Preface

The Department of Infection, Immunity and Inflammation would like to welcome you to the Eighth 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, Immunology, Renal Medicine 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 Professor Peter Andrew for opening the conference, and Professor Andrea Cooper and Dr Primrose Freestone for kindly giving the keynote lectures. We would also like to show our appreciation to Fisher for agreeing to sponsor the event and providing a prize.

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:

Ozcan Gazioglu, Ananthi Ramachandran and Sian Baldock

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Keynote Speakers:

Professor Andrea Cooper –Monday 18th April 2014: 09.40 - 10.25

Dr Primrose Freestone – Tuesday 19th April: 09.30 – 10.15

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Department of Infection, Immunity and Inflammation
8th Annual Postgraduate Student Conference

Tuesday 19th April 2016 , MSB LT2

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Department of Infection, Immunity and Inflammation
8th Annual Postgraduate Student Conference

Wednesday 20th April 2016 , MSB LT2

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Abstracts

Day 1, 18th April

Mohenned Alsaadawi

Development of immunotherapeutics to enhance complement-mediated killing of *P. falciparum*

Supervisor(s): Dr Nicholas Lynch and Prof. Andrew Tobin

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. However, factor H and CR1 could be activated during *P. falciparum* infections and thus accelerating the decay of the C3 convertase while CFHR1 or a polypeptide omitting the N-terminus of CFHR1 can inhibit factor H. My project aims to produce chimeric properdin and CFHR1, which can be used to augment the alternative pathway activity on *P. falciparum* surfaces. Three different forms of humanised mouse properdin were produced in mammalian cell culture and its activity in normal, factor P depleted human serum and wild type mouse serum was tested on zymosan.

Sura Alkhuzaie

Can antibody mediated targeting of *Plasmodium falciparum* surface antigens thrombospondin related anonymous protein (TRAP) and the circumsporozoite protein (CS) enhance killing of *Plasmodium falciparum*?

Supervisor(s): Dr Nicholas Lynch and Prof. Andrew Tobin

Malaria remains the world's most devastating tropical infectious diseases, with as many as 40% of the world population living in perilous areas. It is caused by a parasite called *Plasmodium*, which is transmitted by the bites of infected mosquitoes. In the human body, the parasites multiply in the liver, and then infect the red blood cells. Sporozoites (the stage that infects liver cells) expresses

circumsporozoite (CS) protein and the thrombospondin related anonymous protein (TRAP), which are implicated in recognition of and entry into hepatocyte. These proteins contain highly conserved thrombospondin domain motives (TSP domains), structural motifs also found in thrombospondin-1 and properdin. Previous data show that thrombospondin acts as an effective competitive inhibitor of properdin-dependent complement activation, binding firmly to the same target complex as properdin. Our hypothesis is that the TSP domain containing *Plasmodium* proteins have a similar activity, inhibiting host complement activation and thus protecting the parasite from complement attack. Recombinant TRAP and CS proteins, and fragments thereof, are being produced in prokaryotic and mammalian expression systems and tested *in vitro* for complement inhibitory activity, prior to testing whether they inhibit complement activation on *Plasmodium* itself.

Ramiar Kheder

The role of Vitamin D3 in high fat diet induced fatty liver disease and obesity.

Supervisor(s): Dr Cordula Stover, Dr Michael Browning and Dr James Hobkirk

Abstract: The accumulation of fat in hepatocytes is characteristic of non-alcoholic fatty liver disease (NAFLD). Accumulation of lipids may lead to inflammation; this is called non-alcoholic steatohepatitis (NASH). This study investigates the role of vitamin D3 in diet induced obesity and liver disease on a $LDLR^{-/-}$ / $LDLR^{+/+}$ background using formulated diets.

Material method: LDLR-/-, LDLR+/+ mice. High fat high sucrose diet formulation led to the development of steatosis at 10 weeks in the experimental animals with variation in severity and variable inflammation. Body weight, fat pad weight, liver histology were analysed. Hepatic expression of candidate genes (TNF-α, srebp-1c, TLR4, HMGCR, SR-B1) was performed by qPCR. ELISA was used to quantify serum insulin, Adiponectin, MDA, and IL-6. Liver function and endotoxin levels were also measured.

Result: High fat high sucrose diet led to an increase of body weight, histological features of fatty liver and liver inflammation, insulin resistance, MDA elevation, altered liver function and elevated IL-6. High fat high sucrose diet with supplemented Vitamin D led to findings comparable with the control group that was analysed in parallel (low fat maintenance diet).

Conclusion: High fat high sucrose diet led to the development NAFLD and insulin resistance, and this was ameliorated when a tenfold excess of Vitamin D was present in the high fat high sucrose diet.

Wafaa Khalaf

In vitro generation of antigen specific cytotoxic T cells with potential for adoptive tumour immunotherapy of Multiple Myeloma

Supervisor(s): Dr Michael Browning and Dr Cordula Stover

Multiple myeloma (MM) is a life-threatening malignancy, which is incurable by conventional therapies.

Immunotherapy is a promising approach for treatment of relapsing MM, using antigen specific cytotoxic T-lymphocytes (ASCTL). These ASCTLs can be produced by stimulation of peripheral blood lymphocytes (PBMCs) using hybrid cell lines (made by fusion of professional APC (HMy2; EBV B-lymphoblastoid cell line) and multiple myeloma cells.

Tumour antigen expression profile of three hybrid cell lines generated was determined using real time PCR and flow cytometry. Tumour associated antigens were selected for analysis as inducers of CTL depending on their expression level by the hybrid cell lines and their prevalence in MM patients. The hybrid cell lines were used to induce ASCTL in long term activated T-cell cultures, using PBMCs from healthy HLA-$A2^{+}$ donors, and assessed using HLA-A2-peptide pentamers in flow cytometric analysis, and by interferon-□ and perforin ELIspot assays.

Significantly higher numbers of ASCTLs were produced after multiple rounds of co-culture of the hybrid cell lines with allogeneic PBMCs (in the presence of rhIL-2, rhIL-15 and rhIL-7) compared with their parent tumour cells.

In conclusion, hybrid cell lines generated by fusion of an EBV B-lymphoblastoid cell line and myeloma tumour cells can induce ASCTL in culture from PBMCs from healthy individuals *in vitro*, and may represent a novel strategy for use in immunotherapy of MM and maybe for other haematological malignancies. Future experiments will focus on the ability of these hybrid cell lines to induce ASCTLs from PBMCs from patients with MM.

Bayan Faraj

Observing structural changes in the lipid bilayer of single cell-sized vesicles by pneumolysin using the back-scattered light from optical tweezers

Supervisor(s): Prof. Russell Wallis and Prof. Peter Andrew

Streptococcus pneumonaie is a Gram-positive bacterium, which is commonly known as pneumococcus. Pneumolysin is a key virulence factor, which is produced by all clinical isolates of pneumococcus; the 53 kDa protein contains 471 amino acids

within four structural domains. It is a well-researched pore-forming toxin and a member of the cholesterol-dependent cytolysins (CDCs), so called because they are thought to bind to cholesterol in cell membranes. The main purpose of this part of my project is to explore the structural change induced in the membrane by wild type, truncated and various mutants of pneumolysin. For the latter examples, eight mutations were introduced into the pneumolysin gene with site directed mutagenesis, cloned into pLIECS-93 and purified as the wild type protein. Unilamellar lipid vesicles (liposomes) were prepared as membrane model, comprising cholesterol and the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. Through the combination of two techniques, Raman tweezing and microfluidics, the effect of each protein on the structural change of the lipid membrane was tested. In these experiments, it was possible to isolate a single, cell-sized, liposome from a suspension by optical tweezing. The microfluidic devices enabled the biochemical environment surrounding an optically trapped liposome to be exchanged instantaneously, so the structural change in the lipid bilayer could be monitored as a function of the time following the controlled exposure of the trapped liposome to the protein toxin. We found that the truncated protein (consisting of domain 4 only) did not result in any structural change to the lipid membrane, which was used as the negative control, but wild type pneumolysin and some of the mutants prepared resulted in substantial changes to the structure of the lipid membrane.

Niran Patel

The prevalence of canine oral protozoa and their association with periodontal disease.

Supervisor(s): Prof. Peter Andrew

Periodontal disease is currently one of the most important health concerns for companion animals. If left untreated, it leads to painful periodontal ligament destruction, alveolar bone loss, and eventual loss of teeth. The recent focus of research in canine forms of periodontitis has been the identification and characterisation of the bacterial communities present. However, other microorganisms are known to inhabit the oral cavity and could also influence the disease process.

The objective of this study was to screen for the presence of protozoa within canine plaque samples and to examine their distribution in relation to periodontitis. We employed a novel, broad spectrum 18S PCR designed to target the identification of protists, in conjunction with next generation sequencing analyses. Organisms from the genera Trichomonas and Entamoeba were identified in pooled and categorised

canine plaque samples. Through PCR the overall prevalence of Trichomonads and Entamoebae detected was 56.52 % (52/92) and 4.34 % (4/92) respectively. Using next generation sequence analysis the proportion of Trichomonad sequences found in each of a series of pooled healthy, gingivitis, early stage periodontitis and severe periodontitis samples was 3.51 %, 2.84 %, 6.07 % and 35.04 % respectively. The proportion of Entamoebae found in each of the same pooled samples was 0.01 % (healthy), 0.01 % (gingivitis), 0.80 % (early stage periodontitis), and 7.91 % (severe periodontitis). Statistical analysis concluded both genera of protozoa were associated to animals with periodontal disease. These findings provide the first evidence for the ubiquitous presence of oral protozoa in dog plaque.

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 a major cause of pneumonia worldwide, 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. 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 have impact on pneumolysin function and the impact may differ between strains. There are 18 naturally occurring Ply variants. However the functional significance of these polymorphisms is not known.

In order to study the impact of the pneumolysin (Ply) variations the D39 wild type was used as a background strain. First a recombinant pneumolysin negative mutant was constructed to use as a control. This was followed by site-directed mutagenesis to make the Ply variants.

The method for creation of unmarked point mutation used pORI280 plasmid. The recombinant plasmid contained mutant allele. The plasmid integration with the genome of D39 was achieved through a single crossover strategy. The successful construction of unmarked mutant was verified genetically and phenotypically.

Taiwo Banjo

***Acanthamoeba* Mannose-Binding Protein: structural and functional characterisation of a therapeutic target for *Acanthamoeba* keratitis**

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

Acanthamoeba mannose-binding protein (AcMBP) is a 400 kDa trans-membrane protein and pathogenic factor of the free-living amoeba, **Acanthamoeba castellanii**. *Acanthamoeba* infection causes keratitis, a painful corneal infection that often leads to blindness. AcMBP initiates the adhesion of *Acanthamoeba* onto traumatized corneal surfaces. Therapeutic unresponsiveness of **Acanthamoeba** is attributed to its eukaryotic physiology, making it difficult to target selectively; and its rapid transition into drug-resistant cysts. This study involves the molecular characterisation of AcMBP as a potential target for future anti-*Acanthamoeba* therapeutics.

Previously, I have carried out morphological studies on the different life stages of *Acanthamoeba*; its adhesion towards various surfaces and its cytopathic activity towards cell types. Here, I will describe recent studies to identify the functional domains of AcMBP. Blast searches of the 733 amino acid extracellular region reveal no sequence identity with known domains, with the exception of a small DUF4114 domain (~10 kDa). This region shares low identity with a mannose-binding lectin from *Burkholderia cenocepacia.* Importantly, key carbohydrate-binding residues are conserved. To characterise this domain, the cDNA was expressed in *E.coli* and the protein was refolded from inclusion bodies. Yields are sufficient for structural and functional analysis. Constructs encoding the entire extracellular region together with two truncated forms were also cloned for expression in mammalian cells. Future work will identify the lectin domains and characterise the mechanism of ligand recognition.

Rashed Alghamdi

Studies on the functional significance of Asparaginase and Glutaminase of *Klebsiella Pneumoniae*

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

Klebsiella pneumoniae is a causative agent of several nosocomial and community acquired infections. However, what controls *K. pneumoniae* virulence is incompletely understood. It has been suggested that efficient acquisition and metabolism of host nutrients in the sites of infection are important for the microbe's ability to cause infections. For example, asparaginase and glutaminase

are sets of enzymes that assist *K. pneumoniae* in acquiring necessary nitrogen sources when ammonia (NH_3), the preferable nitrogen source, is low < 1 mM or absent. Asparaginase and glutaminase are involved in the hydrolysis of L-asparagine and L-glutamine to L-aspartate and L-glutamate, respectively, which releases NH_3, the preferred nitrogen source for the microbe. *K. pneumoniae* is also able to use L-asparagine and L-glutamine as the sole nitrogen and carbon sources *in vitro*. It has been found that *K. pneumoniae* contains four putative asparaginase and glutaminase genes (*yneH, ybiK, ansA* and *KPN_01165)*. The aim of this study was to investigate the contribution of these enzymes in *K. pneumoniae* KR3167 survival and virulence by creating isogenic mutant strains in these genes. Growth studies showed that wild type (WT) *K. pneumoniae* has efficient mechanisms to grow in M9 medium supplemented with glucose and either L-asparagine or L-glutamine. In terms of the mutants, there was no significant difference in growth between WT and Δ*yneH.* However, there were significant difference between WT and the *ansA, ybiK,* and *KPN_01165* mutants. Enzyme activity assays showed that the strains Δ*KPN_01165*, and Δ*ybiK* had lower asparaginase activities under the growth conditions compared to the WT.

Xiangyun Zhi

Rgg transcriptional regulators have a role in pneumococcal survival and virulence

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

Streptococcus pneumoniae causes a range of life-threatening diseases in different tissues, suggesting that it has effective mechanisms to sense and respond to environmental stimuli. However, the regulatory mechanisms required for pneumococcal adaptation are poorly understood. The Rgg family proteins are transcriptional regulators, that have been shown to be important for survival and virulence in other streptococci but their role in the pneumococcus is unknown. The pneumococcal type 2 D39 strain has 5 different Rggs, and two of them, SPD_0144 and SPD_0939, are associated with genes coding for a short hydrophobic peptide (*shp)*. It has been suggested that Rgg-SHP circuits are components of quorum sensing systems in Gram positive bacteria. Therefore, the objectives of this study were to determine the role of Rggs in pneumococcal survival and virulence, and to investigate if Rggs interact with their adjacent SHPs.

Site directed mutation of *rgg* genes was done by overlap extension PCR, and the mutants were tested in their ability to utilise mannose, and grow in the presence of H_2O_2. The results showed that Rgg mutants exhibited susceptibility to H_2O_2, and were compromised in their ability to use mannose. Moreover, the expression of

shp0144 and *shp0939* is induced by truncated SHP peptides, and Rggs induce expression of *shp* genes. In addition, Rgg mutants were attenuated in virulence in an experimental murine infection model.

Bushra Shlla

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

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

Streptococcus pneumoniae is responsible for death of approximately 3-5 million people per year due to pneumoniae, meningitis, and bacteremia. The invasive phenotype relies on the expression of different virulence factors that affect the capacity of *S. pneumoniae* to cause disease. The pneumococcal ability to efficiently sense and adapt to diverse environmental conditions are also important for pneumococcal virulence. Transcriptional regulators have a crucial role on pneumococcal adaptation to environmental changes.

Rgg is one of these transcriptional regulators, which plays diverse roles in many bacteria. This research has been conducted to identify the role of Rgg in *S. pneumoniae*. For this purpose, targeted mutation was used for deletion of two Rgg genes, SPD_0999 and SPD_1518, in *S. pneumoniae* D39 by splicing overlap extension method. Furthermore, double SPD_0999 and SPD_1518 mutant was also done. Analysis of Rgg mutant strains indicated that the mutants were attenuated in growth on galactose and mannose compared to the wild type D39, although all strains showed a similar growth profile in BHI. Furthermore, there was no difference between the mutants and wild type in growth on glucose.

In vivo analysis of Rgg mutants showed that SPD_0999 and SPD_1518 are fundamental for pneumococcal virulence. All the mice infected intranasaly with either ΔSPD0999 or ΔSPD1518 and ΔSPD0999/1518 mutant survived significantly longer than the wild type infected cohort, and the bacterial load in mice infected with the mutant strains was less than that of the wild type. Therefore, the available results so far show that Rgg encoded by SPD_0999 and SPD_1518 play a major role in pneumococcal virulence, and studies are underway to determine the mechanism of SPD_0999 or SPD_1518 contribution to virulence.

Iman Abdullah

Mechanistic and phenotypic characterisation of Rgg/SHP communication pathway in *Streptococcus pneumoniae* D39

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

Streptococcus pneumoniae is an opportunistic Gram-positive pathogen causing an array of diseases ranging from mild mucosal infections to life-threating diseases in different host niches. Several putative transcriptional regulators have been predicted to be involved in *in vivo* adaption of this microbe. The Rgg is one of these regulators that operate through interacting with a signal peptide called SHP (short hydrophobic peptide) via the process called quorum sensing system. Although this pathway is studied in several streptococci, its function and mechanism of action still are unclear in *S. pneumoniae*. The pneumococcal type 2 strain D39 has five copies of Rggs, and two of them (SPD_0144 and SPD_0939) are predicted to be associated with *shp* genes encoding for SHP peptides, which regulates its own expression, and is required for Rgg function. Therefore, this study was designed to characterise the Rgg/SHP signaling pathway in *S. pneumoniae* D39 through studying the intermolecular interaction between Rgg144 and SHP144, and identifying the SHP144 residues that are important for Rgg144 activation and binding, and establishing their importance in Rgg144's phenotypic manifestation in pneumococcal biology.

To perform these aims, site directed mutagenesis was used for substitution of SHP144 amino acid residues with alanine (alanine scanning technique), and the effect of amino acid replacements was studied using promoter reporter assays (β-galactosidase assay). The results showed that the mutant strains lacking either Rgg144 or SHP144 displayed lower β-galactosidase activity compared with that of the wild type, and the genetically complemented strains, indicating that Rgg144 and SHP144 are essential for *shp144* expression. Most importantly, amino acids residues at positions 16, 17 and 18 from N-terminus are critical for P*shp144* driven β -galactosidase activity as their replacement with alanine resulted in a pronounced reduction in β -galactosidase activity compared with the activity of strain containing intact copy of *shp144*. These data show the importance of these residues in SHP144 function. Further studies are underway to demonstrate the effects of other residues in SHP144 function and their impacts on Rgg144 conferred phenotype.

Nor Azian Hafneh

Role of Rv2660c during persistence and virulence in *Mycobacterium Tuberculosis*
Supervisor(s): Dr Galina Mukamolova

One key aspect of *Mycobacterium tuberculosis* pathogenesis is its ability to survive stress conditions encountered during macrophage infection and undergo transition into a state of non-replicating persistence (NRP). This forms the basis of global risk of latent tuberculosis, thus substantial research studies are focused on identification of novel "latency-specific" antigens as potential vaccine candidates for prevention of TB reactivation. *rv2660c* encoding a conserved hypothetical protein was shown to be a highly up-regulated gene during *in vitro* starvation model and mice infection. H56: IC31 vaccine constructed with Rv2660c induced a robust immune response in latently TB infected patients and decreased bacterial loads in infected animal models. However, the existence of Rv2660c has been challenged by identification of ncRv12659, a non-coding RNA encoded by a genomic sequence on the opposite strand to *rv2660c*.The aim of this study is to determine the biological role of Rv2660c during mycobacterial growth, persistence and virulence. Unmarked single deletion mutants (Δ*rv2660c* and Δ*rv2661c*) and a double deletion mutant (Δ*rv2660c:2661c*) in *M. tuberculosis* were generated. Their survival and mRNA expression during NRP and exposure to stressful conditions were examined. Our study confirmed that Rv2660c and Rv2661c are dispensable for growth *in vitro*. Initial findings demonstrated Δ*rv2660c:2661c* strain showed a survival defect during oxidative stress. Additional experiments including complementation studies will further demonstrate our previous data that suggested that Rv2660c and Rv2661c may have an important contribution *M.tuberculosis*'s response to stress conditions.

Chris Jenkins

Investigating the lytic transglycosylases of *Burkholderia pseudomallei*
Supervisor(s): Dr Galina Mukamolova and Dr Ed Galyov

Burkholderia pseudomallei is the causative agent of melioidosis, a tropical disease prevalent in South East Asia and Northern Australia. Difficulties with treatment and diagnosis of the disease are enhanced by the ability of *B. pseudomallei* to persist in humans without causing disease for decades. Persisting forms are likely to be in a special non-replicating (possibly non-culturable) state. Remodelling of the cell wall by muralytic enzymes (especially, lytic transglycosylases, LTG) is believed to be important for reactivation of latent infection. These proteins are highly conserved in bacteria and predominantly function in the restructuring of peptidoglycan (PG) for the insertion of large macromolecular structures including secretion systems

and flagella but also PG precursors important in cell elongation and division. Using sequence homology to well characterised LTGs of *E. coli* we have identified 5 putative LTGs in *B. pseudomallei* K96243. Using recombinant Ltg proteins we have shown muralytic activity, solved the structure of LtgE and shown the importance of predicted catalytic sites using site directed mutagenesis. Single, double and triple Ltg mutants result in altered cellular morphology (increased cell length and failure to complete division) and motility. This is the first investigation into the Ltgs of *B. pseudomallei*. Further research could shed light on the function of these proteins in regard to entry and exit of non-culturable or persistent states.

Mariam Mohammed Nur

The role of Mycobacterial Resuscitation promoting factors (Rpfs) in osmotic stress
Supervisor(s): Dr Galina Mukamolova and Dr Primrose Freestone

Tuberculosis (TB) remains a global health threat. It has been estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis*. Its ability to enter a dormant state makes it an exceptionally successful human pathogen. It is postulated that Resuscitation promoting factors (Rpfs) are involved in the reactivation of dormant bacteria via peptidoglycan remodelling, however, precise molecular mechanism remain unknown.

M. tuberculosis has 5 *rpf*-like genes with homologues present in other GC rich microorganisms, such as *M. marinum*. The similar pathogenic characteristics of *M. marinum* to *M. tuberculosis* make it a good model organism to study mycobacterial pathogenesis.

Mycobacteria are subjected to a variety of hostile microenvironments. Recent discoveries in the differential expression profiles of *rpfs* during stress condition have led to the question of whether the different Rpfs are implicated in stress response and TB infection. Although *Rpfs* are associated with a number of different regulatory transcriptional and translational mechanisms; it is not exactly known how expression of these genes is regulated during growth, dormancy and stress exposure.

To explore this, a panel of *rpf* deletion mutants has been generated and validated during growth in standard mycobacterial media and elevated salt conditions. A growth defect phenotype has been observed for *ΔrpfAB M. marinum* mutant in both Sauton's medium and elevated salt conditions. The *ΔrpfA M. marinum* mutant shows a moderate growth defect in the presence of 550mM NaCl compared to WT.

Moreover, *ΔrpfE* and *ΔrpfAE M. marinum* mutants survive better under osmotic conditions. Thus, our findings suggest that Rpfs are important for mycobacterial adaptation to osmotic stress.

Helena White

Screening and Treatment of HIV positive individuals for Latent Tuberculosis Infection

Supervisor(s): Dr Manish Pareek and Prof. Martin Wiselka

HIV positive individuals are at an increased risk both of acquiring tuberculosis (TB) and progressing to active TB disease as a result of reactivation of latent TB infection (LTBI), and screening for LTBI in this cohort in the UK is advocated by two published guidelines from the British HIV Association (BHIVA) and the National Institute of Health and Care Excellence (NICE). It is not currently known whether, or how, HIV centres in the UK implement LTBI screening, and I will present the results of a national questionnaire which I have undertaken, which shows that there is considerable heterogeneity in current practice. There has also not previously been any formal evaluation as to whether LTBI screening is acceptable to individual patients, and I will present the results of a patient questionnaire study which I have undertaken in Leicester, which specifically examines this issue. This study received ethics approval and seeks to correlate the planned intentions and beliefs of individuals relating to accepting LTBI screening and treatment, with the outcomes of the systematic LTBI screening programme which I am currently conducting in Leicester.

Mashael Alruways

Investigation of the role of porin proteins in *Salmonella* acquisition of host iron
Supervisor(s): Dr Primrose Freestone

Iron is essential for the growth and long-term persistence of most human pathogens, and growth within host tissues is dependent on the bacteria being able to steal iron from normally secure iron sources, such as the iron-binding proteins transferrin (Tf) and lactoferrin (Lf) (Freestone et al, 2008). It has previously been shown for *E. coli* that Lf is bound to the bacterial cell by the outer membrane porins OmpC and PhoE (Sallmann et al., 1999). It is therefore of interest to study whether other pathogens within the *Enterobacteriaceae* family similarly utilise their porins to bind Lf, and indeed Tf, and if they do whether this is a new strategy to acquire iron from their host. The hypothesis of this project is that the OmpD and OmpC porins of *Salmonella* are moonlighting as a Tf and Lf binding proteins, and that this capture activity is important to the capacity of *Salmonella* to establish an infection within its host. In this project the *ompD* and *ompC* genes are deleted and an analysis made of the effects of the mutations on *Salmonella* host iron acquisition and virulence.

Giannis Koukkidis

***Salmonella*-Salad Interactions**
Supervisor(s): Dr Primrose Freestone and Dr Suzanne Jordan

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 *Salmonella enterica*. So far, studies have concentrated on improving the hygiene of production and have not considered what happens to the behaviour of *Salmonella* when they enter the actual salad bag. It is known that salad leaves become damaged during processing and that juices are released, so bacteria residing in a salad bag will be bathed in leaf juice.

The intention of our research is to investigate the effect of juices released from damaged salad leaves on the growth, virulence and salad leaf colonisation of *S. enterica*. Our aim is to use this information to develop ways of preventing enteric pathogen attachment to fresh salad produce.

Salmonella responsiveness to salad juices was analysed in water, to reflect the salad bag environment, and in serum- media to model the co-consumption of pathogen and salad leaf. We used assays that measured the effect of salad leaf juice on *S.*

enterica growth, motility and biofilm formation. Light and scanning electron microscopy were used to visualise juice effects on *Salmonella* colonisation of salad leaves and the salad container**.**

Salad juices at more than 1:50 dilutions stimulated *Salmonella* 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 tested significantly increased *Salmonella* biofilm formation and its capacity to colonise and persist on salad leaves, and the salad bag container**.**

Our study shows that even very dilute salad juice can contribute to *Salmonella* colonisation of salad leaves and re-emphasises the importance of preventing enteric pathogen of fresh produce.

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). Prolonged use of antibiotics combined with long stays in hospitals and other healthcare facilities are the main factors responsible for occurrence of the disease.

Treatment of CDAD, particularly by antibiotics is problematic due to the organism's ability to develop resistance; therefore alternative methods of treatment are being sought. Bacteriophages (phages) are viruses that target and infect bacteria, and phage therapy (use of phages 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* interactions in human gut epithelial cells, therefore an *ex situ* model has been developed to mimic phage therapy. This will be used to determine how phages impact the ability of clinically relevant *C. difficile* strains to colonise mammalian cells, to establish how phages influence *C. difficile* growth and biofilm formation and to ascertain the inflammatory responses of epithelial cells that may be triggered by phage. Results have shown that *C. difficile* levels drop in the presence of both phage and epithelial cells compared to with phage alone.

Ahmed Dowah

Identification of Host Receptors for *Clostridium difficile* Bacteriophage

Supervisor(s): Prof. Martha Clokie and Prof. Russell Wallis

Clostridium difficile is responsible for a range of gastrointestinal diseases in the form of C*. difficile* infection (CDI). The emergence of antibiotic resistance led to research into an alternative treatment for CDI. These include the application of bacteriophages, viruses that specifically infect bacteria. A major determinant for phage selection for treatment is phage specificity and an essential part of phage infection is the adsorption process, which involves binding of phage proteins to receptors on the bacterial surface. Therefore identification of the receptors is one of the key aspects to understand the bacteriophage biology. To identify *C. difficile* phage receptors two phages were studied; CDHS1 and CDHM1. They both infect different *C. difficile* strains*,* both phages have morphologically different, and so it's most likely the phages use different receptors to establish infection.

Two main approaches were used to obtain the project aim. First, the chemical nature of the receptors on the surface of the host was determined by treatment of the *C. difficile* cells using chemicals, such as proteinase K, which digest the proteins. After which the phage was incubated with treated cells. The second was to over express the predicted phage proteins that are involved in phage host binding, thereafter using these proteins to identify the corresponding receptors on the *C.difficile* surface.

The obtained results indicate that the phage (CDHM1) was still able to bind to the *C.difficile* even post to the treatment with proteinase K, which may suggest that the receptors are carbohydrate in nature. Currently phage proteins tagged with Green Fluorescent proteins were expressed and binding assays of these proteins with *C.difficile* is ongoing.

Guillermo Rangel Pineros

Exploration of the diversity and ecology of *Streptococcus pneumonia* bacteriophages in The Gambia

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

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,

to unravel their contribution to the success of the pneumococcus in the country and to isolate new lytic pneumophages.

123 nasopharyngeal swabs (NPS), collected from healthy children < 5 years old, were enriched for pneumophages. Phage characterization was conducted by transmission electron microscopy and genome sequencing. The presence of active prophages in the genomes of eight Gambian strains, representing highly prevalent sequence types, was assessed by determination of Mitomycin C minimum inhibitory concentration (MitC-MIC) and the prediction of phage-derived regions in their genomes.

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 Amer

Factors contributing to the control of *Arthrospira fusiformis* in 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) has gradually increased from May to October; this was associated with the increase in the salinity of lake water. From morphological point of view, the H-type of *Arthrospira* morphotype was found to be the less abundant morphotype in the Central basin; this is in agreement with the presence of cyanophages. The findings of microtome assay could support the idea

of presence cyanophages within *Arthrospira* from Lake Bogoria, and the negative staining of the concentrated phages from water samples and culture lysates of infected cultures of *Arthrospira* has reported the presence of three morphotypes of Bacteriophages (myovirus, siphovirus and podovirus), with the greatest number of viruses from the surface of South basin. Chlorophyll fluorescence and optical density (OD) measurements indicating the possibility of lysogenic infections in some strains (*Arthrospira* ND0.4). This study will help us to determine the extent and diversity of viral infection for *Arthrospira* species in alkaline soda lakes in Kenya.

Wafaa Alrashidi

Bacteriophage-Bacteria-Amoeba Interactions

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

In nature, 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 and treatments, plus, multiply within the protozoan cell, producing enough numbers for their transmission through the environment or establishing infections. In this project we will study the implications of lytic phages on opportunistic pathogenic bacteria in the presence of amoebal grazer, in order to bring more understanding on how intracellular survival of bacteria within amoebal cells would assist them to evade killing or infection by bacteriophages. 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 *Pseudomonas aeruginosa* into an amoebal host would protect such bacteria from bacteriophages 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.

Ali Ali

Isolation and Characterisation of *C. perfringens* Bacteriophages
Supervisor(s): Prof. Martha Clokie

Phage therapy rises again after the emergence of multi-antibiotic resistant bacteria including *Clostridium difficile* and *C. perfringens* which represent a health and economic burden. 78 *C. perfringens* strains and 5 bacteriophages have been isolated out of 84 environmental samples. *C. perfringens* strains were confirmed by 16s rDNA amplification and all strains were toxin-typed, most of them were toxin type A. Four unstable bacteriophages that were able to plaque on *C. perfringens* strains were isolated. Five stable bacteriophages that are able to lyse *C. perfringens* were purified and characterised morphologically; three were podoviruses and two were siphoviruses.

The host range for the five bacteriophages were determined, Phage F42 is able to infect two of the 78 *C. perfringens* strains, while phages F43 and F44 were unable to infect any of the 78 *C. perfringens* strains but their isolation host.

The genomes of two phages were sequenced and the DNA of three phages was extracted for the genetic characterisation by complete genome sequencing.

Neda Nezam Abadi

Isolation and characterisation of bacteriophages infecting *Legionella* spp.
Supervisor(s): Dr Ed Galyov and Prof. Martha Clokie

Legionella spp. is the causative agent of Legionnaires' disease, a potentially fatal acute pneumonia with record high morbidity and mortality rates. *Legionella* spp. has been shown to be resistant to many disinfectants, as well as beta-lactam antibiotics, in addition its detection is difficult, often making treatment of this infection challenging. In light of this, bacteriophage, the viral predators of prokaryotes, has been suggested as a potential means for improving both diagnostic identification and therapeutic treatment. Here, we are trying to isolate and characterise bacteriophage against *Legionella* spp. for further development as more specific diagnostic tool, and as an alternative treatment for this pathogen.

A total of 200 water and sediment samples were collected from natural reservoirs and man-made systems, such as cooling towers, homes, spa pools, etc. The samples were first heat-treated and tested for *Legionella* isolation. Subsequently, the same samples were analysed for the presence of free bacteriophages using host range analysis, followed by Transmission Electron Microscopy (TEM).

Seven *Legionella* spp. strains have been isolated from water samples and no isolates were recovered from sediment samples. TEM analysis of these lysates revealed the presence of numerous bacteriophages with diverse morphologies, with *Myoviridae* and *Siphoviridae* being the most common. Consistent with previous work, we observed only zones of confluent lysis on a host lawn following enrichment of samples from different environments (lakes, rivers, ponds, and etc.). No samples yielded single plaques and no zones of lysis could be further propagated. Further work is ongoing to optimise phage purification, propagation, and development for therapeutic and diagnostic purposes.

Iswahyudi Iswahyudi

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.

A mutant strain of *M. tuberculosis* disrupted in *pstP* gene is likely to be impaired in normal signalling via serine/threonine phosphorylation and may therefore show changed behaviour. The aim of this project is to study the function of PstP in *M. tuberculosis* by making an unmarked deletion mutant of *pstP*. But before performing *pstP* gene disruption into slow growing *M. tuberculosis* we decided to test it first in fast growing *M. smegmatis.* Also we decided to check whether over-expression of *pstP* would cause any growth defect. Control and *pstP*-overexpressing *M. smegmatis* were grown and their growth rate and cell length were measured and compared. There was an initial difference in growth of the strains, with the overexpressing strain having an extended lag phase, but the eventual growth rate was not significantly different. To examine whether *pstP* overexpression influences the global phosphoproteome, cell lysates from the two strains will be compared by Western blotting with anti-phosphothreonine antibody. To attempt to disrupt the *pstP* gene in *M. smegmatis*, a plasmid is under construction to make an unmarked deletion by homologous recombination.

Thekra 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

Genomes of the majority of *Clostridium difficile* strains encode prophages, but information on the potential impact of these phages on their hosts is limited. Previous reports revealed that *C. difficile* phages are able to transduce infected cells; however, no studies have shown the impact of this infection during a complete replication cycle. My research is aimed to bridge this gap; I have conducted extensive analysis to determine the impact of phage infection on C. difficile by taking into account all the stages of phage replication cycle. To do this, the growth kinetics of two myoviruses (phiCDHM1 and phiCDHM5) and a siphovirus (phiCDHS1) was first conducted on clinically relevant TL176 and R20291 strains respectively. Preliminary results revealed that the phages complete their life cycle within 20 min and have burst size of 400-1000. Unfortunately, phiCDHM1 and 5 have low plaquing efficiencies on TL76; therefore, further study of these phages on this strain was discontinued. After several optimizations, phiCDHS1 was selected for RNA extraction during the early, mid-log and stationary phases of the phage replication cycle on strain R20291. Total RNA from samples at various times during a one-step growth curve of this virus was isolated. RNAseq analysis will be employed to identify differential gene expression in both the bacteria and the virus. Further analysis of the RNA sequence data will reveal the regulation of virulence genes encoded by this strain.

Hiwa Fatah

Urinary shedding of tubular protein receptors in proteinuric states

Supervisor(s): Prof. Nigel J Brunskill

Background: Proteinuria is clearly associated with the progression of chronic kidney disease but the mechanisms underlying this relationship remain unclear. Recent evidence suggests that altered proximal tubular (PT) handling of filtered proteins may significantly modulate urine protein excretion and progressive renal disease. Megalin and neonatal Fc receptor (FcRn) are endocytic receptors responsible for the PT reabsorption of glomerular ultrafiltered proteins by receptor-mediated endocytosis, and are expressed on the luminal surface of the PT. We investigated the expression and turnover of megalin and FcRn in proteinuria, and the possible mechanisms underlying down-regulation of expression of these receptors.

Methods: Protein overload proteinuria (POP) was induced in uni-nephrectomised mice by intraperitoneal injection of bovine serum albumin (BSA) for 16 days. Controls received saline injections. Expression of megalin and FcRn in kidney was determined by immunocytochemistry (IHC), Western blotting and qPCR. Presence of megalin and FcRn in urine was determined by ELISA. Expression of matrix metalloproteases 3, 7, and 9 in kidney was determined by IHC, Western blotting and qPCR, and γ-secretase by IHC and Western blotting.

Results: Serum total protein concentration was significantly higher in POP animals than controls (39.71±2.78 vs 23.89±0.78 mg/ml). Proteinuric animals had significantly higher overall urinary protein concentrations than controls (61.45 ± 6.03 vs 20.90 ± 0.24 mg/ml). Expression of megalin and FcRn at the protein level in PT was markedly decreased in proteinuric animals compared to control mice as judged by IHC and Western blotting. Conversely gene expression of both receptors in kidney was significantly increased by qPCR. Urinary excretion of both megalin and FcRn was very significantly increased in proteinuric animals compared to the control animals by ELISA (204.40 ± 13.42 and 285.10 ± 28.39 vs 7.27 ± 2.81 and 58.58 ± 21.33 ng/ml respectively). Protein and gene expression of matrix metalloproteases 3, 7, and 9 were significantly increased in the PT of proteinuric animals compared to the control animals. Finally the protein level of γ-secretase was significantly higher in proteinuric animals than controls, by IHC and western blotting.

Conclusions: PT protein receptor turnover is greatly increased in proteinuria, but with a net down-regulation of receptor expression. This is associated with an increase in receptor processing enzymes in PT cells and release of protein receptors into the urine. This reduced protein receptor expression in proteinuria is likely to be an important factor in proteinuric renal disease and offers new therapeutic targets for intervention in proteinuric nephropathy.

Amy Clarke

Developing behaviour change interventions to increase levels of physical activity in patients with chronic kidney disease

Supervisor(s): Dr Alice Smith, Dr Thomas Yates and Dr Jonathan Barratt

Introduction: Physical activity (PA) is beneficial for patients with CKD. However, the majority of patients lead very sedentary lifestyles. Self-management programmes have successfully increased PA in other chronic disease groups; however, these programmes are not routinely available for patients with CKD. Following the MRC framework for the development of complex interventions this work applies a

systematic approach to the development of a psychological intervention to increase levels of PA in patients with CKD.

Methods: I applied a systematic approach to the development of an intervention to increase PA levels in patients with CKD:
1) Review of the literature;
2) Survey (N=1715, CKD stages 1-5) to describe levels of physical functioning, PA behaviours and psychological determinants of exercise;
3) Qualitative study (N=36, CKD stages 1-5) to explore beliefs regarding exercise among patients with CKD;
4) The person based approach to the planning, design and development of a PA intervention;
5) Final revisions and iterations;
6) Pilot study.

Results: The survey demonstrated widespread physical inactivity amongst CKD patients (>80%). Qualitative inquiry identified the following barriers to PA: low self-efficacy, poor physical function, fear of injury/aggravating disease and lack of informational support. The "person-based approach" provided a systematic framework to aid the development of a behaviour change intervention. Qualitative research and consultations with patient partners was invaluable to this research and ensured that development was user-led at all stages.

Conclusion: The next phase will be to pilot the intervention, with assessment of user experience via semi-structured interviews upon completion.

Safia Blbas

The role of phosphorylation events in regulation of the cachexia mediator SNAT2 in L6 rat skeletal muscle cells

Supervisor(s): Dr Alan Bevington and Dr Cordula Stover

Problem: Metabolic acidosis is common in CKD and stimulates muscle protein wasting which may further enhance chronic inflammation. In vivo, this muscle wasting by acidosis also requires the presence of glucocorticoid (GC). Metabolic acidosis is thought to act by inhibiting the pH-sensitive System A amino acid transporter protein SNAT2, resulting in impaired global protein synthesis and enhanced global proteolysis.

Purpose: SNAT2 is strongly activated by amino acid depletion (AAD), in a pathway dependent on protein phosphorylation (MAP kinase activation). Furthermore, recent evidence suggests that the human SNAT2 protein can be phosphorylated.

Therefore, the aim of this study was to investigate (a) how glucocorticoid and AAD influence SNAT2 activity in cultured skeletal muscle cells, and (b) the possible role of phosphorylation events in these effects.

Design: The L6 rat muscle cell model was used to study the effects of AAD, the glucocorticoid Dexamethasone (DEX), and drugs influencing protein phosphorylation, on SNAT2 transport activity. The activity of this transporter was measured from the rate of α-[1-14C]-MeAIB transport into the cells. To obtain an experimental system in which the effect of direct SNAT2 phosphorylation on its function could be assessed; human SNAT2 was cloned and expressed in the readily transfected cell line HEK293A.

Findings: In L6 myotubes, (DEX) and AAD have effect on SNAT2 activity, suggesting both Tyr and Ser/Thr phosphorylation events may be important in regulating SNAT2's activity. The effect of DEX is functionally important through its effect on protein anabolic signalling pathways such as AKt. In eGFP-tagged SNAT2 transfected HEK293A cells, as demonstrated by eGFP fluorescence and by immunoblotting cell lysates with anti-GFP antibody. Strongly enhanced α-[1-14C]-MeAIB transport into the cells indicated that the expressed construct was functionally active.

Conclusion: SNAT2 regulation by glucocorticoid or AAD may act through a combination of Tyr and Ser/Thr phosphorylation events, possibly on the SNAT2 protein itself. Expression of functionally active SNAT2 in HEK293A cells provides an experimental model in which to test this possibility.

Yan Song

Encouraging a Healthier Lifestyle in Patients with Dialysis: A Multicultural Perspective

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

Background: Physical activity or exercise is recommended for haemodialysis (HD) patients to optimize health, function and quality of life, but the majority lead very sedentary lives. Patient-centred strategies to address this behaviour are required. Therefore, the study is to explore the functional capacity and physical activity (PA) status of HD patients between UK and China, and to gain an understanding of psychology determinants and patient attitudes towards PA and exercise. Additionally, Exploring HD patients' dietary behaviours and nutritional status is the purpose of this study as well.

Methods: A cross-sectional study using convenience sampling is conducted at HD units in Leicester and Nantong, using 6 validated questionnaires. Functional

capacity, habitual PA levels and leisure time exercise are assessed by Duke Activity Status Index (DASI), General Practice Physical Activity Questionnaire (GPPAQ) and Leisure Time Exercise Questionnaire (LTEQ) respectively. A Self-Efficacy Questionnaire (SEQ), Stages of Change Questionnaire (SOCQ) and the Dialysis Patient-Perceived Exercise Benefits and Barriers Scale (DPEBBS) are used to assess exercise-related self-efficacy, patients' readiness to change a health-related behavior, motivators and barriers. HD patients' dietary habits are assessed using the 24-hour diet recall. In addition, qualitative interviews are used to gain more details of patients' perception towards exercise and nutritional status.

Results: 189 and 212 patients completed the questionnaires in Leicester and Nantong respectively. 43 Chinese HD patients participated in the nutrition study. Interviews about exercise and nutritional status were conducted with 28 participants.

Conclusion: Data analysis is expected to end a couple of months later allowing for more conclusions to obtain

Dina Nilasari

The Role of KCa 3.1 in Tubular Interstitial Inflammation and Interstitial Fibrosis
Supervisor(s): Dr Jonathan Barratt, Prof. Peter Bradding and Dr Karen Molyneux

Introduction It is known that fibrosis plays an important role in the progression of CKD to end stage renal failure (ESRF). Despite much research, the mechanisms controlling renal fibrosis are not fully understood. The intermediate-conductance Ca^{2+}-activated potassium channel, KCa3.1, has emerged as an important regulator of fibroblast proliferation in renal and other diseases. The aim of this study was to investigate the expression of KCa3.1 in cultured human mesangial cells (MC), podocytes and proximal tubule epithelial cells (PTEC) and determine the effect of blocking KCa3.1 on the *in vitro* response of human MC.

Methods: To determine whether human MC, podocytes and PTEC constitutively express KCa3.1, cell lysates were subjected to western blotting. To evaluate the role of KCa3.1 in the pro-inflammatory and pro-fibrotic response of human MC to TGF-? and IgA1 serum starved human MC were incubated with IgA1 and TGF-?, with or without the KCa3.1 blocker, TRAM-34, for 24 hours.

Results: Human MCs, podocytes and PTECs constitutively express KCa3.1. IgA1 induced an up-regulation of KCa3.1 expression in human MC and PTEC which could be inhibited by the KCa3.1 blocker, TRAM-34, suggesting this effect was in part mediated by KCa3.1 itself. Human MC exposed to IgA1, as expected, developed a

pro-inflammatory phenotype with secretion of IL-6. Administration of TRAM-34 inhibited the IgA1-mediated release of IL-6 by human MC.

Conclusion: Expression of KCa3.1 by human MC, podocytes and PTEC, the upregulation of KCa3.1 in human MC and PTEC by IgA1 and TGF-□ and the inhibition of IgA1 induced human MC activation suggest a role for this channel in the glomerular and tubulointerstitial fibrosis and may offer a novel therapeutic target in renal fibrosis.

Keyur Shah

Title: TBC

Supervisor(s): Dr Michael Nicholson and Dr James Burton

Sian Baldock

Does the cystic fibrosis phlegm feed the *Pseudomonas*?

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

Introduction: *Pseudomonas aeruginosa* is an important pathogen in cystic fibrosis (CF) due to its ability to colonise the airway by adhering to mucins within stagnant mucus. However, what remains to be elucidated is how pathogens survive in the airway.

Aims: To investigate the growth of *Pseudomonas* laboratory strains, PA01 and PA14, in medium supplemented with paediatric/adult CF sputum and purified mucin. This is in order to elucidate whether CF mucin provides a nutrient source for *Pseudomonas*.

Methods: Sputum samples were solubilised in guanidine hydrochloride and purified via caesium chloride/guanidine hydrochloride density gradient centrifugation. Fractions containing mucins were dialysed, lyophilised, re-suspended, and sterilised via autoclaving. Strains were grown in samples with M9 medium. Growth was assessed via CFU counts. Specific growth rates were calculated at log growth phase.

Results: PA01 and PA14 have an increased growth rate in adult (n = 5) compared to paediatric (n = 5) CF sputum ($p<0.0001$, one-way ANOVA) whilst PA01 has an increased growth rate of 1.336 m (hour-1) in adult vs. 0.8320 m ($hour^{-1}$) in paediatric mucin ($p<0.05$, one-way ANOVA).

Conclusions: This is the first report of a difference in growth rate of *Pseudomonas aeruginosa* strains in adult vs. paediatric CF sputum. Currently the mechanism remains to be defined. Growth rate differences could be due to differences in mucin metabolism or sputum composition, such as the presence of growth promoting factors. It is therefore necessary to discover whether decline in lung function due to respiratory infection is partly due to an alteration in composition with age.

Nidhal Gharbawi

Lung Function in Children of Different Origins

Supervisor(s): Dr Caroline Beardsmore and Dr Erol Gaillard

Background: Within Leicester, the largest ethnic minority groups have their origins in the Indian subcontinent. They have lower levels of lung function than the white population, but the differences do not appear to relate to either socioeconomic status or body proportions.

Aim: My project aims to investigate whether differences in lung function between white and south Asian children can be explained in part by differences in strength of respiratory muscles ((maximum inspiratory and expiratory pressures (MIP and MEP)). Also, we aim to investigate effects of physical activity on lung function and respiratory muscle strength.

Methods: Spirometry, MIP and MEP and anthropometry were measured in children aged 5-11 in their primary schools. A questionnaire determined which children engaged in exercise (defined as spending more than 10 minutes at a time in vigorous physical activity), and whether they spent more than six hours per day in sedentary activity.

Results: Sixty-four children (52 south Asian) were studied; 19 reported no exercise, 34 children spent >6hr per day in sedentary activities. We obtained MIP on 55 and MEP on 60 children. There was no significant relationship between respiratory muscle strength and height. MIP and MEP were higher in children engaging in exercise, with a trend for higher respiratory pressures in the less sedentary children (Table).

MIP and MEP with activity			
	Exercise	No exercise	p
MIP	6.85(0.42)	5.57(0.40)	0.037
MEP	6.42(0.32)	5.15(0.40)	0.013
	Not sedentary	Sedentary	
MIP	6.71(0.44)	6.26(0.49)	0.25
MEP	6.30(0.32)	5.80(0.41)	0.17

Mean (SD) MIP or MEP (kPa)

Conclusion: MIP and MEP show considerable variability in healthy children. Preliminary data suggest that physical activity in children may influence respiratory muscle strength, but a bigger sample size is required.

Eva-Maria Rick

The fungal microbiome in severe asthma and its clinical significance

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

Asthma is a chronic airway disease affecting > 300 million people worldwide, causing symptoms such as breathlessness. Patients with severe asthma require high doses of medication or suffer from symptoms despite optimal treatment. Allergic asthma is triggered by allergens such as fungi. Sensitisation to *Aspergillus fumigatus* is associated with decreased lung function in patients with asthma, but the lung fungal microbiome is unclear due to insensitive detection methods. This study uses both culture and high-throughput sequencing of the fungal nuclear ribosomal operon to identify fungi from different lung compartments.

Yeasts were most commonly cultured, especially from sputum. The highest culture rates of *A. fumigatus* were found in patients with asthma sensitised to *A. fumigatus* and/or *Penicillium chrysogenum*, of which 89% were IgE-co-sensitised to at least one other fungus. Preliminary sequencing data have been produced for 61 sputum and 29 bronchoscopy-derived samples. Sequencing yielded more species than detected by culture, including uncommon fungi in asthma such as *Asterotremella* or *Nectriaceae*. *A. fumigatus* was the most prevalent and abundant fungus in all samples. The data showed high similarity concerning the most prevalent fungi (*Candida albicans*, *Cladosporium spp., Aspergillus niger*), but less so for less prevalent fungi and when sequence abundance was considered rather than prevalence. *Candida dubliensis* was the only fungus detected in > 30% of sputum samples of asthmatics (39%) but not in healthy controls. Other fungi such as *A.niger* or *Malassezia restricta* were at least 1.5 times more prevalent in the asthmatics.
These preliminary findings provide the basis for our future work.

Marie-jo Medina

Keep your hands to yourself

Supervisor(s): Dr Manish Pareek and Prof. Peter Andrew

Background: RSV is the leading cause of nosocomial infections in paediatric wards, particularly in children under 6 months of age and in health professionals in charge of their care. Adults are generally infected at a rate of 5-10% per year. During epidemics, however, approximately 60% of paediatric staff acquire nosocomial RSV, and at least 27% pass it on to patients and other staff. In the first observed natural virus transmission study, presented here are inter-epidemic data on RSV nosocomial infection in a local healthcare facility.

Methods: The TraVerse method of natural respiratory virus transmission was employed to determine RSV transmission rates in a hospital setting, using healthy young adults as proxy healthcare workers attending to symptomatic paediatric inpatients. Viral load was detected using RT-PCR and disease severity was determined by analysing symptom diary cards.

Results: A natural RSVB transmission rate of 14% was determined in the inter-epidemic periods during Jan 2013 to May 2015. Direct infection by hands was highly instrumental in the spread of virus and severity of symptoms, occurring within 10 minutes of interaction with a diseased child.

Conclusions: RSV nosocomial infection occurs rapidly and results in severe symptoms, particularly when contact with contaminated hands has transpired. Reported adult infection rates outside epidemic periods may have been under-estimated.

Tariq Daud

The role WNT5a in Th17 asthma

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

Background: Asthma maybe characterised by distinct tissue molecular phenotypes (Choy, Hart et al. 2015). However the process of repair and remodelling remains ambiguous in this context.

WNT5a acting through the non-canonical axis exhibits functional cross-talk with TGF-β1, which may influence repair and remodelling.

Aims and objectives: We sought to evaluate the expression of WNT5a and TGF-β1 in the asthmatic epithelium, stratified by pathological phenotype and whether WNT5a potentiates epithelial repair and remodelling via cross talk with TGF-β1.

Methods: Endobronchial biopsies from a previously described cohort of subjects (9 healthy and 23 asthmatics) in whom the gene signature profiles for Th2 and Th17 activity were available were studied. Tissue Sections were immunostained for WNT5a and TGF-β1. Cultured BEAS-2B epithelial cells were evaluated for markers of epithelial to mesenchymal transition (EMT) and SMAD2/3 nuclear translocation post stimulation with TGF-β1 [10ng/ml] or WNT5a [1ug/ml].

Results: Quantitative thresholding displayed an increase in epithelial WNT5a in asthma ($p=0.0085$). Interestingly this was constrained to patients with a Th17 signature endotype ($p=0.0188$). Additionally, we found a significant correlation between TGF-β1 and WNT5a immunostaining in the epithelium ($R^2=0.5818$, $p=<0.0001$). Stimulation of BEAS-2B cell with TGF-β1 increased the expression of

markers of EMT, WNT5a and ROR2 expression. Similarly, both TGF-β1 and WNT5a were shown to induce SMAD2/3 nuclear translocation, which was inhibited by Box-5.

Conclusions: WNT5a protein is increased in the airway epithelium in patients with asthma displaying a mucosal Th17-dependent gene signature. Additionally, we show potential in vitro evidence of TGF-β1-WNT5a cross-talk via the SMAD2/3 axis.

Panayiota Stylianou

The Functional Relevance of Tensin1 in COPD aetiology

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

Background: Chronic obstructive pulmonary disease (COPD) constitutes a major cause of morbidity and mortality. A recent genome wide association study (GWAS) showed significant association of the TNS1 gene (which encodes tensin1) with COPD. A non-synonymous single nucleotide polymorphism (SNP) (W1197R) in the TNS1 gene is associated with airflow obstruction in GWAS.

Aim: To examine the mRNA, protein expression and the prevalence of W1197R of tensin1 in structural cells from healthy subjects and patients with COPD.

Methods and Materials: Lung resections were immunostained for tensin1 protein expression. Cultured human airway smooth muscle cells (ASM) were evaluated for tensin1 expression using qRT-PCR and immunofluorescence. Healthy subjects and patients were genotyped using Restriction Fragment Length Polymorphism (RFLP) in ASM cells.

Results: Immunohistochemical staining on lung resections (n=11) demonstrated increased tensin1 expression in the airway smooth muscle (p=0.0073) and lamina propria (p=0.0121) in COPD donors when compared to healthy controls. Expression in the apical airway epithelium was similar in both groups. A similar level of tensin1 mRNA and protein expression was detected in ASM cells, obtained from COPD donors when compared to healthy controls (n=7). Confocal immunofluorescence staining revealed co-localisation of tensin1 with a-SMA, a protein found on actin microfilaments (Overlap coefficient=0.8). RFLP revealed the presence of W1197R in healthy controls (n=7) but not in COPD donors (n=7) (p=0.0291).

Conclusion: We have showed that tensin1 protein expression is increased in the lamina propria and ASM in COPD airways. In addition, co-localisation of tensin1 with αSMA was shown suggesting the interaction of these two proteins. Preliminary data suggest the W1197R SNP associated with COPD in GWAS is present

predominantly in healthy subjects suggesting that the polymorphism is a protective factor for COPD.

Abdulrahman Alzahrani

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 TNFa-induced production of the different chemokines CCL5, CXCL10 and CXCL8.

Results: Supernatants from 30 minutes 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.

Jamie McCarthy

A role for interleukin-15 in human type 2 innate lymphoid cell function?

Supervisor(s): Prof. David Cousins and Prof. Chris 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+, $c\text{-}kit^{int}$, $KLRG1^{int}$ 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.

Adam Luke Smith

Mouse tracheal airway smooth muscle cells express functional P2X receptors.

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

Asthma is an inflammatory disease that results in airway remodelling and obstruction, and airway smooth muscle (ASM) hyperresponsiveness. The trigger(s) responsible for airway smooth muscle hyperresponsiveness remain(s) unclear. Extracellular nucleotides are known to cause the contraction of different smooth muscles including *vas deferens* and bladder, thereby making them good candidates for causing ASM hyperresponsiveness. In addition, the concentration of extracellular nucleotides is raised in asthmatic airways. The receptors for extracellular nucleotides, named P2 receptors, are composed of 2 subfamilies: the ligand-gated ion channels P2X receptors (7 genes have been cloned: P2X1-P2X7) and the metabotropic P2Y receptors (8 genes identified: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11-14). We hypothesise that functional P2 receptors are expressed in mouse ASM, and contribute to the abnormal contraction of asthmatic airways. This study investigates the presence of P2X receptors in mouse tracheal ASM using real-time PCR, western blot and immunohistochemical approaches. Quantitative

PCR experiments give the P2X receptor transcript expression profile P2X4 >>P2X6 ≈ P2X7 >>P2X5 >>P2X1 ≈ P2X2 ≈ P2X3 (n=4) in mice. In addition, P2X1, P2X4 and P2X7 receptor proteins were detected in this tissue by western blot, and were found to co-localise to the ASM by immunohistochemistry.
In conclusion, mouse tracheal ASM cells express both P2X receptor transcripts and protein (in particular P2X1, P2X4 and P2X7). Preliminary data suggests that these receptors are functional in these cells, and that they play a physiological role in the tissue.

Zahraa Al-isawi

The Potential Protective Effect of C-peptide on Vascular Dysfunction in Diabetes
Supervisor(s): Prof. Nigel Brunskill, Dr. Alan Bevington and Dr. Gary Willars

Introduction: Diabetes (DM) is characterized by long-term microvascular and macrovascular complications. Changes in red blood cell (RBC) functions have been implicated in the development of diabetic vascular complications.
Microparticles (MPs) are fragments from the plasma membrane, its shedding from the RBC membrane is a normal physiological process, however, an increase in its level has been observed in DM. Elevated MP level might play a role in the development of endothelial dysfunction.
C-peptide, the connecting peptide between A and B chains of proinsulin, is a promising agent to ameliorate the DM vascular complication. The aim of this study is to investigate the potential protective effect of C-peptide on MP generation from RBCs exposed to high glucose level.

Methods: Initially, to refine MP isolation technique, induction of MP generation from RBCs was performed by stimulation of purified RBCs from healthy volunteers by Ca^{+2} ionophore. Then, differential centrifugation was used to separate MPs from intact RBCs. Characterization of RBC-derived MPs was performed with RBC marker by western blotting. To mimic the hyperglycemic condition in diabetic patients, RBC suspensions prepared from healthy volunteers were incubated with normal or high glucose. Nanoparticle tracking analysis was used to estimate the particle sizes and concentrations.

Results: Incubation of 45% hematocrit percentage (Hct%) RBCs with high glucose concentration, 25 mM, for 1 hr. to 6 hrs did not show any significant difference compared to those incubated with normal concentration, 5 mM. Increasing the time to 24 hours resulted in a significant increase in particle numbers in samples incubated with 5 mM glucose. In contrast, decreasing the Hct% to 10% showed an

elevation trend in particle numbers at 25 mM compared to that at 5 mM, therefore, optimizing the incubation conditions is still on going.

Conclusion: The observed elevation in particle levels in samples with 45% Hct% and incubated for 24 hours is probably due to energy depletion that resulted from glucose consumption from the medium. However, an i*n-vitro* incubation of RBCs with glucose for a short time might not reflect the effect of long-term hyperglycemia in diabetic patients. Therefore, the future work will be focused on using an animal model to study the effect of DM on MP generation.

Heather MacKinnon

Developing a Self-directed programme to increase health through Physical Activity in chronic Kidney disease (SPARK)

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

Engaging in physical activity (PA) reduces cardio-vascular risk and improves Quality of Life, however the CKD patient population remains inactive. This project will create a self-directed intervention promoting PA for people with CKD not requiring renal replacement, and hopes to produce the positive results seen in other chronic diseases.

The initial intervention comprised a 6-week walking and strength training programme, grounded in the Theory of Planned Behaviour and delivered using Motivational Interviewing, supported by written material and telephone calls.

This nascent design was iteratively reviewed by two expert panels, one PPI session, two patient focus groups (n=10; 5male; mean age= 68years; mean eGFR=42.4mL/min/1.73m^2). Group sessions were digitally recorded, professionally transcribed and analysed in NVivo. Transcripts were subject to deductive thematic analysis, identifying themes at a semantic level.

Initial feasibility testing was conducted (n=8, 6male; mean age=66 years, mean eGFR=38mL/min/1.73m^2). Participants attended 4sessions (outcome measures pre/post intervention; Motivational Interviewing session; feedback interview) and were encouraged to engage in self-monitoring using pedometers and activity diaries.

Intervention updates included: extending intervention from 6 to 8 weeks, adding pedometer use and reformatting educational material.

Initial testing demonstrated:

- good recruitment rates (8/14(57%) consented)
- excellent retention rates (100% completion/attendance).

Engagement was measured via PA diaries completion:

- Diary completion varied from 10%-100% (mean=83%)
- walks completed on intervention days 9%-73%(mean=41%)
- strength training sessions completed 0%-94%(mean=30%)

This theory-based intervention has been modified based on Expert and Patient feedback and the feasibility data is favourable. Potential limitations include inaccurate diary-keeping and small sample size, however encouraging trends are seen.

Ruth Hartley

Title: TBC

Supervisor(s):Prof. Christopher Brightling and Dr Salman Siddiqui

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