Preface

The Department of Infection, Immunity and Inflammation would like to welcome you to the Seventh 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, 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 Russell Wallis, Professor David Cousins for kindly giving the keynote lectures.

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:
Eleana Hadjisolomou, Emma Whittle, and Sonia Suleman,

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Abstracts

Day 1, 13th April

Hanan Alrashidi

New aspects of control of complement activation
Supervisor(s): Prof. Robert B. Sim and Prof. Wilhelm Schwaeble

The complement system in human blood represents a major component of innate immunity. The role of the complement system is to recognise foreign materials in contact with the blood. These materials can be microorganisms, synthetic particles, or damaged and altered self-components, such as apoptotic and necrotic cells. Complement can be activated by three different main pathways, the classical, alternative and lectin pathways. The classical pathway activation is achieved through the binding of the protein C1q to targets and it can be controlled by inhibitors such as C1INH. Factor H is well-known as an inhibitor of the alternative pathway but since it can bind to many of the same ligands as C1q it might compete with C1q and therefore is involved in classical pathway control. Different target molecules, which activate the classical pathway, show variable binding to both of these complement proteins. Since the concentration of C1q and Factor H can vary widely between individuals, the C1q: FH ratio may influence the degree of classical pathway activation in response to different targets.

Ahmed Abdullah Ahmed

Assessment of the role of the lectin pathway of complement activation in the pathophysiology of thrombosis using experimental models of inflammatory diseases
Supervisor(s): Prof. Wilhelm Schwaeble

DIC, septic shock, haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) all have in common that they cause serious diseases due to inflammation related coagulopathy in association with complements activation.
Wild type mice C57BL/6 were used in all HUS mouse model experiments and in FITC-photo illumination thrombosis induction. ADAMTS-13 knockout mice were used in the TTP mouse model experiments. In HUS model all mice were injected

with shigatoxin2 and Lipopolysaccharide with anti-MASP-2 antibodies, anti-properdin antibodies or control non-blocking antibodies. In TTP mouse model VWF and LPS from *E.coli* O111:B4 with monoclonal inhibitory anti-MASP2 antibodies, iso control antibodies or saline were given ip to the mice. Intravital microscopy with FITC photo illumination were used to induce thrombosis in the capillary blood vessels of the cremaster muscles of C57BL/6 male mice after injecting them with the inhibitory monoclonal anti-MASP2 antibodies or iso control antibodies.

There is no significant difference in the survival among all tested mouse groups used in HUS model. Treatment of mice with monoclonal anti-MASP-2 antibodies prolonged the time required for blood vessel to be completely occluded and shows that MASP -2 inhibition provides a significant therapeutic degree of MASP-2 dependent protection from thrombotic pathologies. ADAMTS 13-Knockout mice given VWF, LPS with the inhibitory anti-MASP2 antibodies preserved higher number of platelet counts than the control group.

Absence of Shigatoxin receptors in murine endothelial cells makes the phenotype of HUS in rodents different from human therefore, blocking of complements has no significant importance in the cure of mice injected with stx2 and LPS and the mice are likely to die from tubular necrosis or neurotoxicity rather than glomerular thrombosis. Induction of thrombosis in Cremaster blood vessels or platelets adhesions during TTP induces endothelial cell death and activation of thrombosis. Complement system specially the lectin pathway has interaction with coagulation system and may initiate a viscous cycle. Therefore, inhibition of the lectin pathway may interrupt this cycle. Therapeutic blockade of the complement system by monoclonal anti- C5 antibodies (Eculizumab) has recently been shown to provide an effective therapeutic approach in the treatment of HUS in human. This encouraged testing of more complements blocking antibodies to assess their ability to treat such thrombotic events.

Youssef Alaofi

The impact of the classical, lectin and alternative pathways of complement activation on protective immunity against Streptococcus pneumoniae infection following vaccination with established pneumococcal vaccines.

Supervisor(s): Prof. Wilhelm Schwaeble and Prof. Peter Andrew

The complement system plays a major role in the immune response to *Streptococcus pneumoniae* infections. My work aims to determine the impact of the classical, the lectin and the alternative pathways of complement activation the immunity following vaccination with pneumococcal polysaccharide vaccines. I am

studying the differences in the immune response to pneumococcal vaccines, which are PneumovaxII (pneumococcal polysaccharide vaccine), Prevenar13 (pneumococcal polysaccharide conjugate vaccine) and the carrier protein of Prevenar13 (CRM_{197}). I am comparing these results with those achieved using CRM_{197} which elicits an immune response of a different kind than that achieved with pneumococcal polysaccharide vaccines. In addition to the immunisation route, WT mice, C1q-/- and MASP-2-/- mice were immunised subcutaneously (s.c. / i.p.) with 20µg of CRM_{197} or with 1µg of PneumovaxII. These experiments aimed to insure and compare the antibody response to CRM_{197} and to PneumovaxII in WT mice and complement deficient mice deficient of the components, $C1q^{-/-}$ and $MASP\text{-}2^{-/-}$ mice. When immunised with single or 3 spaced doses of CRM_{197} or PneumovaxII all mice generally responded with high antibody titres for this vaccine protein compound (CRM_{197}) and for PneumovaxII. The results indicate that strong increase in the antibody response to CRM_{197}, occurs immediately after third immunisation (at day 21). However, the strong antibody response to PneumovaxII, occurs immediately after first immunisation. The result also suggests that the antibody response to CRM_{197} does not involve C1q (the classical pathway) or MASP-2 (the lectin pathway). However, the antibody response to PneumovaxII involved C1q (the classical pathway).

Ibtihal Al-karaawi

The role of CL-11, a novel recognition component of the lectin activation pathway of complement in pneumococcal infection

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

The Lectin activation pathway of complement is initiated by lectin pathway specific recognition molecules MBL, ficolins, and CL-11. These recognition subcomponents recognize the wide range of carbohydrates on microbial surfaces and activate the complement system via the lectin pathway effector enzyme MASP-2. The key role of lectin pathway activation to fight S. pneumoniae has been demonstrated by Prof. Schwaeble's team using a mouse line deficient of the lectin pathway effector enzyme MASP-2 and in mice deficient of the lectin pathway recognition component Ficolin A. CL- 11, an only recently discovered recognition subcomponent of the lectin pathway was shown to have a role in the activation of the lectin pathway by binding to bacteria, fungi and the influenza A virus. My work aims to define the role of CL-11 in the immune defense lectin pathway against S. pneumoniae. In vitro studies was indicated that CL-11 can activate the lectin pathway of complement on the surface of S. pneumoniae. So far, I compared the susceptibility of CL-11 deficient mice with that of sex, age and strain mutated wildtype control which

revealed a dramatically high mortality (80% CL-11 -/- and 20% wild type) in CL-11 deficient mice. This work identified CL-11 to be a critical component of the immune response to S. pneumoniae infection.

Taiwo Banjo

Biology, adhesion & molecular characterisation of *acanthamoeba castellanii*: the role of mannose binding protein

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

Acanthamoeba castellanii is a ubiquitous, opportunistic, free-living-amoeba (FLA) causing *Acanthamoeba keratitis* (AK), an aggravating-vision-threatening clinical infection of the cornea. Attachment to cell surfaces is mediated by *Acanthamoeba* Mannose-Binding Protein (MBP), thereby inducing the secretion of proteases essential to invade/degrade hosts' tissue. The project aims to characterise different life forms of *Acanthamoeba* and investigate their modes-of-binding to contact lenses, human epithelial cells; molecular characterisation of *Acanthamoeba* MBP and ultimately to develop therapeutic-leads designed to prevent host tissues infection.

Conditions for generating different life stages of *Acanthamoeba* were examined and confirmed by cell culture & microscopy (phase-contrast/florescence). Adhesion and cytopathic assays of different life forms of *Acanthamoeba* were carried out using various surfaces. The inhibitory effects of mannose on *Acanthamoeba* adhesion/cytopathology were also investigated.

Currently, I have isolated three di**f**ferent life stages of *Acanthamoeba castellanii* namely the trophozoites (infective form), mature cysts (dormant form) and a recently discovered form called protocysts. The rapid, reversible differentiation of protocysts to trophozoites circumvents the mature cysts. All three forms bind differently to various surfaces (contact lenses, micro-titre plates and Hep2/Vero epithelial cell lines) in the order: trophozoites >mature cysts >protocysts. Cytopathic assays on epithelial cells showed that protocysts rapidly destroy the monolayers via swift differentiation into the infective trophozoites.

Future work would be directed at studying MBP; aiming to develop inhibitors as potential therapeutic-leads to block adhesion and/or to trap the *Acanthamoeba* protocyst form, which is more likely susceptible to drugs than the mature cysts that are resistant to all existing treatments.

Key words: *Acanthamoeba*, protocysts, adhesion, cytopathology, mannose-binding, affinity chromatography.

Bayan H A Faraj

Characterisation of the interactions of the *Streptococcus pneumoniae* toxin, Ply, with soluble molecules of the immune system

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

Streptococcus pneumonia (pneumococcus) is an opportunistic Gram positive pathogenic bacterium that causes many diseases, including pneumonia, meningitis and septicaemia. All clinical isolates produce a toxin called pneumolysin (Ply) that is a key virulence factor of the bacterium. Ply kills host cells by forming pores in cholesterol-containing membranes and also activates part of the host's immune system called the complement cascade. Both activities are necessary for the full impact of the toxin, *in vivo*. Complement activation normally protects the host, however, activation on Ply subverts the immune system, making the host more vulnerable to the pneumococcus. This study focuses on the interactions between Ply and soluble molecules of the immune system that have been identified as potential binding partners, specifically L-ficolin, a component of the lectin pathway of complement activation and IgGs, which initiate the classical pathway. I will present the results of experiments aimed at investigating the binding between recombinant Ply and full length human L-ficolin and with human antibodies (whole IgG as well as monoclonal IgG isotypes). Analysis using protein fragments has been used to identify the binding sites on interacting components. The results shed new light on how Ply interacts with the host's immune system to activate complement.

Ramiar Kamal Kheder

The role of properdin in steatohepatitis (non-alcoholic fatty liver disease), a pre cancer disease.

Supervisor(s): Dr Cordula Stover, Dr Michael Browning, Dr Wen Chung

A histopathological study of non-alcoholic steatohepatitis (NASH) liver specimen showed myeloperoxidase positive neutrophils around steatotic hepatocytes and colocalised properdin and C3c, contrasting very clearly with healthy liver. C3 activation fragments had previously been detected in histopathological analysis of non-alcoholic fatty liver disease (NAFLD), a pre-form of NASH. This study investigates the role of properdin in diet induced obesity and liver disease by comparing properdin deficient and wild type mice on a $LDLR^{-/-}$ background (prone to develop metabolic syndrome). A Western diet formulation led to the

development steatosis (10-12 weeks) in the experimental animals with variation in severity and variable inflammation. Initial results from histology, Immunohistochemistry, glucose measurement and Oil O red staining will be presented. The role of properdin in the increase body weight, fat pad weight is under investigation. Further diets (high fat high sugar) are planned.

Izzat Al-Rayahi

The Role of Properdin in Tumour Development and Cell Recruitment.
Supervisor(s): Dr Cordula Stover, Dr Mike Browning and Dr Lee Machado

Properdin, as the only positive regulator, amplifies complement activation and has been implicated in the tumour response in human lymphoma and carcinoma. This project investigates the role of properdin in a syngeneic tumour model using properdin deficient and properdin wild type mice. A melanoma cell line (B16F10) was used, and cells were implanted in the flank to produce tumourous growth within 15 days. Tumour weight was recorded and the cell response in the experimental animals analysed and compared to naïve mice where appropriate. Tumour cells, bone marrow cells and splenocytes were analysed for percentage of T-regulatory, MDSCs ($CD45^+$, C5aR, $CD8^+$, $CD4^+$) and intracellular presence of immunoregulatory cytokines IL-10, TGF-β and IL-17. There was a tendency for smaller tumour weight in properdin deficient tumour bearing mice. There were fewer MDSCs (negative regulators of immune response) in tumour tissue of LDLR-/- properdin deficient mice. The percentage of T-regs in tumour was higher in properdin deficient compared to wildtype mice. This implies a marginal role only for properdin in determining the tumour size and cell recruitment in the wildtype experimental mice. Lastly, bioluminescence imaging in combination with a luciferase expressing variant of B16F10 was used to investigate the growth of tumour over time for both genotypes.

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 Rggs role 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 galactose, 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 galactose. In addition, Rgg mutants were attenuated in virulence in an experimental murine infection model.

Fayez Abdullah I Alghofaili

***Streptococcus pneumoniae*-Stress Hormones Interactions**
Supervisor(s): Dr Hasan Yesilkaya and Dr Primrose Freestone

Streptococcus pneumoniae remains one of the most important human bacterial pathogens causing wide range of mild to life-threating diseases. It is also a commensal microorganism colonising up to 40% of people depending on age. Fundamental aspects of its ability of transition from colonising to infecting state as well as bacterial-host interactions remain poorly understood. In the field of microbial endocrinology, it has been well established (mainly in Gram-negative bacteria) that stress hormones, such as epinephrine, dopamine and norepinephrine, and their derived inotropes play an essential role in determining the outcome of bacterial infections. Recent evidence has found that pneumococci are highly inotrope responsive. Therapeutic levels of norepinephrine stimulate pneumococcal growth and markedly alter expression of genes involved in metabolism and virulence. However, it is not known how the pneumococci sense, process and respond to inotrope signals. This project aims to elucidate the mechanism by which these hormones increase pneumococcal growth and identify the genetic cascade responsible for recognition and processing of inotrope signals, including the receptors involved. It, also, aims to evaluate the role of stress hormones in the pneumococcal transition from colonisation to invasive mode. Catecholamines growth stimulating effect on type 2 D39 strain has been shown and all 13 known pneumococcal two-component systems have been successfully mutated and are ready to be tested along with the wild type in the presence and absence of stress hormones.

Anfal Shakir Motib

Functional Characterisation of PlcR Regulators in Streptococcus *pneumoniae*.

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

Streptococcus pneumoniae is a major cause of mortality and morbidity around the world; it causes several serious invasive infections. Virulence factors that lead to increase the pathogenicity of this bacterium are regulated in response to different environmental stimuli.

Transcriptional regulators have a major impact on pneumococcal adaptation to its environmental. One of them is PlcR. This research has been devised to identify the role of PlcR in pneumococci. In order to do this, site direct mutation was used for deletion of two PlcR genes, SPD_1745 and SPD_1786, in *S. pneumoniae* D39 by splicing overlap extension method. Moreover, double SPD_1745 and SPD_1786 mutant was also made. Then PlcR deficient strains were tested *in vitro* and *in vivo.*

In vivo analysis of PlcR mutants showed that SPD_1745 is essential for pneumococcal virulence as the absence of this gene lead to total loss of virulence. All the mice infected intranasaly with either SPD_1745 or SPD_1745- SPD_1786 mutant survived the infection and no bacteremia could be detected, while wild type infected group died within 48 hours, moreover, no difference in virulence between the wild type and SPD_1786 could be seen. *In vitro* analysis showed that under microaerobic conditions, the growth profile of pneumococcal strains that were grown on glucose and galactose showed a significant difference compared to the wild type D39, although all strains displayed similar growth profile in BHI. Moreover, some enzymatic activities were tested to study the role of PlcR in virulence and they appeared there was significant difference between the mutants and wild type in neuraminidase, Hyaluronidase and β-galactosidase activity. Therefore, the available results so far show that PlcR encoded by SPD_1745 plays a major role in pneumococcal virulence, and studies are underway to determine the mechanism of SPD_1745 contribution to virulence.

Hasan Faisal Hussein Kahya

Studies on Pneumococcal Esterases

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

The genome of pneumococcal strains contains 4 putative esterase genes (SPD_0534 (*estA*), SPD_0932, SPD_1239, and SPD_1506 (*axe*)). The lipolytic enzymes coded by

these genes have been reported to be important for bacterial physiology and virulence in other microorganisms but their role in *S. pneumoniae* is not known. Therefore, the objective of this study was to determine esterases' role in pneumococcal biology by testing isogenic mutants and recombinant esterases in microbiological, biochemical and *in vivo* assays.

The results showed that the highest level of pneumococcal esterase activity could be obtained with p-Nitrophenyl acetate (pNPA), indicating that the pneumococcus has esterase specific for short acyl chains. All mutants displayed significantly less esterase activity than the parental strain, and EstA was found to be responsible for main esterase activity. The purified recombinant EstA and Axe had optimal activity against pNPA as a synthetic substrate compared to other p-nitrophenyl esters. In addition, EstA and Axe activity against tributyrin (triglyceride) and acetylated xylan revealed that EstA, but not Axe, can catalyse tributyrin, and both of enzymes could use acetylated xylan as substrate. It has been found in this study that EstA and Axe can catalyse Bovine Sub-maxillary Mucin (BSM), which is an organic highly acetylated substrate, and the acetate release increases in a time dependent manner. We also found that pre-treatment of BSM by EstA or Axe increases sialic acid release by neuraminidase. Esterases' role in potentiation of neuraminidase activity was demonstrated further by testing double esterase-neuraminidase mutants (*ΔestAnanA* and *ΔaxenanA*) in medium containing BSM as the sole carbon source. The replacement of S^{121} in EstA and S^{181} in Axe to alanine deactivated the catalytic activity of esterases. Furthermore, it was revealed that *ΔestAnanA* showed a significant attenuation in growth compared to *ΔnanA* in this medium but no difference in growth could be seen between *ΔaxenanA* and *ΔnanA*. In addition the mutation of *estA* alone or in combination with *nanA* reduced the pneumococcal colonisation and virulence significantly after intranasal infection. qRT-PCR results showed that the expression level of *estA* and *axe* were significantly up regulated when exposed to BSM.

Zaaima Al-Jabri

Investigation of integrase genes in *Acinetobacter* and characterization of novel resistance islands in *Acinetobacter baumannii*

Supervisor(s): Prof. Marco Oggioni and Dr Kumar Rajakumar

Acinetobacter baumannii is an opportunistic pathogen causing a wide range of hospital-associated infections. In the last few years, it has developed multi-drug resistance to broad spectrum antibiotics. The genomic plasticity of *A. baumannii* has significantly contributed to its capability of acquiring new resistance

determinants. This study aims to characterize two different resistance islands in two distinct loci; G8 and G62, and explore the structure and function of integrase genes (*int*) that are vital for integration and excision of the foreign DNA into the bacterial chromosome. In addition, a variety of bioinformatics tools have been applied to analyze the structure of integrase proteins at DNA as well as a protein level including InterPro and Swiss Model. The analysis shows that these integrase genes are highly similar in different *A. baumannii* strains in terms of structure. To further facilitate our understanding of the possible functions of the integrase genes, cloning of the integrase genes into pWSK129 plasmid was performed and the activity of the integrase is analyzed in both *E. coli* and *A. baumannii* backgrounds, under its native and an inducible promoter. The activity of the integrase is detected through a PCR assay and further qPCR assay allowed relative quantification between different conditions and backgrounds. These results support the significance of *int* genes in resistance islands mobility. Further molecular approaches such as allelic exchange are being implemented to investigate the role of these genes in the knock out mutants of *int* genes and their genomic islands.

Mohammed Al Madadha

A Study of the Role of Genomic Islands in Steering *Klebsiella* towards New Pathogenicity Niches and their Relationship with the Fic Domain.

Supervisor(s): Dr Edouard Galyov and Dr Kumar Rajakumar

Klebsiella pneumoniae is one of the major causes of mortality and morbidity in the hospital setting, its impact on the human health is further highlighted in its ability to cause a multitude of infections in various tissue types pointing to its high virulence and adaptation mechanisms. It has gained its fame as the major cause of Carbapenem Resistant *Enterobacteriaceae* (CRE) infections worldwide.

Along with its arsenal of known virulence factors, *Klebsiella' s* genome is quickly being recognized for its plasticity by acquiring many its virulent factors through horizontal gene transfer (HGT) including fitness genes, pathogenesis genes, antimicrobial resistance genes and others.

Genomic islands is one of those mechanisms of HGT that is being shown to have been underestimated for contributing for genome plasticity, recent reports have shown that these islands may have a role for niche adaptation, aiding this pathogen to differentiate itself to thrive in various niches.

The Fido family of protein, those that contain the conserved Fic motif (HPFXXGN[G/K]) is a prevalent virulence factor employed by many bacterial pathogens. Its role is being increasingly recognized by their effect on the regulation of the eukaryotic cells' cytoskeletal system and other regulatory mechanisms where bacterial pathogens are employing this mechanism to circumvent host's defences.

This study is aimed to describe the Fido family of proteins in *Klebsiella* by identifying and categorizing these proteins, and study their role in the pathogenesis and disease progression and the relationship of these proteins and HGT.

Ros Abdul Aziz

A hospital-based matched case-control study to identify clinical outcome and risk factors associated with *Klebsiella pneumoniae* blood stream infection in Leicestershire.

Supervisor(s): Dr Ed Galyov and Dr Kumar Rajakumar

Klebsiella pneumoniae (*Kp*) is a bacterial pathogen of worldwide importance and a significant contributor to multiple disease presentations associated with both nosocomial and community acquired disease. The cases are increasing and few effective antibiotics are currently available to treat patients. Although *Kp* is the second most common cause of Gram-negative bloodstream infections (BSI), its epidemiology has not been defined in a nonselected population. To our knowledge, no study has specifically examined risk factors for K. pneumoniae infections or its impact on mortality in Leicestershire. We sought to describe the epidemiology and clinical outcomes associated with K. pneumoniae and to determine the antimicrobial resistance and virulence mechanisms. Risk factors associated with *K. pneumoniae* infections were investigated by a matched case-control study for the period of July 2011 to October 2012 at UHL NHS Trust and cases did not cluster in time and space. A cohort study was also performed to evaluate the association between *Kp* BSI and in-hospital mortality. Clinical data were obtained from medical records and variables were analysed as risk factors. Accumulation of selected antibiotic resistant genes and virulence properties of *Kp* isolates were analyzed by PCR and DNA sequencing. Genetic relatedness of the isolates were evaluated by MLST, cps typing and PFGE. We observed decreased antibiotic susceptibility among Kp isolated from the patients that showed phenotypes compatible with certain enzyme production. Molecular epidemiology revealed that isolates belonged to several distinct clones, although one clonal complex was predominant. Most cases could not be linked to a specific patient-to-patient transmission event or to a common source.

David Ngmenterebo

Type Six Secretion Systems, T6SSs, "*the spring loaded nano-daggar*" mediate virulence in pathogenic *Klebsiella pneumonia*

Supervisor(s): Dr Kumar Rajakumar and Dr Yassine Amrani

Type Six Secretions systems is the most recent secretion systems discovered in well 25 % Gram negative bacteria. T6SS is often considered as a reminiscent of T-phage due the high protein and structural homology between both. It is a "spring-loaded nano-daggar" that has been implicated in the virulence of many proteobacteria though its role is yet to be explored in some opportunistic pathogens such as *Klebsiella pneumoniae*, the second most common pathogen involved in nocomial infection.

It is therefore crucial to examine the virulent role of T6SSs in *K. pneumoniae* considering that fact that its significant contributor of pneumonia-related and urinary tract infections. We explored the virulent role of the identified putative T6SS gene clusters in pathogenic *K. pneumoniae* HS11286, a multi-drug resistant strain and *K. pneumonaie* NTUH-K2044, hypermucoid strain. Together with existing data, we used genomic *in silico* mining techniques to identify and define the boundaries T6SS clusters, thus facilitating isogenic mutants generation. Several *in vitro* and *in vivo* phenotypic assays/models were employed to measure T6ss-mediated virulence in *K. pneumoniae*.

Here we report T6SS-dependent antagonistic behaviour of *K. pneumoniae* in both intra- and inter-species competition. T6SSs play a significant impact on *K. pneumoniae* biofilm formation and DNA mobilization. T6SS promotes phagocytic resistance and intracellular multiplication towards J774 but internalization of A549 cells. A *Galleria mellonella* model revealed that, the survival of the larvae and recovery of *K. pneumoniae* post-inoculation was T6SS dependent. All these data together suggest that T6SS plays a role in the virulence of pathogenic *K. pneumonia*

Robeena Farzand

Distribution and roles of integrative and conjugative elements (ICEs) in *Klebsiella pneumonia*

Supervisor(s): Dr Kumar Rajakumar & Prof. Mike Barer

Background: Integrative and conjugative elements (ICEs), a subset of genomic islands, are self-transmissible MGEs (mobile genetic elements) that comprise (i) ICE integration and excision module, (ii) ICE conjugation module, (iii) ICE regulation module and (iv, optional) accessory genes module. These mobile DNA fragment can be transferred to new host by the process of conjugation. This project is designed

to explore the distributions, roles and mobilization associated properties of ICEs in different strains of *K. pneumoniae*. Because the genomes of *K. pneumoniae* are highly variable and have been found to harbour many strain specific genomic islands.

Methods: For distribution and structural analysis of ICEs, *tR*IP PCR and PCR mapping strategies were used, respectively. For mating pair formation and DNA mobilization of ICE*Kp*1, different versions of plasmid pACYC184-oriT (*oriT* was amplified from ICE*Kp*1 of KR1730) were constructed. Various knockouts were done by using lambda red recombination system.

Results: In this study, It was found that 3 out of 22 strains of *Klebsiella pneumoniae* (from LRI Hospital) were positive for ICE*Kp*1 like MGE. The conjugation results confirmed that frequency of DNA mobilization of *Kp* HS1186 strain is higher than reported previously. Results observed regarding self-transmissibility of ICE1 (*Kp* HS11286) suggest, that further study of ICE1 transfer will provide an important body of knowledge related to spread of virulence factors.

Discussion: Conspicuously, our preliminary data regarding mobilization of ICEs and co mobilization of plasmid seems to be promising, and needs to be repeated for statistical significance.

Hastyar Najmuldeen

Superoxide Dismutase Activity is Important for Oxidative Survival, Biofilm Formation of *Klebsiella pneumonia*

Supervisor(s): Dr Hasan Yesilkaya

Bachground: *Klebsiella pneumoniae* is the causative agent of several nosocomial and community acquired infections. Some of its virulence determinants have been identified but it is not known how it copes with damaging effects of reactive oxygen species. Superoxide dismutase (SOD) is responsible for removal of toxic superoxide radicals, and *K. pneumoniae* genome contains three superoxide dismutase genes coding for Mn-, Fe- and CuZn- co-factored SODs, *sodA*, *sodB*, and *sodC*, respectively. This work is designed to evaluate inducibility of *sod* genes during oxidative stress, and the importance of each SOD in oxidative survival and biofilm formation of *K. pneumoniae*.

Materials and Methods: Lambda Red system and Flp-recombinase mediated excision were used to construct markerless isogenic *sod* mutants. Single, double, and triple SOD mutants were characterized phenotypically through growth studies in oxygenated environment, by biofilm formation assay using polystyrene microtitre

plates, and by microscopy.

Results: Phenotypic characterization of single, double and triple isogenic SOD deficient mutants indicated that *sodB* alone or in combination with *sodA*, have a major impact on total SOD activity, and growth in oxygenated environment. It was also found that *sodA* and *sodC* are inducible whereas *sodB* is constitutively expressed. Deletion of Cu/ZnSOD and FeSOD genes in single and double mutants are increased biofilm formation. Finally, mutation of *sodA,* but not *sodB* and *sodC,* has significantly reduced colony size.

Conclusions: Our results demonstrate that all *sod* genes contribute to total SOD activity in *K. pneumoniae,* and SOD is important for the microbe's survival in oxidizing environment. We also demonstrated that SOD activity is important for biofilm formation, which is linked to *K. pneumoniae* virulence. Further work is underway to demonstrate SOD's role in *K. pneumoniae* infection.

Ali Abdulkareem Ali

Isolation and characterization of *Clostridium perfringens* bacteriophage and design of improved Second generation *Clostridium difficile* Bacteriophage
Supervisor(s): Prof. Martha Clokie

Clostridium difficile (C. difficile) and *Clostridium perfringens (C. perfringens)* represent a health and economic burden as they cause diseases for humans and farm animals. Huge economic loss is incurred as a result. Little research has been carried out on the development of the phage therapy for both pathogens. This project aims to isolate, characterise and examine the feasibility of using *C. perfringens* bacteriophages or their gene products for the treatment and control of *C. perfringens* associated diseases. It also aims to genetically improve existing *C. difficile* phages and phages of *C. perfringens* that will be isolated, using site-directed mutagenesis to excise/replace undesired gene/s and potentially add useful one. To isolate *C. perfringens* phages 20 faeces and soil samples were collected; 18 *C. perfringens* strains were isolated and additional 9 *C. perfringens* strains were screened. All were confirmed to be *C. perfringens* by specific 16-23S rDNA amplification. These strains were induced using MitomycinC and Norofloxacin to isolate temperate phages and enriched to isolate free phages. Seven bacteriophages were isolated; Clonal stock for three phages prepared and four were unstable and could not be purified. Treatment of *C. difficile* with Sodium-dodecyl sulphate and mutanolysin was performed to prepare cells for electroporation. Down-stream, up-stream flanking regions and a tetracycline cassette were amplified to prepare insert for homologous recombination.

Saroa Rashid

Isolation and characterization of *Clostridium difficile* and the bacteriophages from the environment
Supervisor(s): Prof. Martha Clokie

Clostridium difficile is a gut pathogen that is able to cause prolonged hospital associated diarrheal infection, usually after antibiotic treatment. Little is known about *C. difficile* strains from outside Europe, the USA and Australia. Thus, in order to increase our knowledge of the biogeography and diversity of these strains, 60 strains were isolated from soil and sediment samples which collected from different

geographical areas in Kurdistan region of-Iraq. These strains were subjected to PCR ribotyping and, diverse ribotypes were detected including common types found in the UK (001, 078 and ribotype 035) and others which are very rare the in UK (091 and 604). Furthermore, additional strains were found that are distinct from previously described types, suggesting they are novel strains. Multi Locus Sequence Typing (MLST) analysis performed from sequenced genome of 14 strains representing diverse sequence types. The increasing emergence of antimicrobial resistance strains of *C. difficile* has led to demand for novel antimicrobial agents. Although our lab has a collection of phages they are limited in their ability to infect key ribotypes of *C. difficile* including the problematic ribotype 078. 16 new phages were isolated from the same region to determine if *C. diffcile* phages isolated from this area could infect ribotype 078. Host range results showed that the phages have wide host ranges and they could be considered as generalist phages, due to wide host ranges both within the bacterial community from which they were isolated. Phages have wide host range and are able to infect most clinically relevant ribotypes in UK including 078. The new phages has sequenced and characterized.

Malgorzata Wegrzyn

Is strain variation significant in the response to conditions applied to induce the sputum phenotypes of *Mycobacterium tuberculosis*?

Supervisor(s): Prof. Michael Barer

Tuberculosis (TB) is one of the oldest human diseases and remains in our populations causing high mortality rates. Sputum provides a source of bacterial population ready for transmission to a new host and therefore must be targeted by antibiotic treatment. Transcriptome analysis of bacilli expectorated in sputum identified a slow or non-replicating growth pattern with lack of aerobic respiration. The cytological analyses revealed mycobacterial cells rich in lipid bodies (LB). LBs are rarely observed in rapidly growing cells under laboratory conditions, suggesting that LB formation might play a role in survival and transmission adaptation.

Several models have been developed to replicate conditions, which *Mycobacterium tuberculosis (Mtb)* encounters *in vivo*. Two of these have been applied in this study to induce a sputum phenotype of *Mtb*. Following earlier work in our laboratory, it was hypothesised that difficulties to replicate the reported gene expression pattern could be due to interstrain variation. The extensively studied H37Rv strain has been passaged for many years outside the host in laboratory conditions. It was concluded

that it may not be a suitable representative of sputum isolates. Thus, recently isolated clinical strains such as Beijing and CH were included.

Initial work focussed on validation of a suitable normalisation procedure for quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) transcriptional profiles. The data normalised to *SigA* was then compared with the sputum transcriptome. The results confirmed distinct gene expression patterns between strains and also suggested that none of the studied strains may fully replicate the previously observed sputum transcriptome.

Mutaib Mashraqi

Assessment of the role of haptoglobin (HP) as a novel opsonin in the immune system against *Staphylococcus aureus*

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

Haptoglobin is an acute-phase plasma protein. It is a marker for many inflammatory-related diseases. Its best known function is to eliminate haemoglobin from plasma via phagocytosis and to prevent loss of iron through the kidneys, thereby protecting the kidneys from damage and sequestering iron. It was also shown in an *in vitro* study that haptoglobin binds lipoteichoic acid (LTA) of *Staphylococcus aureus*. Therefore, this project will characterise this interaction as well as assess the role of haptoglobin in the immune defence against *S. aureus* using mouse models.

Wafaa Khalaf

In vitro generation of 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 one of the most serious life-threatening haematological malignancies, which is incurable by conventional therapies such as chemotherapy and radiotherapy. Following Stem cell transplantation 44% of patients entered complete remission. Adoptive T cell immunotherapy is an alternative approach for treatment of this disease.

Production of autologous myeloma antigen specific cytotoxic T lymphocytes (MASCTL) may be the way to increase the time to relapse of myeloma patients. These cells can be produced by stimulation of peripheral blood lymphocytes (PBMCs) from healthy volunteers and MM patients with hybrid cell lines (made by

fusion of professional antigen presenting cell (APC) HMy2 (EBV B-lymphoblastoid cell line) and different myeloma cells).

Flow cytometric analysis for APC markers, including MHC (class I and class II) and co-stimulatory molecules such as CD80 and CD86 was carried out on the hybrid cell lines. CFSE staining and flow cytometric analysis was used to demonstrate allogeneic stimulation of T cells in vitro. Also, reverse transcription PCR and real time PCR have been used for detection of the antigen profile of the hybrid cell lines, and the level of protein expression of these antigens by the used hybrid cell lines have been also determined. Selection of some of the myeloma antigens was done (depending on their expression level by the hybrids and their ratio in MM) for using to examine the specificity of MASCTL.

Future work will focus on the ability of the hybrid cell lines to stimulate MASCTL, using the identified myeloma antigens expressed by the hybrid cell lines, as a novel possible strategy for treatment of MM.

Samy Alghadban

Role of complement activation in chronic kidney disease

Supervisor(s): Prof. Nigel Brunskill, Prof. Wilhelm Schwaeble

Introduction: Renal fibrosis and inflammation are prominent features of late stage kidney disease. It is evidenced that the activation of the complement in mice increased renal fibrosis in a model of tubulointerstitial injury (Fearna *et al.*, 2011). However, particular complement components involved are not yet well defined. Current study aims to define the complement pathway(s) involved in the injury in a chronic kidney disease model.

Methods: 8 weeks old WT and MASP-2 deficient mice were surgically nephrectomised unilaterally then allowed seven days to recover. To induce protein overload nephropathy, mice were given i.p. doses of low endotoxin bovine serum albumin diluted in saline on daily basis. Doses concentration were increased incrementally from 2 mg/gm body weight in the first dose to 15 mg/gm body weight in the seventh dose and continued at this concentration to 15 doses total. Kidneys were collected at the end of the experiment. Formalin fixed, paraffine embedded, 5 micron kidney sections from each mouse were deparaffinised and stained with F4/80 anti-macrophage antibody, TGFβ, TNFα and TUNEL assays. 30 Images from each section were analysed using computer-based image analysis software to compare between groups.

Results: Computer-based analysis of sections showed a significant reduction in macrophage infiltration in MASP-2 deficient mice compared to wild type (P value = 0.0345). Similarly, TGFβ and TNFα staining were significantly reduced (P values = 0.0303 and 0.026 respectively). TUNEL assay also showed highly significant reduction in apoptotic cells populations in MASP-2 deficient mice compared to wild type (P=0.0001).

Discussion: Absence of the lectin pathway functional activity in mice might confer protection against renal scaring in proteinuric kidney disease model.

Dalia Alammari

Can Myeloma light chain activate kidney proximal tubular cells to become pro-inflammatory cells?

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

Introduction: Multiple Myeloma (MM) is a cancer of plasma cells and leads to excessive presence of free light chain (FLC) in blood. The over production of FLC and the associated light chain proteinuria often cause renal failure. Renal failure occurs as a result of decreased renal function or as direct toxic effect on the proximal tubular cells (PTCs). The excess of FLC causes PTCs to increase their endocytosis via megalin and this might trigger inflammation detrimental to the kidney.

Purpose: The project pursues the hypothesis that FLC overload-mediated renal toxicity comes from the production of inflammatory cytokines and H_2O_2. In addition, the complement system may become activated via the alternative pathway.

Method: Monoclonal LC was isolated and purified from the urine of a myeloma patient. HK2 cells, which are human proximal tubular epithelium cells (PTECs), were stimulated with the FLC for up to 72h, when cell viability is not compromised. Various cytokines and H_2O_2 production were determined in the supernatants by ELISA and enzymatic test, protein level by western blot and mRNA level by RT-PCR.

Results: Exposing the PTECs to a relevant pathological FLC concentration for prolonged time has a toxic effect and leads to damage of the cells.In addition; the results show up regulation in the production of different cytokines and chemokines such as IL6, IL8, and MCP-1. In addition, there is an increase in the H_2O_2 production and complement C3component.

Conclusion: Increased FLC endocytosis leads to production of inflammatory cytokines and this is likely to contribute PTECs injury (possibly by apoptosis) in multiple myeloma patients.

Chris Jenkins

Investigation of non-culturable *Burkholderia pseudomallei*
Supervisor(s): Dr Galina Mukamolova, Dr Edouard Galyov

During persistent infection, *B. pseudomallei* is likely to be in a slow-replicating (possibly non-culturable) state. Bacterial muralytic enzymes, especially lytic transglycosylases (LTG), are believed to contribute to reactivation of latent infections. LTGs are highly conserved in bacteria and predominantly function in remodelling of cell wall by restructuring peptidoglycan (PG), facilitating the insertion of large macromolecular structures including secretion systems and flagella and also aiding bacterial division. This project aims to investigate the role of LTGs in resuscitation of non-culturable *B. pseudomallei* and to assess their contribution to bacterial virulence and stress response. Based on sequence homology to well-characterised LTGs of *E. coli*, we have identified 5 putative LTGs in *B. pseudomallei* K96243. Recombinant *B. pseudomallei* LTGs were purified and shown to cleave PG. Furthermore we established in vitro conditions under which *B. pseudomallei* produce non-culturable morphologically intact forms, and are currently assessing if LTGs contribute to resuscitation of such bacteria.

Dr Zinah Zwaini

The inflammatory response of renal proximal tubular epithelial cells in conditions mimicking ischemia reperfusion injury
Supervisor(s): Dr Cordula Stover and Dr Bin Yang

Successful kidney transplantation is a life-saving procedure to patients with irreversible chronic renal failure. Despite various obstacles facing this surgery, preserving donor kidney and consequent ischemia reperfusion injury (IRI) are still major challenges affecting renal function as well as short and long term prognosis of transplant surgery. This study pursues the possible mechanism of IRI and its relation to complement cascade specifically alternative pathway activity. It also characterises changes in damage associated inflammatory gene expressions and ultrastructural analysis. The study is based on analysis an in vitro IRI model of PTEC of normal human kidney (HK-2) after exposing them to condition simulates IRI. The ischemia is induced via incubating cells in hypoxic chamber and Locke's buffer for 6

h before reperfusing them for 24 and 48 h. Microarray Proteom Profiler was used to analyse a group of inflammatory and pro inflammatory mediators .The array nicely shows that while some proteins are downregulated, implying perhaps a compromised state of the cell, others are upregulated. This shows the complexity of reaction that is captured in the in vitro model, and underlines the justification to first attempt a replacement strategy of animal modelling, where a multitude of factors impact on the target cells.

Dr Chee Kay Cheung

TGF-β1 release from PTEC is stimulated by galactose-deficient polymeric IgA1
Supervisor(s): Dr Jonathan Barratt, Dr Karen Molyneux and Prof. Nigel Brunskill

Introduction: IgA nephropathy (IgAN) is the commonest primary glomerulonephritis and a leading cause of end stage renal disease. Progression is dependent upon tubulointerstitial injury and not on severity of mesangial IgA1 deposition. We aimed to ascertain whether filtered IgA1 had a role in promoting tubulointerstitial fibrosis.

Methods: Total IgA1 was purified from serum from healthy individuals and patients with IgAN by affinity chromatography, then separated into monomeric and polymeric IgA1 (mIgA1 and pIgA1) by size exclusion chromatography. Human HK2 PTEC were incubated with 100μg/mL mIgA1 and pIgA1 for 48h. Supernatants were tested for TGF-β1 and IL-6 release, and cell lysates for RNA extraction. The galactosylation profile of the individual IgA1 preparations was tested by their ability to bind to the GalNac specific lectin *Helix Aspersa*. Proximal tubule uptake of IgA was tested in Munich Wistar Frömter rats by intravital 2-photon microscopy.

Results: Both pIgA1 and mIgA1 upregulated TGF-β1 release from PTEC, with pIgA1 having the strongest effect. pIgA1 also caused an upregulation in TGFβ1 mRNA expression. No significant increase in IL-6 release was observed. pIgA1 had a lower galactosylation profile versus mIgA1, and the level of IgA1 galactosylation inversely correlated with TGF-β1 release. igA uptake by the proximal tubule was upregulated in an in vivo model of proteinuria.

Conclusions: PTEC TGF-β1 production is significantly upregulated by IgA1, with a stronger effect observed with galactose-deficient polymeric IgA. Leakage of this form of IgA1 into the urinary space through the damaged glomerulus may drive tubulointerstitial fibrosis in IgAN.

Safia Blbas

The role of acidosis-sensing in the regulation of chronic inflammation by skeletal muscle

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

Introduction: Many chronic inflammatory diseases (including chronic kidney disease -CKD) are characterised by loss of muscle (cachexia). Metabolic acidosis is common in CKD and stimulates muscle wasting which may further enhance chronic inflammation. In vivo muscle wasting by acidosis also requires the presence of glucocorticoid. Metabolic acidosis may act by inhibiting pH-sensitive amino acid transporter protein SNAT2 which may be regulated by phosphorylation. Therefore, the aim of this study is to investigate how glucocorticoid reinforces the inhibitory effect of acid on SNAT2 in skeletal muscle, and the role of SNAT2 phosphorylation in this inhibition.

Methods: A cell culture model of rat skeletal muscle (L6-Z myotubes) was used to study activity of SNAT2 in response to glucocorticoid (Dexamethasone 500nM-DEX) and other manoeuvers which affect SNAT2 activity. HEK-293A cells were transfected with Tagged SNAT2 cDNA constructs. The activity of SNAT2 was measured by the amount of α-[1-^{14}C]-MeAIB transported into the cells and corrected for total protein content using Lowry assay.

Results: Incubation of L6 myotubes with 500nM DEX for 4h significantly inhibited the activity of SNAT2 by 60% and this effect was blunted (but not abolished) by Phosphoprotein tyrosine phosphatase (PTPase) inhibitor Vanadate (100µM). Amino acid starvation (which may act on SNAT2 through Ser/Thr phosphorylation) reproducibly abolished DEX's inhibitory effect on SNAT2. Transfecting HEK-293A cells with SNAT2 cDNA constructs increased α-[1-^{14}C]-MeAIB transport activity six fold compared with wild type control.

Conclusions: 1) Glucocorticoid may act on SNAT2 by mechanisms inducing PTPases and direct effects on Ser/Thr phosphorylation of SNAT2 protein itself.
2) Cell lines transfected with SNAT2 cDNA constructs may be useful models in which to determine the molecular basis of glucocorticoid's action on SNAT2.

Douglas Gould

Aerobic and Resistance Exercise Training in Pre-Dialysis Chronic Kidney Disease
Supervisor(s): Dr Alice Smith and Prof. Nigel Brunskill

Introduction: Patients with chronic kidney disease (CKD) have reduced physical function and exercise capacity, which strongly associates with mortality. The progressive loss of skeletal muscle and altered muscle metabolism commonly reported in CKD patients may contribute to this. However the synergistic relationship between skeletal muscle dysfunction and measures of exercise capacity and physical function are under investigated in this population. Furthermore, exercise interventions are capable of improving physical function in CKD patients; whilst resistance training significantly improves muscle size and strength. However it is unknown if combining resistance exercise with more traditional aerobic exercise will produce the same effects.

Methods: Patients with CKD stage 3b-5 (eGFR <45 ml/min/1.73m^2) not receiving dialysis are recruited from outpatient clinics at Leicester General Hospital. Following a six-week control period patients are randomised to receive one of two 12-week exercise interventions, consisting of aerobic exercise alone or in combination with resistance training. Exercise sessions are performed 3 times per week for the 12-week period. Measures of skeletal muscle size and strength, body composition, exercise capacity and physical function are performed at 3 time points. Skeletal muscle biopsies are collected from consenting patients under fasting conditions on 3 separate occasions.

Results: To date 25 patients have been recruited, with 7 completing the study protocol. Furthermore, 3 patients have been excluded due contraindications to exercise following a maximal exercise test and 1 patient has been lost to follow up.

Conclusion: patient involvement is expected to end Jan 2016 allowing for data analysis to commence**.**

Violeta Diez Beltran

The role of System L amino acid transporters in pancreatic β-cells.
Supervisor(s): Dr Alan Bevington and Dr Karen Molyneux

Type 2 Diabetes mellitus is due to a decline in β-cell mass and function in conditions of insulin resistance. Growth factors, insulin and nutrients, such as glucose and amino acids play an important role in β-cell expansion, activating the Mammalian Target of Rapamycin (mTOR) to control cell growth and proliferation of mammalian

cells. mTOR signalling is modulated by BCAAs and L-leucine seems to have a bigger effect. The uptake of BCAAs into β-cells is controlled by the System L amino acid transporters (LAT1) and its expression is elevated in islets from obese diabetic mice. Thus the uptake mechanisms of these transporters in β-cells may have significant effect in the development of T2D.

Defining the role of LATs in the activation of mTORC1, determining the effect of nutrients on LAT1 expression and establishing the significance of amino acid delivery by LAT1 for the activation of mTORC1 signalling, is possible to describe the effect of LAT1 on β-cell**s** and whether changes on its expression may influence the onset of T2D.

The addition of L-leucine on INS1E cells and rat islets of Langerhans significantly increased the mTORC1 activation and the L-leucine uptake across the membrane assisted by LATs, which was inhibited by a L-leucine analogue (BCH). Consequently, the overexpression of LAT1 in HEK293 cells increased the L-leucine uptake and the mTOR activation.

Understanding the role of LAT1 and the development of T2DM may reveal novel therapeutic targets or potential nutritional interventions, which could be used for the treatment or possible prevention of T2D.

Jaspreet Sahota

The development of phages as a treatment for Cystic fibrosis associated *P. aeruginosa* infections

Supervisor(s): Prof. Martha Clokie

Pseudomonas aeruginosa has established itself as the leading cause of morbidity and mortality in cystic fibrosis sufferers. This versatile pathogen is remarkably difficult to treat due to its ability to display various antibiotic resistance mechanisms, which have now been shown to target even the first line antibiotics used to treat *P. aeruginosa*. In addition, once it has become established as a chronic infection, due to the formation of biofilm, it is essentially impossible to eradicate with conventional treatments. Hence there is a need for alternative therapies, the most promising of which is bacteriophage therapy.

The bacteriophages used were obtained from commercial Eastern European phage cocktails not available in, or tested on UK *P. aeruginosa* strains before. The phages were tested on their abilities to kill planktonic culture in cocktails and with antibiotics. The same combinations were also tested against biofilms on their ability to both prevent and degrade biofilms, when applied as both liquid and aerosol

preparation. In addition, the phage with the largest host range (killing 98% of all tested strains) was nebulised as a prophylaxis in both healthy and cystic fibrosis ciliated nasal epithelium tissue culture models.

Multiple phages and antibiotic are able to kill *P. aeruginosa* culture and significantly prevent bacterial regrowth. The same combinations can also significantly reduce/degrade the biofilm independent of the method of delivery used. When nebulised as a prophylaxis, the phage is able to successfully prevent an infection and reduced the formation of biofilm.

Bethan Barker

Exploring the interplay between sputum bacterial load and quadriceps strength in stable COPD and at exacerbation.

Supervisor(s): Prof. Chris Brightling and Prof. Michael Steiner

Introduction: Quadriceps weakness is a common systemic manifestation of chronic obstructive pulmonary disease (COPD) associated with increased morbidity. The aetiology of quadriceps dysfunction in COPD remains unclear.

Hypothesis: Airway bacterial load and inflammation are related to quadriceps dysfunction in stable COPD and at exacerbation.

Methods: Within an observational study at 3 UK centres, spirometry, quadriceps strength (quadriceps maximal voluntary contraction [QMVC]), 6 minute walk distance [6MWD]), symptom scores, sputum bacterial load and blood C reactive protein (CRP) were obtained from stable COPD patients. Data were collected from a sub-group of patients at exacerbation onset, 2 weeks and 6 weeks post exacerbation.

Results: 278 stable patients (66% male) were recruited. Quadriceps strength (QMVC %predicted) correlated with symptom scores, 6MWD and sputum bacterial load. Multiple linear regression revealed that only 6MWD and sputum bacterial load contributed independently to quadriceps strength (r^2=0.21, r=0.45, p<0.01; β= -0.17, p=0.04 and β=0.38, p<0.01 respectively). Exacerbation episodes were captured from 55 patients. At exacerbation onset symptoms, airway inflammation and CRP increased. There was a small but statistically significant reduction in QMVC which returned to pre-exacerbation values within 2 weeks, and no significant change in 6MWD between stable and 2 week post exacerbation values.

Conclusions: Sputum bacterial load is an independent predictor of quadriceps strength in stable COPD patients. In out-patient exacerbations of COPD there is a

small drop in QMVC at exacerbation onset but this effect is short-lived. This provides evidence against community managed COPD exacerbations having a role in quadriceps muscle function decline in COPD.

Marie-jo Medina

Beware: Babies are hazardous to your health!

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

Background: Viruses are the leading agents of respiratory infections in all age groups and are a major cause of morbidity and mortality worldwide. Rhinoviruses are one group of several viruses that infect the human respiratory tract that are of particular importance in healthcare settings.

Rhinoviruses cause 35-60% of common colds in humans, and are the most common respiratory pathogens in the first year of life. Symptoms of infection are generally mild and self-limiting. However, frequent infection result in the most time lost at work and school, and the greatest numbers of GP consultations. It is estimated that one to two years of an average person's life is spent in misery over a common cold. Person-to-person transmission of rhinoviruses is thought to occur through aerosol inhalation and direct 'hand-to-surface-to-hand contact.' Rhinoviruses can remain active on surfaces for several hours; however, transfer onto host hand, nose, and eyes could occur within just a few seconds.

Aim: The aim of this study is to prospectively investigate the transmission of rhinoviruses from LRI paediatric inpatients to healthcare workers (HCWs) in a natural healthcare setting.

Methods: Thirty-minute interactions between children aged 0-6 years and medical students acting as proxy HCWs. Swab samples of 743 nose and throat (nt), hand, face, clothing and toys were collected for RT-PCR analysis and DNA sequencing.

Results: Transmission rates were 20%, 28.6%, 28.6%, and 14% for nt, hand, face, and toys, respectively.

Conclusions: The findings may be used in intervention studies on respiratory virus transmission to HCWs.

Sian Baldock

Does the cystic fibrosis phlegm feed the *Pseudomonas*?

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

Cystic fibrosis (CF) is the most common autosomal recessive disease affecting multiple organ systems. Notably it affects the respiratory tract where decline in lung function as a result of chronic respiratory infections is responsible for the reduced life expectancy of patients. *Pseudomonas aeruginosa* is an important pathogen in CF due to its ability to colonise the airway by adhering to mucins within stagnant mucus secretions. In healthy individuals mucus is effectively cleared via mucociliary clearance, which is abnormal in CF. This allows for chronic colonisation of the airways making eradication difficult. However, what remains to be elucidated is how pathogens survive in the airways. Previous studies have demonstrated that pathogens such as *Streptococcus pneumoniae* express glycosidases enabling utilisation of mucin, a glycoprotein component of mucus present within expectorated sputum samples, as a nutrient source for survival. This project aims to investigate the differences in growth, as assessed by CFU counts using the Miles and Misra method, of *P. aeruginosa* CF clinical isolates by culturing in medium supplemented with paediatric and adult sputum samples previously dialysed to remove low MW sugars, lyophilised, and comparing with the growth in sputum from healthy controls. This is in order to elucidate whether survival of pathogenic bacteria in the airway is due to differences in mucin composition providing more favourable growth in the airway of CF patients compared to healthy controls. Results have demonstrated that laboratory strain growth rate is increased in adult CF sputum compared to paediatric CF sputum indicating alteration of mucin composition with disease progression.

Joseph Morley

Drug resistance and virus infection in clinical and environmental *Aspergillus fumigatus* populations

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

Aspergillus fumigatus is a ubiquitous, spore-forming filamentous fungus. As a saprotroph, *A. fumigatus* plays a key role in the degradation of organic matter and nutrient cycling. As well as this environmental importance, *A. fumigatus* is also an opportunistic pathogen of humans and is among the most common fungal respiratory pathogens. It has been noted in recent years that antifungal drug resistance is becoming an increasingly common problem. As fungal cells are

eukaryotic, there are only a limited number of drugs available to tackle fungal infections and so any resistance developing to these few drugs is a cause for concern. *A. fumigatus* is found at high concentrations in compost and because many agricultural fungicides are based on similar chemicals to the antifungal drugs used clinically, it has been suggested that industrial composting sites may be a source of this resistance. This project aims to further understand the origin of drug resistance in *A. fumigatus* and the genetic mechanisms behind the resistant phenotypes.

Mycoviruses have been identified in fungal populations for many years, but there is little understanding of their importance and the influence they may have on their hosts. The majority of known mycoviruses are characterised only as segments of viral dsRNA. Their function and impact on their hosts remains unknown for the majority of these viruses. I am looking at the prevalence and composition of virus populations within clinical and environmental populations of *A. fumigatus* and attempting to ascertain their role in the life history of this important microbe.

Panayiota Stylianou

Exploring 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 in developed countries. A recent genome wide association study showed significant association of the TNS1 gene (which encodes tensin1) with COPD. A non-synonymous single nucleotide polymorphism (SNP) (rs2571445) in the TNS1 gene is associated with airflow obstruction in genome wide association studies (GWAS), however its pattern of expression and (patho)physiological role in the airways is not known. Tensin1, a 220 kDa cytoplasmic phosphoprotein, is involved in migration, transformation and cytoskeletal organization.

Aim: The aim of this study is to examine the mRNA and protein expression of tensin1 in structural cells from healthy subjects and patients with COPD, asthma and idiopathic pulmonary fibrosis (IPF).

Methods: Tensin1 expression was examined via qRT-PCR, western blotting and immunohistochemistry.

Results: A similar level of tensin1 mRNA and protein expression was detected in human airway smooth muscle (ASM) cells and human bronchial epithelial cells obtained from COPD and asthmatic donors when compared to healthy controls.

Immunostaining for tensin1 on bronchial biopsies and lung resections from asthmatic and COPD donors respectively and respective healthy controls was consistent with the in vitro results obtained for the cellular expression of tensin1 mRNA, with positive tensin1 immunoreactivity detected in apical bronchial epithelium, connective tissue cells, ASM cells and vessels. A significant increase of tensin1 immunostaining was detected in the airway smooth muscle and lamina propria in COPD donors when compared to healthy controls. In addition, we found decreased mRNA and protein expression in human lung fibroblasts obtained from IPF donors when compared to healthy controls.

Conclusion: In conclusion, we have showed that tensin1 is expressed in ASM cells, airway epithelial cells, and parenchymal lung fibroblasts. Tensin1 protein expression is increased in the lamina propria and ASM in COPD airways, while expression is downregulated in IPF-derived parenchymal fibroblasts.

Lorna Latimer

Skeletal Muscle Dysfunction and Mechanisms of Adaptation to Exercise Training in COPD and Healthy Ageing

Supervisor(s): Prof. Mick Steiner and Prof. Paul Greenhaff (University of Nottingham)

Exercise capacity is adversely affected by both chronic disease and ageing. Alterations in skeletal muscle physiology contribute to this decline in physical performance. Exercise capacity and skeletal muscle function can be improved through exercise training of various modes. The description of muscle and whole-body adaptation to exercise training in COPD and ageing is incomplete and may inform improvements to strategies to improve exercise capacity.

Early data from my work describe the acute impact of two muscle training interventions: percutaneous electrical stimulation (PES) and resistance exercise (RE) on quadriceps muscle gene expression in COPD. Patients underwent 30min of quadriceps PES or 5x30 maximal isokinetic knee extensions at 180°/s. Resting vastus lateralis muscle biopsies were performed immediately before and 24hr after PES or RE. Microfluidic low density microarray cards were used to assess the expression of 384 targeted transcripts by RT-PCR. Significant change in expression from baseline was determined using the ΔΔCT method with a False Discovery Rate (FDR) of <5%.

PES and RE altered mRNA abundance of 18 and 68 genes respectively (FDR <5%), of which 14 were common to both contraction modalities and of the same magnitude of fold change. Biological functions of up-regulated genes included inflammation, hypertrophy, muscle protein turnover & muscle growth, whilst down-regulated genes included mitochondrial and cell signalling transcripts. Thus, voluntary RE

produces more profound acute changes in muscle mRNA abundance than PES, but there is a commonality of response around genes associated with muscle anabolism.

Rachid Berair

Airway structural remodelling in asthma: Functional relevance; and suitability as a target for therapy

Supervisor: Prof. Chris Brightling

Asthma remains a major health problem with significant morbidity, mortality and economic costs. Airway remodelling is one of the defining characteristics; however, it is still poorly understood. The relevance of the different components of remodelling is not fully known and, importantly, the effects of asthma therapy targeting remodelling are not fully explored. I hypothesise that remodelling in asthma: is a significant determinant of the clinical and physiological domains; could be assessed by computed tomography (CT); and, could and modified, with beneficial effects, by PGD2-antagonism and by Thermoplasty. In my studies remodelling was measured in endobronchial biopsies and the association with clinical and physiological domains of asthma was examined. Remodelling was also compared to CT-derived morphometry and densitometry. Lastly, dynamic changes in remodelling are examined in response to novel asthma therapies, namely, bronchial thermoplasty and CRTh2 antagonism. There was significant correlation between airway smooth muscle content (ASM%) and vascularity with obstructive spirometry markers and patients with fixed airflow obstruction had increased ASM% and vascularity. Epithelial thickness and ASM% correlated with segmental asthma-related CT morphometry markers.

Vascularity and to a lesser extent ASM% were significantly associated with CT-derived air-trapping indices. In a double-blind RCT, in addition to improvement in clinical and physiological endpoints, treatment with PDG2-antagonism improved submucosal eosinophils, mesenchymal fibrosis, epithelial integrity and Surprisingly ASM%. All these results have shown that some aspects of remodelling are physiologically relevant and are associated with asthma-related CT markers. Furthermore, some elements of the remodelling are reversible with PDG2-antagonism with accompanying clinical benefit.

Tariq Daud

The role of WNT5a in airway remodelling in asthma

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

Background: Asthma is a heterogeneous disease characterized by variable airflow obstruction and airway hyper-responsiveness (AHR). The underlying pathology of asthma centres on chronic airway inflammation and subsequently airway remodelling. These features have shown to negatively correlate with lung function. More importantly, current treatments display limited efficacy in reducing airway remodelling features most notably aberrant ECM deposition.
WNT signalling can be divided as (1) canonical (β-catenin dependent) or (2) non-canonical (β-catenin independent). Recent evidence highlights increased gene and protein expression of WNT5a (a non-canonical ligand) in fibrotic lung diseases. Furthermore, WNT5a appears to mediate its fibrogenic effects with the ability to cross-talk with TGF-β1, a profibrotic cytokine also increased in asthma.

Results: We initially optimised and validated a WNT5a antibody, to be utilised for detection in GMA-embedded bronchial biopsy sections, western blotting and immunofluorescence. To investigate the expression pattern of WNT5a, 4 healthy subjects and 16 asthmatic subjects were analysed by immunohistochemistry. The data showed that in asthmatic subjects there is an increase in WNT5a ($p=0.0573$) and TGF-β positive cells ($p=0.0449$) and have strong correlation ($p=<0.0001$) in the submucosa/lamina propria. Additionally, we developed a semi-quantitative score (SQS) that shows WNT5a protein expression is increased in asthmatic epithelium ($p=0.0024$).

Conclusion: Evidence in the literature supports the notion that asthmatic epithelium appears damaged, suggesting that; WNT5a protein increased in asthmatic epithelium as observed by our group, tries to recapitulate a developmental repair program. This will be further tested *in vitro* utilising an ORIS cell migration system and a PCR profiling assay on primary bronchial epithelial cells.

Adelina Gavrila

Bypassing corticosteroid insensitivity in airway smooth muscle cells using a plant derivative

Supervisor(s): Dr. Yassine Amrani and Prof. Chris Brightling

Preclinical models of human conditions including asthma showed the therapeutic potential of a dissociated glucocorticoid (GC) receptor (GR α) ligand called

compound A (CpdA). Whether CpdA inhibits GC insensitivity, a central feature of severe asthma, has not been addressed. We showed that CpdA suppresses production of GC-resistant chemokines. Our aim was to further investigate the mechanisms underlying CpdA sensitive pathways in airway smooth muscle (ASM) cells. ASM cells were treated with TNFα/IFNγ with or without CpdA and the modulatory effects on chemokine expression were investigated by ELISA and qPCR. Activation of GRα by CpdA was assessed by qPCR, immunostaining and receptor antagonism (RU486). The effect of CpdA on the transcription factor IRF-1 was investigated using immunoblot, immunostaining and siRNA knockdown. CpdA dose-dependently inhibited the production of fluticasone-resistant CCL5, CX3CL1, and CXCL10 induced by TNFα/IFNγ (protein and mRNA). CpdA failed to induce mRNA expression of the GRE-inducible gene Glucocorticoid-induced Leucine Zipper (GILZ), while transiently inducing MAPK phosphatase 1 (MKP-1) (mRNA, protein). CpdA inhibitory action was not associated with GRα nuclear translocation or prevented by RU486 antagonism. We show that CpdA inhibits cytokine induced IRF-1 activation and nuclear translocation, previously associated with reduced steroid sensitivity in ASM cells. Furthermore, IRF-1 siRNA knockdown reduced cytokine-induced CCL5 and CX3CL1 production. In conclusion, CpdA suppresses production of GC-resistant chemokines via IRF-1 dependent and independent mechanisms. Thus, targeting CpdA sensitive pathways in ASM cells represents an alternative therapeutic approach for GC insensitivity treatment in asthma.

Michael Ghebre

Asthma and chronic obstructive pulmonary disease overlap: biological exacerbation clusters

Supervisor(s): Prof. Chris Brightling and Prof. John Thompson

Background: Asthma and chronic obstructive pulmonary disease (COPD) are heterogeneous diseases.

Objective: We sought to determine, in terms of their sputum cellular and mediator profiles at exacerbation, the extent to which they represent distinct or overlapping conditions.

Methods: Moderate-to-severe asthma and COPD subjects were prospectively recruited to a single-centre. Sputum mediators were available in thirty-one asthmatic and seventy-three COPD subjects assessed at exacerbation. Biological subgroups were determined using factor and cluster analyses on 22 sputum cytokines. The patterns of clinical parameters, and sputum mediators were

assessed across the identified clusters and the change of the characteristics between stable and exacerbation were investigated.

Results: Three biological clusters at exacerbation were identified: cluster 1- asthma (n=11) and COPD (n=21) with high blood and sputum eosinophil count, and high Th2 cytokines (IL-5, IL-13, CCL-13, CCL-17 and CCL-26); cluster 2-asthma predominant, asthma (n=15) and COPD (n=18), high in Th1 cytokines (IFN-g, CXCL-10, CXCL-11 and CCL5) and; cluster 3-COPD predominant, COPD (n=34) and asthma (n=5), with high blood and sputum neutrophil count and high pro-inflammatory mediators (IL-1β, IL-6, IL-6R, TNF-α, TNF-R1, TNF-R2 and VEGF) and increased bacterial colonisation. Compared to stable state the eosinophil and neutrophil counts increased in cluster 1 and 3 respectively.

Conclusions: Sputum cytokine profiling of asthma and COPD exacerbations reveals three biological clusters with different overlapping proportions of each disease.

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