the CF lungs, accessing its host's iron sources within lung fluids is essential. My research has shown that *P. aeruginosa* is apparently using its outer membrane proteins to capture and steal Fe from the normally secure high affinity ferric iron binding proteins transferrin (Tf) and lactoferrin (Lf), whose function is to make lung secretions Fe limited and so hostile to bacterial growth.

By applying a number of proteomic and microbial methods it was found that one of the principal outer membrane porins of *P. aeruginosa* PA01 and PA14 lab strains and *P. aeruginosa* CF clinical, OprF, is a Tf and Lf binding protein. It was also found that the membrane located motility protein flagellin of *P. aeruginosa* can also anchor Tf. Use of ^{55}Fe-transferrin to examine *P. aeruginosa* ability to uptake Tf-iron showed that *P. aeruginosa* CF isolates were able to acquire more iron in comparison with the lab strains. Interestingly, CF clinical isolates expressingthe highest level of OprF were able to acquire the highest levels of Tf iron, but conversely made less of the siderophore pyoverdine (which can also sequester Tf iron). Investigation of the growth of *P. aeruginosa* lab strains and clinical isolates in iron limited host like serum-medium culture were also carried out, and also showed differences between lab strains and CF isolates.

Giannis Koukkidis

E.coli-Salad Interactions

Supervisor(s): Dr Primrose Freestone & Dr Suzanne Jordan

Introduction: Fresh produce such as salad leaves are an important part of a healthy diet but in recent years have been associated with infection by enteric pathogens such as *E. coli* and *Salmonella enterica*. In July 2016 a UK *E. coli*-rocket outbreak made >150 people ill and killed 2. However, despite calls for improvements in salad leaf hygiene until our recent publication, few studies had investigated pathogenic *E. coli* behaviour when actually within a bagged salad. Salad leaves become damaged during processing and juices are released, so bacteria residing in a salad bag will be bathed in salad leaf juice.

Aims: The intention of my research is to investigate the effect of juices released from damaged salad leaves on the growth, virulence and salad leaf colonisation of *E. coli*. The overall intention is to use this information to develop novel ways of preventing enteric pathogen attachment to fresh salad produce.

***Methods**: E. coli* responsiveness to salad juices was analysed in water, to reflect the salad bag environment, and in more host-like serum-media to model the co-consumption of pathogen and salad leaf. Assays measured the effect of salad leaf juice exposure on *E. coli* growth, motility and biofilm formation (features important in fresh produce colonisation and persistence). Light and scanning electron microscopy were also used to visualise juice effects on *E. coli* colonisation of salad leaves and the plastic salad bag container.

Results: Salad juices at >1/50 dilutions significantly stimulated *E. coli* growth in all media tested. In serum-media, juices enhanced growth by several logs via provision of host iron from serum-transferrin. In water, leaf juices from all salad leaves as well as the fluid within the bag salad significantly increased *E. coli* biofilm formation and its capacity to colonise and persist on salad leaves, and the salad bag container.

Conclusion: This study shows that even very dilute salad juice can contribute to *E. coli* colonisation of salad leaves and re-emphasises the importance of preventing enteric pathogen contamination of fresh produce.

Ozcan Gazioglu

The genetic basis of thermoregulation in *Streptococcus pneumoniae* D39

Supervisor(s): Dr Hasan Yesilkaya and Prof. Peter Andrew

Streptococcus pneumoniae is a commensal Gram-positive bacterium that causes a wide range of local, such as otitis media, and systemic infections, such as pneumonia and meningitis. Virulence gene expression in most pathogens is modulated by several environmental stimuli. Temperature is one of the key environmental stimuli that varies in different parts of body, and during health and disease. Previous studies in other human pathogens showed the importance of thermoregulation in virulence, but mechanism of temperature control in *S. pneumoniae* is still unclear. To determine the genes involved in thermal adaptation, the genes that are differentially expressed at different temperatures were identified by microarray analysis, and selected ones were mutated by site-directed mutagenesis. In addition, the responsiveness of selected differentially expressed genes to different temperatures were evaluated by reporter assays. It was found that the pneumococcus has altered phenotype at 34°C and 40°C for production of glycosidase and haemolytic activity, which are important for colonization and invasive disease. The pneumococcal transcriptome is highly responsive to the changes in temperature as detected by microarray analysis. At 34°C, out of 2085 genes, 491 were differentially regulated, while 189 genes were differentially regulated at 40°C relative to 37°C. The results showed that promoter

activity of tested genes significantly reduced at lower (34°C) and higher temperature (40°C) relative to ambient temperature (37°C). Furthermore, the growth rate and yield of mutants were significantly lower than D39. The cell sizes of D39 and mutants were significantly reduced when temperature is lower or higher than 37°C. Phenotypic assays also suggested that without putative thermoregulatory genes, neuraminidase and haemolytic activities of *S. pneumoniae* were significantly reduced in a temperature-dependent manner.

Abdalla M. Hamed

Characterisation of the putative *Enterobacteriacae* catecholamine hormone receptors

Supervisor(s): Dr Primrose Freestone and Dr Hasan Yesilkaya

Bacteria, due to their prolonged coexistence with animals and plant have evolved systems for sensing their host's signal molecules. Stress hormones related to fight or flight such as norepinephrine, epinephrine and dopamine have been shown to be used as environmental cues which affect the growth and virulence of microbial normal flora as well as pathogenic bacteria. It has been suggested that bacteria such as *E. coli* and *Salmonella* had evolved two component system (TCS) to recognize catecholamine stress signal hormones and they are presumed to be acting as putative adrenergic receptors; these are the QseC and QseE TCS. In this study, investigation of catecholamine responsiveness and overall physiology of these TCS were carried out focused on three aspects; growth, virulence and protein expression. It was found that in terms of growth stimulation that wildtype *E. coli* and *Salmonella* and their *qseC* and *qseE* mutants responded to the catecholamines very similarly. Also, attachment and biofilm formation of the *qse*C and *qse*E the mutants were mostly unchanged from wildtype, as also were their membrane and cytoplasmic protein profiles. So far, the only differences in the phenotypes of *qse*C and *qse*E mutants from wildtype have been in how they grow in culture media in the presence of different carbon and nitrogen sources, which suggests a metabolism rather than catecholamine sensing role for the *E. coli* and *Salmonella* QseC and QseE TCS.

Emad Mohameed

The influence of strain background and variation in protein structure on the roles of pneumolysin in infectious diseases due to *Streptococcus pneumoniae*

Supervisor(s): Prof. Peter Andrew and Dr Hasan Yesilkaya

Streptococcus pneumoniae (pneumococcus) is major worldwide cause of pneumonia, bacteraemia, meningitis and otitis media. The pneumococcus has many virulence

factors, but a key factor is a pneumolysin. This toxin has the ability to form pores in the host cell membranes that cause alteration in cell functioning or cell lysis.

To study the impact of the pneumplysin (Ply) variation a group of the pneumolysin variation was chosen, according to their haemolytic activities. First a recombinant pneumolysin negative mutant was constructed. This will be followed by site-directed mutagenesis to make the Ply variations, which then will be transformed into pneumococcal strain D39. The amino acid sequence of pneumolysin (Ply) is generally conserved but recently some variations in its sequence have been described, some of these associated with changes in activity. We hypothesised that these sequence variants will change pneumococcal virulence but the impact may differ between strains.

Bushra Shlla

Investigating the role of stand alone Rgg transcriptional regulators in *Streptococcus pneumoniae*

Supervisor(s): Dr Hasan Yesilkaya and Prof. Peter Andrew

Rgg is a group of transcriptional regulators found in Gram positive bacteria, and they play diverse roles including in metabolism and virulence in range of bacteria. However, their roles in the important human pathogen *Streptococcus pneumoniae* are not known in detail. To assess the functional importance of Rgg homologs in *S. pneumoniae*, targeted mutation was used for deletion of two *rgg* genes, SPD_0999 and SPD_1518 (rgg0999 and rgg1518). *In vitro* analysis showed that under microaerobic conditions, the mutants were attenuated on mannose and galactose compared to the wild type, whereas their growth profiles were similar to that of wild type in BHI (Brain Heart Infusion) and CDM (Chemically Defined Medium) containing glucose. *In vivo* analysis of Rgg mutants showed that both SPD_0999 and SPD_1518 are required for pneumococcal virulence. All mice infected intranasaly with either Rgg mutant survived significantly longer and had less bacteria in their blood compared to the wild type. Moreover, reporter assays showed that SPD_1518 expression was significantly induced by galactose while SPD_0999 expression was not induced with any of the tested sugars. Therefore, so far the available results show that Rgg encoded by SPD_0999 or SPD_1518 play a major role in pneumococcal virulence, and studies are underway to determine the mechanism of SPD_0999 and SPD_1518 contribution to virulence

Ahmad Alzamami

Oligomerisation of the complement regulator, properdin

Supervisor(s): Prof. Russell Wallis and Prof. Wilhelm Schwaeble

The complement system provides protection against invading pathogens through lysis and opsonisation and by triggering the inflammatory response. One of the three activation pathways called the alternative pathway activates spontaneously on pathogen surfaces and depends on regulators to protect host cells. Properdin is the only positive regulator of the complement system. It stabilises a key enzyme called the alternative pathway C3 convertase extending its half-life by up to 10-fold. Properdin consists of seven thrombospondin type I domains and circulates as dimers, trimers, and tetramers of polypeptides connected to each other via head-to-tail contacts. Recent work indicates that recombinant properdin provides protection against *Neisseria meningitides* and *Streptococcus pneumonia* and that the larger oligomers are more effective in protection.

The aim of this project is to understand how properdin oligomerises. The stategy I have chosen is to produce the full length and the truncated recombinant properdin systems in mammalian and bacterial expression systems. Full-length properdin produced in CHO cells oligomerises in the same way as the wild-type protein. Truncated forms containing the putative oligomerisation domains showed only weak self-association when produced in bacteria, whereas equivalent fragments produced in CHO cells formed larger oligomers suggesting that posttranslational modifications may play a major role in oligomerization.

Rafah Al-Zubaidi

The confounding modulatory effect of β- estrogen in analysing mouse melanoma tumours and cells.

Supervisor(s): Dr Cordula Stover and Dr Lee Machado

Genetic, inflammatory and environmental factors enhance the development of cancers, but the impact of experimental diet on *in vivo* study outcomes is under appreciated. Many different formulations of commercial rodent diets are designed and available, differing in their ingredient to meet the needs of animal's production, growth and maintenance. Soy meal is rich in phytoestrogen and it is one of the major sources of protein in chow diet. *Isoflavones* are phytoestrogens and are found to exert estrogenic or anti- estrogenic effects through the interaction with estrogen receptors. This may lead to interference with measurements of different studies involving estrogenic activity and may affect the interpretation of animal model. The

role of these hormones on melanoma is not clear. However, animal experiments showed a dependence of melanoma growth on hormones.

The present study aimed to understand the effect of β- estradiol *ex vivo* on cultured Murine melanoma cell line (B16F10) after hormone treatment. In order to do so, firstly the effect of estrogen on cell viability will be assessed by growth assays (MTT and Crystal violet assay). Next, RT –PCR to show expression of estrogen receptor in B16F10 mRNA. Finally, qPCR analysis will be used to quantify a possible difference in the expression level of estrogen receptor at different periods after treatment with 180nM β – estradiol.

Zeayd Fadhil Saeed

The effect of properdin gene deletion on bone mineral density in C57BL/6 mice

Supervisor(s): Dr Cordula Stover and Prof. Jonathan Barratt

The complement properdin is a serine protein of complement proteins components, which has the role to increase the amplification lop of the alternative pathway of complement system.

The aim of this study is to investigate whether the properdin absent has an effect on bone mineral density.

Males and females of C57BL/6 (6months old), the lumbar vertebra were harvested and subjected to Micro-Computed tomography and analysed using Analysed 12.0 software to measure bone microarchitecture parameters.

The result showed that there is no significant difference in properdin knockout mice comparing to WT mice, regarding to Bone Mineral Density.

Luay Al-Kanan

Characterisation of collectins CL-Kl and CL-L1 and their role in the innate immune system

Supervisor(s): Prof. Russell Wallis and Dr Shaun Heaphy

The complement system is an important part of the innate immune response and serves as a bridge between innate and adaptive immunity. It plays an essential role in protection of the body against microbes by direct lysis of pathogens or by opsonization and recruitment of leukocytes. Complement is activated by three pathways: Classical (CP), Lectin (LP) and Alternative pathways (AP). The LP is

activated by mannan-binding lectin (MBL), ficolins, and collectin kidney1 (CL-K1 aka CL-11), and collectin-liver1 (CL-L1 aka CL-10) in association with three proteases: MASPs-1, -2 and -3.

CL-L1 and CL-K1 are recently described members of the lectin pathway, however relatively little is known about their role in host defence or their sugar specificities. It has been reported that they form heterooligomers in serum, called CL-KL, although CL-K1 and CL-L1 have different tissue distributions, so some free CL-K1 and CL-L1 may also be produced. The aim of my project is to compare the roles of CL-K1, CL-L1 and CL-KL in complement activation, and characterise each protein with respect to its ligand specificity, oligomerisation and complement activation, by using recombinant human CL-L1, CL-K1 and CL-KL.

To achieve this aim, I have cloned the full-length cDNAs of CL-L1 and CL-K1 in mammalian cells Although CL-K1 is secreted, no CL-L1 was detected in the media of producing cells. I am currently attempting to co-express CL-K1 and CL-L1 to see in CL-LK is produced. To examine the structure and sugar specificity of CL-L1, I have expressed the carbohydrate recognition domain (CRD) in *E. coli*. Protein can be refolded from inclusion bodies. Future work will focus on characterising the different forms of collectin with respect to their sugar specificity, structure and ability to activate complement.

Jamal Almitairi

Structural characterization of complement component C1s interacting with its associated classical pathway recognition molecule.

Supervisor(s): Prof. Russell Wallis and Dr Shaun Heaphy

The C1 complex initiates the classical pathway of complement via binding of its recognition molecule, C1q, to immobilised antibodies and pathogen-associated molecular patterns. C1q is bouquet-like structure with six trimeric subunits each composed of three polypeptides, C1qA, C1qB and C1qC. It associates with two serine proteases C1r and C1s, as a heterotetramer, $C1r_2s_2$. C1r and C1s comprise N-terminal CUB1-EGF-CUB2 domains linked to CCP1-CCP2-serine protease domains. The proteases bind to Hyp-Gly-Lys-Xaa-Gly-Pro motifs located on the collagen-like stalks of C1q via their CUB domains. Overall there are six binding sites for C1q, two on each C1r polypeptide and one on each C1s. In order to understand how C1q binds to C1r and C1s, I have crystallised the CUB1-EGF-CUB2 region of C1s with different synthetic peptides derived from the collagen-like domain of C1q. Complexes have been analysed with individual peptides as well as mixtures of different peptides. Using this

strategy I have identified the interactions that stabilise the complex and have established the likely order of the three chains of C1q. These are the first complexes of a protein bound to collagen heterotrimers.

Eva-Maria Rick

The lung fungal microbiome in patients with asthma – overlooked allergens?

Supervisor(s): Dr Catherine Pashley and Prof. Andrew Wardlaw

Sensitisation to *Aspergillus fumigatus,* and isolation of filamentous fungi from sputa of asthmatics by culture, have been associated with reduced lung function. However, the inherent insensitivity of culture means fungi having a detrimental effect on health could be missed.

Aims: To determine the lung mycobiome in patients with asthma and healthy controls using amplicon-based high-throughput sequencing (HTS), and to characterise the allergens from any fungi of interest identified.

Methods: Patients with asthma were grouped into IgE-sensitised to fungi or non-fungal sensitised. A third group comprised healthy controls. HTS data from the internal transcribed spacer region 2 were obtained using the Illumina MiSeq platform and QIIME for bioinformatics analysis.

Results: The preliminary data from sputum (82 patients) and bronchoscopy-derived (18 patients) samples showed that the five fungi with the highest sequence abundances and qPCR-adjusted HTS data were the same, independent of fungal sensitisation or sample type.
Aspergillus section *Nigri* was more abundant by HTS in sputum samples from asthmatics compared to healthy controls (P=0.0323). *Aspergillus* section *Nigri* samples from culture were represented by *A. niger* and *A. tubingensis*. Proteins and RNA were extracted from *A. tubingensis*, *A. niger* and *A. fumigatus* (control) species for allergen characterisation. Immunoblots of the protein extracts incubated with human sera have been investigated using both fluorescent and chemiluminescent detection systems.

Conclusions: HTS is a sensitive method to assess the lung mycobiome. The main fungal species detected were comparable between different patient groups and between different sample types. *Aspergillus* section *Nigri* could comprise an underestimated allergenic source.

Day 3, 29th March

Sally Majd

A feasibility study of the randomised control trial of asthma tailored-PR (AT-PR) versus usual care (UC) in individuals with severe asthma

Supervisor(s): Dr Rachel Evans and Prof. Peter Bradding

Introduction: Adults with severe asthma suffer from exertional breathlessness. Exercise training can improve breathlessness in other chronic lung diseases. However, there is limited evidence regarding the safety and efficacy of pulmonary rehabilitation (PR) (where exercise is a key component) for patients with severe asthma.

Aim: The primary aim was to understand the feasibility of performing a randomised controlled trial of AT-PR vs UC.

Method: Patients with severe asthma (MRC dyspnoea ≥ 2) were recruited and randomised 2:1 to AT-PR and UC. The primary outcomes were recruitment and retention rates. All patients performed an incremental shuttle walk test (ISWT), maximal incremental treadmill (ITM) and cycle ergometer tests (ICE), completed the asthma control questionnaire (ACQ) and chronic respiratory questionnaire (CRQ), before and after 12 weeks of AT-PR or UC.

Results: 61 participants (38 female, mean [SD], age 54 [13]yrs, BMI 32 [7]kg/m^2, FEV_1 1.95 [0.74]l, FEV_1/FVC 69 [11]%) out of 238 eligible patients were recruited (26% recruitment rate) and 51 were randomised to AT-PR/UC (34/17). Retention rate for PR and UC were 62% and 53% respectively. AT-PR group improved in their ISWT distance compared to UC and improvement was seen inVO_{2pk} on ICE post AT-PR (Table 1). ACQ score and the dyspnoea, fatigue and mastery domains of CRQ improved in AT-PR compared to UC (Table 1).

Conclusion: This study provides data to inform the design of a larger randomised controlled trial as AT-PR maybe effective for patients with severe asthma.

Table 1: Comparison of exercise performance, asthma control and health related quality of life between AT-PR and UC.

	PR Group			UC Group			
	Baseline (n=21)	Post (n=21)	Mean Diff (95% CI)	Baseline (n=9)	Post (n=9)	Mean Diff (95% CI)	Diff in Change BTW Groups
VO_{2pk} (ml/min/kg) ITM	22.5 (6.2)	23.3 (7.6)	0.8 (-0.5 to 2.1)	22.9 (3.9)	22.3 (2.4)	-0.6 (-2.8 to 1.7)	1.3
VO_{2pk} (ml/min/kg) ICE	17.6 (6.0)	18.8 (6.9)	1.2* (0.1 to 2.3)	16.9 (4.3)	16.1 (2.8)	-0.8 (-3.4 to 1.7)	2.0
ISWT Distance (m)	417 (172)	450 (199)	33* (3 to 63)	443 (121)	403 (104)	-40* (-72 to 8)	73†
ACQ	2.07 (0.80)	1.92 (0.77)	-0.16 (-0.44 to 0.13)	1.51 (0.86)	1.92 (0.99)	0.41 (-0.10 to 0.93)	0.57†
CRQ Dyspnoea	3.06 (1.20)	4.10 (1.31)	1.04* (0.5 to 1.57)	3.72 (1.47)	3.60 (1.14)	-0.12 (-0.89 to 0.65)	1.15†
CRQ Fatigue	3.74 (1.09)	4.70 (1.17)	0.96* (0.35 to 1.58)	4.72 (1.74)	4.36 (1.56)	-0.36 (-1.08 to 0.37)	1.32†
CRQ Emotional Function	5.10 (1.36)	5.39 (1.07)	0.28 (-0.13 to 0.69)	6.14 (0.70)	6.06 (0.75)	-0.08 (-0.29 to 0.14)	0.36
CRQ Mastery	4.73 (1.29)	5.40 (1.11)	0.68* (0.25 to 1.10)	6.11 (0.98)	6.03 (0.77)	-0.08 (-0.68 to 0.51)	0.76†

Data presented in mean (SD)
**represents significant within group differences (p<0.05)*
†represents significant between group differences (p<0.05)
PR, pulmonary rehabilitation; UC, usual care; VO_{2pk}, peak oxygen uptake; ITM, maximal incremental treadmill test; ICE, maximal incremental cycle ergometer test; ISW, incremental shuttle walk test; ACQ, asthma control questionnaire; CRQ, chronic respiratory questionnaire

Alex Bell

Parametric Response Map Registered CT Feature and Small Airway Physiology Analysis in Asthma

Supervisor(s): Prof. Salman Siddiqui and Prof. Alexander Gorban

Introduction / Background: Asthma is a disease characterized by spatiotemporal ventilation heterogeneity (VH). We hypothesized that imaging biomarkers of VH, extracted from parametric response map (PRM) registered inspiratory and expiratory CT scans in asthma, would be associated with asthma severity and small airway physiology.

Aims / Objectives: We aimed to evaluate PRM based CT features including a novel spatially regionalized approach, stratified axial analysis (SAA), in a cohort of asthma patients and healthy volunteers. We hypothesized that SAA biomarkers would associate with VH biomarkers Sacin and R5-R20.

Methods: 41 patients with asthma and 11 healthy age-matched volunteers underwent inspiratory (expiratory) volumetric CT scanning at TLC (FRC). CT

biomarkers, notably SAA based inferior-superior ventilation slope (SAAz), were calculated. Linear discriminant analysis (LDA) was utilized to understand how spirometry, clinical and CT feature sets could relate to asthma severity, via VH marker discrimination.

Results: SAAz was found to provide the best single feature discriminator for both Sacin and R5-R20. LDA demonstrated that CT based feature sets can contribute significantly to VH discrimination. Polar analysis of SAAz revealed statistically significant ($p < 0.05$) ventilation gradient reversal [Figure 1].

Conclusions: We developed a novel stratified axial-based CT imaging biomarker of inferior-superior ventilation gradient in asthma, which associates with small airway markers of VH.

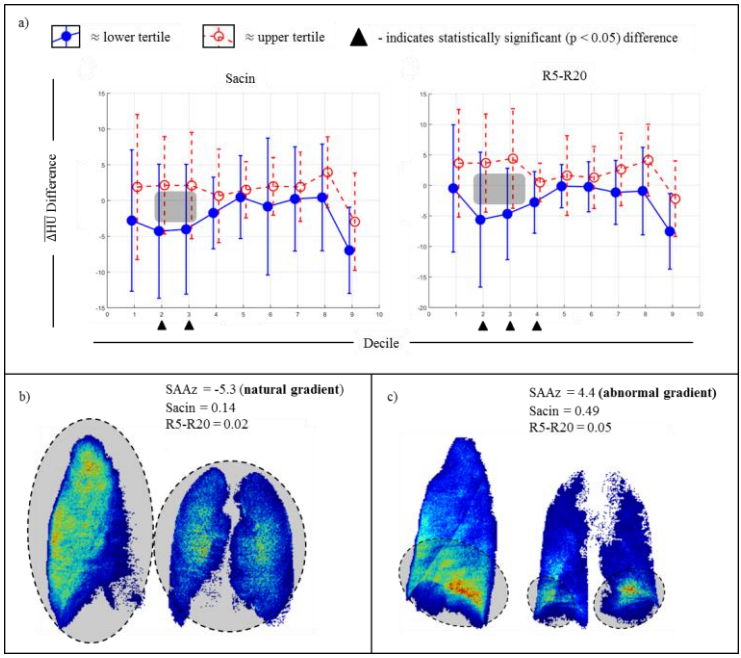


Fig. 1 – Stratified axial analysis (SAA) with respect to inferior-superior axis (SAAz) highlights importance of ventilation gradient reversal in VH markers Sacin and R5-R20, localized to the basal regions of the lungs. (a) Polar analysis demonstrates statistically significant ($p < 0.05$, Wilcoxon rank-sum test) contributions to SAAz occur at inferior deciles of the lungs. (b, c) PRMfsad lateral (left) and PRMemph coronal (right) min-max projections illustrate respective voxel spatial distributions. (b) Subject with biologically natural SAAz, shows homogeneous

disease distribution. (c) Subject with abnormal SAAz, shows heterogeneous disease distribution, preferentially distributed to basal regions.

Nidhal Gharbawi

Lung Function in Children of Different Origins

Supervisor(s): Dr Caroline Beardsmore, Dr Erol Gaillard & Dr Maria Viskaduraki

Background: Previous studies have compared south Asian (SA) children with their white counterparts and shown a reduction in lung function (forced vital capacity, FVC) of 9-13%. Ethnic differences in respiratory muscle strength could potentially explain this difference.

Aim: To investigate differences in respiratory muscle strength between white and SA children.

Methods: We measured height, weight, and spirometry. FVC was expressed as %predicted, based on predicted values for white children (Rosenthal et al Thorax 1993; 48:794). Respiratory muscle strength was assessed by measuring maximum inspiratory and expiratory pressures (MIP and MEP, respectively). For both measurements, the child breathed through a pneumotachograph attached to a shutter. To measure MIP, after several quiet breaths, the child exhaled maximally and the shutter was activated. The child made an inspiratory effort against the shutter and peak pressure was recorded. The test was repeated several times. Measurements of MEP were similar, except that the child inhaled maximally and then made a forceful expiratory effort.

Results: We studied 251 healthy children aged 5-11y. We obtained valid MIP measurements on 194 (44 white, 150 SA), and valid MEP on 219 (53white, 166 SA). There was no significant difference between unadjusted MIP and MEP in white and SA children. This finding was unchanged after adjustment for age, height and weight (Table).

	White	S Asian	P
Mean MIP (adjusted)(kPa)	7.39	7.00	0.242
Mean MEP (adjusted)(kPa)	6.39	6.47	0.759
Mean FVC(% pred)	104	92	<0.000

Conclusion: We did not find significant differences in respiratory muscle strength between two ethnic groups. The differences in FVC remain unexplained.

Rehab Bagadood

Effects of obesity on corticosteroid sensitivity in health and allergic inflammation
Supervisor(s): Dr Yassine Amrani and Dr Cordula Stover

Background and purpose: Asthma is a lung disease that is characterized by chronic inflammation and reversible airflow obstruction. Obesity is now recognized as an important risk factor for asthma. Patients who are overweight and obese are more likely to suffer from severe asthma, poor asthma control and poor quality of life. Obesity is also associated with a poor response to therapy including corticosteroids, although the underlying mechanisms are still unknown. *We investigated whether obesity reduces the anti-inflammatory response of corticosteroids*.

Methods: Splenocytes were isolated from normal and high fat diet fed mice (n=7 male and female C57BL/6 mice fed for 12 weeks), treated with TCR activators in the presence of different concentrations of dexamethasone (10^{-10} to 10^{-6}M) and their *in vitro* proliferation and cytokine responses measured using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and ELISA assays respectively.

Results: The high-fat-fed mice exhibited increased body weight compared with the lean mice in both males and females (50-55%; $P<0.05$). Treating splenocytes with anti-CD3/CD28 antibodies for 3 days stimulated a dose-dependent increase in cell proliferation (14-fold increasing; $P<0.01$) and production of TNFα and IL-2 (480 ± 41.7pg/ml and 1324 ± 64.9pg/ml; $P<0.01$) when compared to unstimulated cells. Dexamethasone showed a similar inhibitory pattern (IC_{50}) in the proliferation and cytokine response in splenocytes from both lean and obese mice.

Conclusions: The study shows that obesity does not affect the ability of dexamethasone to suppress TCR-induced responses in splenocytes isolated from both male and female mice. Whether this also applies to a mouse model of allergic inflammation remains to be determined.

Felicity Easton

TRP Channel Regulation of smooth muscle hyperresponsiveness in Asthma
Supervisor(s): Prof. Peter Bradding, Dr Yassine Amrani and Prof. Andrew Wardlaw

Inhibiting bronchoconstriction is a key goal in asthma therapy. Exaggerated and sustained bronchoconstriction resulting from airway hyperresponsiveness is initiated by a broad range of stimuli (direct and indirect), but sustained by the recruitment of extracellular Ca^{2+} through plasmalemmal Ca^{2+} ion channels. Ca^{2+}carrying ion channels

therefore represent a potential therapeutic target to attenuate bronchoconstriction and provide an effective treatment strategy for asthma.

A variety of Ca^{2+}-carrying channels have been proposed as potential Ca^{2+} influx pathways in human airway smooth muscle (ASM), but inhibitors of these channels do not appear to moderate $[Ca^{2+}]_i$ or attenuate ASM contraction in patients with asthma.

Transient Receptor Potential (TRP) channels are permeable to Ca^{2+} and display polymodal activation; sensitized to chemical, nociceptive, thermal and mechanical stimuli. However, the role of TRP channels in human ASM cells is poorly defined and the Ca^{2+} influx pathways remain elusive.

We aim to investigate Ca^{2+} carrying TRP channel expression and function in ASM cells from asthmatic patients compared to healthy subjects. To-date we have confirmed the presence and relative expression of mRNA for TRPA1, TRPV1, -V2, V4, TRPM7 and all TRPC channels using QRT-PCR. Of the identified TRP channels, definition of their functional role in agonist-dependent ASM Ca^{2+} responses and ASM contraction will be elucidated by patch clamp electrophysiology, the FLIPR Ca^{2+} assay and gel contraction assays using both selective antagonists and gene silencing with shRNAs.

Adam Smith

Cultured Mouse Tracheal Airway Smooth Muscle Cells Express Functional P2 Receptors

Supervisor(s): Dr Catherine Vial, Prof. Andrew Wardlaw, Prof. Peter Bradding

Introduction: Asthma is an inflammatory disease that results in airway remodelling and obstruction, and airway smooth muscle (ASM) hyperresponsiveness. In asthmatic airways, there is a rise in extracellular nucleotide concentration. Extracellular nucleotides activate P2 receptors, known to regulate contraction and proliferation of many smooth muscle types. P2 receptors are divided into P2X and P2Y receptors, ligand-gated ion channels and metabotropic GPCRs respectively. We hypothesise that functional P2 receptors are expressed by ASM cells and contribute to airways function and the pathogenesis of asthma.

Aims: To identify and characterise the functional P2 receptor subtypes expressed in ASM.

Methods: A widefield epifluorescence microscopy approach for calcium imaging using fura-2 AM (2□M) was used in conjunction with pharmacological tools to identify functional P2 receptors expressed in cultured mouse tracheal-ASM (T-ASM) cells (passage 1).

Results and discussion: In cultured mouse T-ASM cells, ATP, UTP, ADP and UDP (100μM) induce fast and transient $[Ca^{2+}]_i$ rises, while ATP (1mM) induced a 'biphasic' response consisting of a sharp peak and a sustained $[Ca^{2+}]_i$ rise. The use of selective pharmacological tools (based on our qPCR data) indicates the presence of the functional P2 receptor subtypes P2Y1, P2Y2 and P2X7.

Conclusion: These data show that cultured mouse T-ASM cells express functional P2X7, P2Y1 and P2Y2 receptors. The physiological role of these receptor subtypes (e.g. contraction and proliferation) in ASM cells is currently under investigation. The reason other P2 receptors detected by qPCR are not functional remains to be investigated (e.g. expression loss, internalisation).

Jamie McCarthy

A role for human type 2 innate lymphoid cells in asthma exacerbation?
Supervisor(s): Prof. David Cousins and Prof. Christopher Brightling

Rhinoviruses are the most common cause of viral exacerbations of asthma. Infection of human bronchial epithelial cells (HBECs) by rhinovirus induces production of the T-helper 2 (Th2) cytokines Interleukin (IL)-25 and IL-33. Group 2 innate lymphoid cells (ILC2s) produce IL-13 and IL-5 in response to IL-25 or IL-33 in combination with common gamma chain cytokines (e.g. IL-2, IL-7). ILC2s and HBECs express CRTh2, the receptor for Prostaglandin D_2 (PGD_2) as such it may play an important role in asthma exacerbations.

We aim to investigate the relationship between rhinovirus infection, HBECs and ILC2s to identify the molecular and cellular interactions that occur. In particular the nature and source of common gamma chain cytokine and the role of PGD_2/CRTh2.

ILC2s were identified in human peripheral blood as Lineage-, CD123-, CRTh2+ cells. The cells were further phenotyped as $CD45^{Hi}$, CD127+, CD161+, CD25+, c-kit^{int}, $KLRG1^{int}$ and $CD126^{int}$. IL-15 is a common gamma chain cytokine that is increased in humans during rhinovirus infection. Multicolour flow cytometry was used to examine the effect of IL-15 on human peripheral blood ILC2s. Based upon phosphorylation of STAT5, ILC2s were unresponsive to IL-15 treatment directly *ex vivo*; including co-

stimulation with IL-15Rα. Similarly, ILC2s did not express IL15Rα (CD215) *ex vivo*. However, stimulation with IL-33 in a purified cell culture of ILC2s leads to an increase of IL15Rα mRNA. Further experiments will investigate combinations of cytokine stimulations to examine IL-15 responsiveness of human ILC2s.

Mohammed Alshammari

The role of IL-33, ST2 and Type 2 innate lymphoid cells in asthma

Supervisor(s): Prof. David Cousins and Dr Yassine Amrani

Background: Type 2 innate lymphoid cells (ILC2s) are a recently identified lymphoid cell population that express the type 2 cytokines, in particular interleukin (IL)-5 and IL-13 in response to IL-25, IL-33 and TSLP. SNPs in both IL-33 and the IL-33 receptor (ST2/IL1RL1) have been implicated in asthma. However, the biology of IL-33 and its interaction with ST2 is complex and poorly understood. We hypothesise that activation of ILC2s by IL-33 via ST2 is a critical driver of the type 2 immune response in asthma. The initial aims of the study are to express ST2 in HEK293 cells using plasmid with GFP, validate antibodies against human ST2 for use in flow cytometry and test the validated antibodies on ILC2 cells.

Methods: HEK293 cells were transfected with ST2 in pCDH-CMV-MCS-EF1-copGFP. ST2 expression was assessed using antibodies to allow detection by flow cytometry. PBMCs were freshly isolated from donors and were stained for multiple cell surface markers. ILC2s were defined as Lin- CD123- CRTh2+.

Results: The monoclonal and the polyclonal anti-human ST2 were both able to detect membrane bound ST2 on transfected HEK293 cells. Remarkably, we were able to detect ST2 expression on a peripheral blood cell population that has not been defined previously.

Discussion: We have validated antibodies to human ST2 to assess its expression on freshly isolated PBMCs in particular ILC2s. Also, we detected ST2 expression on unknown population defined as Lin+ CD123+ FcεRI- CD3- CRTh2- ST2+. The identity of these cells requires further confirmation and investigation.

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Airway smooth muscle and mast cell interaction modulates corticosteroids sensitivity

Supervisor(s): Dr Yassine Amrani and Prof. Peter Bradding

Background: Corticosteroids are the main anti-inflammatory therapy that is used to treat asthmatic patients. However, a proportion of patients affected by the severe form of asthma do not properly respond to corticosteroids and the underlying mechanisms are unknown. Airway smooth muscle cells (ASMCs) are capable of regulating immune response in asthma by the secretion of various pro-inflammatory mediators. Interestingly, recent studies showed that the pro-inflammatory function of ASMCs was insensitive to corticosteroids in severe asthma. As infiltration of mast cells in ASM bundle is feature of asthma and mast cells and ASM shows a bidirectional functional interaction, we hypothesised that mast cells may regulate the ASM corticosteroids insensitivity in severe asthma.

Methods: Healthy ASMCs were pre-treated with supernatants of non-activated or activated human lung mast cell (HLMC). ASMCs were then washed and treated with or without fluticasone before being stimulated with tumor necrosis factor alpha (TNFα). ELISA and gene expression were used to assess the effect of fluticasone on TNF-induced production of the different chemokines CCL5, CXCL10 and CXCL8.

Results: Supernatants from activated mast cells significantly reduce fluticasone-dependent repression of CXCL10 and CCL5 induced by TNFα by 27.52 and 21.12 % respectively, although no effect was seen on the net production of chemokines.

Conclusion: These studies show that the reduced corticosteroid sensitivity seen in asthma may result from a suppressive action of mast cells on the anti-inflammatory effects of corticosteroids on ASMCs.

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