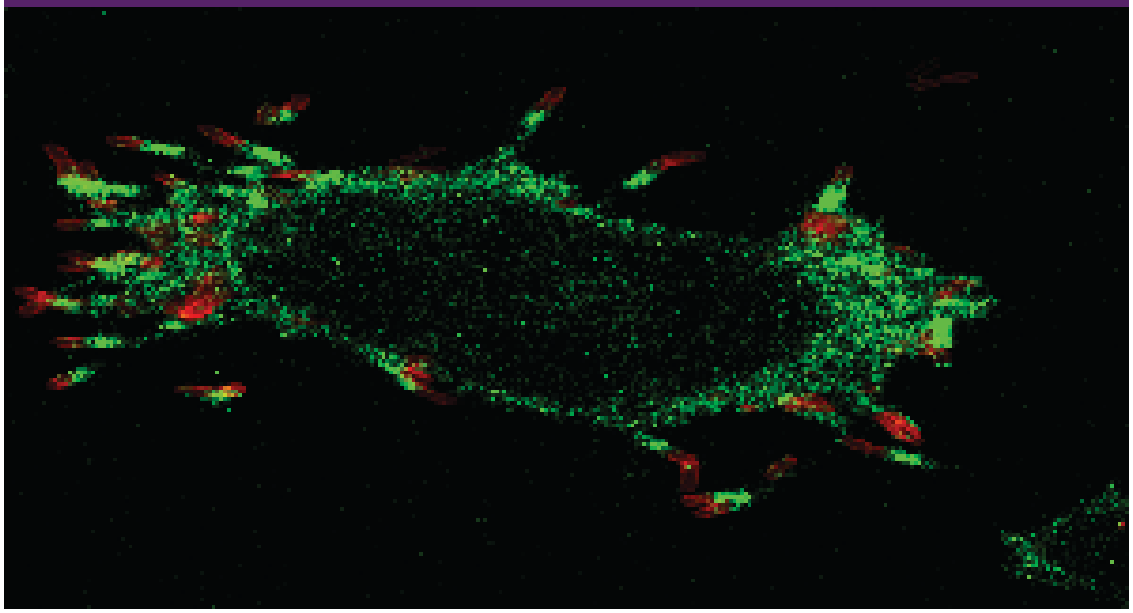
University of Leicester

THE AWARDS 2008



Department of Infection, Immunity & Inflammation

2nd Annual Postgraduate Student Conference

7th - 9th April 2010
Bennett Building

Programme and Abstracts

Preface:

Each year our postgraduate students are expected to present a seminar that informs the audience about their research. The main opportunity for them to do so is in the Easter break, when the absence of undergraduates frees up space in the lecture theatres. Last year the students organised the seminars in a conference format, which transformed the event into something rather special. Refreshments were provided; there were two guest speakers; a programme of abstracts; and the result was a transformation in the atmosphere and a much greater level of engagement in the event. We are delighted that the students are working along similar lines in 2010, and we extend thanks on behalf of the Department to those who have taken the lead in the organisation. Arine Ahmad, Andrew Bell, Luisa Crosatti, Nawal Helmi, Depesh Pankhania, Fathima Casim Sahib Mohamed Sharaf and Heidi Wan have shared the preparatory work and we congratulate them on their commitment.

The conference provides a great opportunity to learn what is happening in all parts of our diverse department. We have a tendency to remain cloistered in our own laboratories, working in our own research areas, but for three days we are privileged to learn about the work of our students and colleagues. We strongly encourage everyone who attends to provide feedback and constructive criticism to all who present, and to ask questions of them, thereby helping them to develop vital skills in presenting their work. Above all we hope everyone who participates enjoys the conference and the networking it provides.

Dr Caroline Beardsmore and Dr Roger James
Postgraduate Tutors for the Department of III's

Contents:

Programme of Speakers

Wednesday, 7th April 2010	
9.30-9.35	Conference Opening
9.35-9.45	TBA
	LT5 - Chair: Dr. Izabella Pawluczyk
9.45-10.15	Sarah Hosgood (P25) "Ischaemia reperfusion injury in kidney transplantation; The role of preservation"
10.15-10.45	Elvina Chrysanthou (P28) "The lectin pathway of complement activation in cerebral ischaemia and reperfusion injury"
10.45-11.15	Sumit Gupta (P21) "Scanning the asthmatic airway: defining the relationship between physiology, inflammation and airway structure in asthma using computed tomography"
11.15-11.45	Tea/Coffee Break (in the foyer)
	LT5 - Chair: Ewan Harrison
11.45-12.15	Caroline Spink (P18) "Analysis of the effects of plant-derived factors on the growth and physiology of lactic acid bacteria (LAB) and other microbial species"
12.15-12.45	Jon van Aartsen (P12) "Characterization of a *Klebsiella pneumoniae* genomic island bearing a novel type 1-like fimbrial operon"
12.45-13.15	Fathima Farveen Casim Sahib Mohammed Sharaff (P15) "Environmental influences on bacterial biofilm formation"
13.15-14.15	Break for lunch (not provided)
	LT5 - Chair: Dr. Natalie Lazar Adler
14.15-14.45	Marialuisa Crosatti (P8) "Virulence in *Pseudomonas aeruginosa* clinical isolates"
14.45-15.15	Kate Hargreaves (P16) "Isolation and characterization of bacteriophages from environmental *Clostridium difficile* isolates"
15.15-15.45	Abbie Fairs (P19) "Fungal exposure, sensitization and airways colonization in asthma"
15.45-16.15	Tea/Coffee Break (in the foyer)

Thursday, 8th April 2010	
	LT5 - Chair: Dr. Daniel Neill
9.30-10.00	Salwa Abdalla (P7) "Mechanisms of Biofilm formation by *Listeria monocytogenes"*
10.00-10.30	Mohammed Almaghrabi (P15) "The isolation of *Streptococcus pneumoniae* lytic phages and their *in vivo* and *in vitro* interactions"
10.30-11.00	Muhammad Adnan (P7) "Structure function studies of pneumolysin"
11.00-11.30	Tea/Coffee Break (in the foyer)
	LT5
11.30-12.30	**Dr. Ruth Sauder** (Respiratory Medicine, Glenfield Hospital) "Airway Smooth Muscle Hyperplasia in Asthma; potential mechanism for airway smooth muscle/progenitor cell recruitment/differentiation"
12.30-13.30	Break for lunch (not provided)
	LT5 - Chair: TBA
13.30-14.00	Eman Yousuf Abu-Rish (P27) "Investigation of the intracellular pathways of Toll-like receptor signalling in human B cells"
14.00-14.30	Chris Furze (P29) "Engineering new biological activities into members of the collectin family of animal lectins"
14.30-15.00	Heidi Wan (P24) "Assessing the Role of Structural Cells in Asthma Progression/Exacerbation Using a 3-Dimentional Ex Vivo Cell Model"
15.00-15.30	Tea/Coffee Break (in the foyer)
	LT5 - Chair: Dr. Umakhanth Venkatraman Girija
15.30-16.00	Eva Horvath-Papp (P10) "The genetics of aminoglycoside resistance in *Acinetobacter baumannii*"
16.00-16.30	Janet Nale (P17) "ISOLATION AND CHARACTERIZATION OF TEMPERATE BACTERIOPHAGES OF THE HYPERVIRULENT *Clostridium difficile* STRAIN R027"

Friday, 9th April 2010	
	LT5 - Chair: Dr. Barbara Rieck
9.30-10.00	Manjith Narayanan (P21) "Lung alveoli continue to form till early adulthood in humans: new evidence from 3He magnetic resonance"
10.00-10.30	Katy Roach (P22) "The Role of the K+ channel Kca3.1 in Pulmonary Fibrosis"
10.30-11.00	Latifa Chachi (P18) "TNFα signaling in chemokine secretion in airway smooth muscle: Modulation by corticosteroids and IFNγ"
11.00-11.30	Tea/Coffee Break (in the foyer)
	LT5 – Host: Prof. Barer
11.30-12.30	**Dr. Catherine Rees** (Division of Food Sciences, University of Nottingham) "New tricks to combat an old foe; bacteriophage detection of Mycobacteria"
12.30-13.30	Break for lunch (not provided)
	LT5 - Chair: Dr. Helen O'Hare
13.30-14.00	Jessica Loraine (P13) "Investigation into and Isolation of Muropeptides from Modified *E. coli* Peptidoglycan"
14.00-14.30	Andrew Bell (P8) "Analysing the properties of tubercle bacilli in sputum"
14.30-15.00	Arine F. Ahmad (P14) "Detection of pathogenic *Balamuthia mandrillaris* by culture-independent polymerase chain reaction (PCR)"
15.00-15.30	Tea/Coffee Break (in the foyer)
	LT5 - Chair: Dr Jinyu Shan
15.30-16.00	Amanda Jane Sutcliffe (P23) "Airway Smooth Muscle from Asthmatic subjects exhibit oxidative stress"
16.00-16.30	Mahmoud Tawfick (P30) "Using Recombinant DNA Technology in Vaccine Development"
16.30-17.00	Concluding Remarks

Microbial Physiology and Pathogenesis

Salwa Abdalla

"Mechanisms of Biofilm formation by *Listeria monocytogenes*"

Bacterial food contamination is considered to be a crucial health problem worldwide. *Listeria monocytogenes* causes problems in food processing industries because of its ability to survive and grow over a wide range of environmental conditions. Furthermore *L. monocytogenes* can attach to and produce biofilm on wide variety of different surfaces in the food-processing environment, allowing the bacterium to be resistant to antimicrobial and sanitizing agents.

This report covers investigations of the ability of wild type of *L. monocytogenes* strain and *Tn917* transposon mutants to attach to abiotic surfaces at different of temperatures. A published assay was modified to determine attachment to polystyrene surfaces over two hours. The attached cells were detected using crystal violet staining.

The results showed that certain transposon mutants have a considerable reduction in the level of attachment, and this reduction was a temperature-dependent. In addition, these changes in the attachment level were evident at 18 ^{0}C and 30 ^{0}C but not at 37 ^{0}C. The identity of the transposon insertion site in these mutants is being actively pursued. In conclusion, the present data have revealed that there is a temperature-dependent involvement of some genes in surface attachment.

Muhammad Adnan

"Structure function studies of pneumolysin"

The pneumococcus is one of the most important human pathogens, causing life threatening invasive diseases such as pneumonia and septicemia, especially in young children, the aged, cigarette smokers and immunocompromised. The pneumococcus produces many virulence factors among which pneumolysin is very prominent, Pneumolysin is a 52KD protein toxin, having four domains and 471 amino acids. It is produced in the cytoplasm of all serotypes of *Streptococcus pneumoniae*. It is not only cytotoxic to mammalian cells but also activates the classical pathway of the complement system. It is released from the pneumococcus on lysis or in the late stationary phase of growth and has

no terminal signal for secretion. Pneumolysin is a leading candidate for the next generation of pneumococcal vaccines and therefore understanding its mode of action is of great interest. The aim of this project is to isolate and purify domain 4 as it is attached to the rest of the molecule via a single exposed polypeptide. The plan is to put a protease cleaving site (ENLYFQG/S) at the junction of domain 4 with rest of the molecule in such a way so that after cleavage of the molecule with TEV protease we can purify domain 4 for use in structural studies (NMR), to study interaction with the complement system and to test if it is a suitable vaccine.

Andrew Bell

"Analysing the properties of tubercle bacilli in sputum"

Mycobacterium tuberculosis is responsible for the pulmonary infection Tuberculosis. The World Health Organisation estimates that one third of the world's population is currently infected, and approximately 2 million people die as a result of tuberculosis each year. The tubercle bacilli are able to remain latent in a host for decades, before reactivating when the host immune system is weakened. It is thought that lipid metabolism plays an important role in the long term survival of *M. tuberculosis*, and so a better understanding of lipid storage and utilisation is sought.

Despite recent advances in molecular biology, examining smears of sputum by microscopy is still one of the primary means of diagnosis. Sputum samples are the only source of tubercle bacilli for laboratory based study, but are of variable quality and quantity.

By analysing the lipid content of tubercle from sputum samples by fluorescence microscopy it is hoped to gain a greater insight into both transmission of the bacterium and life inside a granuloma.

In addition recent work on improving existing staining techniques has cast doubt on the efficiency and sensitivity of the most widely used fluorescence stains for acid-fast bacilli.

Marialuisa Crosatti

"Virulence in *Pseudomonas aeruginosa* clinical isolates"

Pseudomonas aeruginosa (*P. aeruginosa*) is the leading causes of nosocomial pneumonia and poses a serious threat for immune-compromised patients. The most

commune route of infection is the respiratory tract but the prognosis worsen when the bacteria moves from the lung into the bloodstream. *P. aeruginosa* strains encompass several virulence factors from production of pyocyanin that damages tissue, and alginate compounds, to mask surface antigens, to translocation of Type III Secretion System (TTSS) effectors (ExoU, ExoS, ExoY, ExoT) into target cells.

We collected strains from patients at the Leicester Royal Infirmary from either sputum samples or from blood samples: strains isolated from blood are considered more virulent than the ones isolated from sputum. We propose to study the virulence of these clinical isolates using invertebrate model of infections and molecular biology techniques.

We tested the strains in *Caenorhabditis elegans* (*C. elegans*) high-throughput assay where *P. aeruginosa* strains were grown in King's media and then on Nematode Growth Media agar (NGM) in 24well plates. *C. elegans* worms (N2 strain) were synchronized and 4 were seeded for each well. At the end of the experiment, a score was assigned to each well indicating the degree of progeny present. Higher score meant more progeny and therefore less virulence. Blood-derived strains had a statistically significant lower score than sputum-derived strains (P-value for t-test for independent samples was below 0.001). Therefore they were in average more virulent for *C. elegans* than sputum samples.

P. aeruginosa may produce green-blue pigments both in vivo and in vitro including pyocyanin. During the high-throughput experiments, we recorded the colour of clinical isolates in King's media and on NGM agar. Almost half of sputum isolates did not show any pigment formation in both media while all blood isolates made green-blue pigment in at least one of the media. As consequence, "green-blue" strains had in average a lower score compared to the other strains indicating a possible connection between pigment production and virulence in *C. elegans*.

Finally, we characterized a subset of clinical isolates using polymerase chain reaction (PCR) to determine the presence of four TTSS effectors genes: *exoU*, *exoS*, *exoT* and *exoY*. Both blood and sputum strain showed an high prevalence of *exoS*, *exoT* and *exoY*. *exoU* was more prevalent in blood isolates and sputum but the difference was not statistically significant.

Eva Horvath-Papp

"The genetics of aminoglycoside resistance in *Acinetobacter baumannii*"

Acinetobacter baumannii is a non-fermentative Gram-negative bacillus, normally found in water reservoirs and soil. It has now become increasingly recognised as a major nosocomial infection, causing ventilator-associated pneumonia, skin and wound infections, urinary-tract infections, meningitis and septicaemia. Prior to the 1970s, *A. baumannii* was not considered to be a major problem, but its unique ability to gain resistance to antibiotics has resulted in increase in the prevalence of drug-resistance. A large proportion of strains (up to 30% in the U.K.) are now classed as multi-drug resistant (MDR), or in some of the worst cases, pan-drug resistant (PDR) strains have now been reported. These resistance determinants are caused by a range of genetic determinants such as multidrug efflux pumps, target modification, porin deficiency, and modifications of the antibiotic itself.

Aminoglycosides are a class of antibiotics that were until recently, used very successfully against *A. baumannii*. However, within the last 20 years, their widespread use has resulted in increased resistance. Some resistance genes, such as streptomycin, that they are no longer effective against *A. baumannii*. Yet, in many instances, it has been observed that these genes have persisted in the gene pool, despite the active selection forces being removed.

The aim of this project is to understand why these genes have been preserved. By analysing the antibiotic resistance of clones with single copy aminoglycoside resistance genes, compared to clones with two such genes, it is hoped that an advantage of preserving previous resistance genes will be discovered.

Previous work in the lab from a genomic library identified a gene possibly involved in amikacin resistance. Current investigations have included cloning this gene from known amikacin-resistant *A. baumannii* genes, into pGEM-T Easy (cloning confirmed by digestion). After testing the clones for amikacin resistance, there was no significant difference in resistance observed when compared to control strains of *E. coli*.

An attempt to recover the gene through the use of a marker rescue assay on a genomic library was made, although as yet, there have been no significant results. Current aims in the project involve recreating the original clone artificially through Long-Range PCR cloning. Through the recreation of the clone, it is hoped that the amikacin resistance can be characterized.

Future work will be centred on analysing the difference of antimicrobial susceptibility between clones possessing only one, or two independent aminoglycoside resistance genes. It is hoped that comparing the difference will shed light on the persistence of resistance genes no longer actively selected for in clinical environments.

Baye Gelaw Tarekegn

"Association between exhaled Nitric oxide in patients with pulmonary tuberculosis and lipid bodies within tubercle bacilli in sputum in Gondar health institutions, north-west Ethiopia"

Tuberculosis (TB) is a disease of antiquity, caused by *Mycobacterium tuberculosis* complex. It is a major public health problem, with around 9 million new cases and 2 million deaths estimated to occur each year globally.

Ethiopia ranks eighth among the worlds 22 countries with a high tuberculosis burden. According to WHO global TB report 2006, the country had more than 267,000 TB cases in 2004, with an estimated incidence rate of 353 cases per 100,000 people.

Lipid bodies (also known as lipid droplets, adiposomes) are dynamic organelles with key roles in regulating storage turn over of lipids in different cells and organisms.

Sputum has been traditionally thought to contain active growing tubercle bacilli. However, recent studies rejected the commonly held belief that smear-positive sputum is dominated by aerobically replicating *Mycobacterium tuberculosis*. Survey on clinical samples revealed that lipid bodies are universal features of tubercle bacilli in sputum. A number of conditions including hypoxia, Nitric oxide (NO) exposure, PH, heat and cold shock were shown to promote lipid body formation in *Mycobacterium tuberculosis* in vitro. The formation of lipid bodies in NO exposed *Mycobacterium tuberculosis* was shown to correlate with the level of antibiotic tolerance. Antibiotic tolerance was thought to be a result of transitory growth arrest.

In vitro, *Mycobacterium tuberculosis* formed lipid bodies after 4 hour NO treatment and this treated populations of bacteria was significantly more tolerant of isoniazid and rifampicin than cultures treated without NO (control groups). However, the level of nitric oxide in vivo and its association for the synthesis of lipid bodies on mycobacteria is not fully elucidated. Therefore, it is highly important to understand weather endogenous NO has

contribution for *Mycobacterium tuberculosis* dormancy on sputum smear positive tuberculosis patients.

Jon van Aartsen

"Characterization of a *Klebsiella pneumoniae* genomic island bearing a novel type 1-like fimbrial operon"

Klebsiella pneumoniae (*Kp*), a clinically important nosocomial pathogen, harbors a fimbrial operon which is highly homologous to the well-described phase variable and virulence-associated type 1 fimbrial *fim* operon present in *Escherichia coli*. However, exclusive to the *Kp fim* cluster is the *fimK* gene. FimK possesses an N-terminal DNA binding helix-turn-helix domain and a C-terminal EAL domain, which may be involved in the hydrolysis of cyclic-di-GMP, a bacteria-specific intracellular messenger. Variations in intracellular cyclic-di-GMP levels are known to affect biofilm formation, virulence, motility and the production of surface structures. Previous work showed that *Kp fimK* null mutants displayed increased biofilm formation and increased surface expression of type 1 fimbriae.

We identified a similarly organised locus (*fim2*) in KpGI-5, a novel 14.0kb genomic island located downstream of the *met56*-tRNA gene in *Kp* strain KR116. Interestingly, the *fim2* locus codes for FimK2, a protein of ~290 amino acids with a C-terminal region predicted to contain an intact EAL domain similar to that of FimK (Blastp identity 54%, expect value $3e^{-7}$). Upstream of *fimK2* we identified novel homologs of fimbrial proteins encoded by the *Kp fim* operon (Blastp identity 61% to 88%). Based on similarities to these subunits, it is likely that the *fim2* locus encodes a fimbrial structure which plays an as of yet unidentified role in *Kp* infection and/or host colonization.

This project aims to examine whether or not acquisition of the KpGI-5 *fim2* locus affects *Kp* virulence traits. Additionally, the contribution of the novel regulatory proteins FimK and FimK2 to specific virulence phenotypes will be investigated.

To date, we have sequenced and analysed two fosmid clones that encompass the entire KpGI-5 island. To investigate the effects of FimK and FimK2 on *Kp* phenotype, *fimK* and *fimK2* single mutants and a *fimK fimK2* double mutant were created in two distinct strain backgrounds. These derivatives are currently being analysed by electron microscopy, wax moth larvae infection models and a crystal violet-based biofilm quantification assay.

Numerous attempts have been made to express and purify FimK and FimK2 fused to 6xHis or GST, but all fusions remain insoluble. Moreover, a panel of *Kp fim* and *fim2* locus mutants have also been produced to assess the contribution of each locus to *Kp* virulence phenotypes. These strains are also being analysed using methods similar to those mentioned before. Additionally, experiments are underway to express the *fim* and *fim2* loci in the afimbriate *E. coli* strain HB101

Jessica Loraine

"Investigation into and Isolation of Muropeptides from Modified *E. coli* Peptidoglycan"

Millions of people, every year die from tuberculosis, in addition to this roughly one third of the Earth's population are infected with the causative agent; Mycobacterium Tuberculosis. It can take many years or a life time before symptoms present, the bacteria present in individuals who are asymptomatic are believed to be in a dormant state. Rpfs (resuscitating protein factors, muralytic proteins) which were first identified in Micrococcus Luteus and are produced by actively growing bacteria, were found to induce resuscitation to the vegetative state. The Mtb genome was found to contain five homologous Rpf genes (RpfA-E), addition of the corresponding protein (in vitro to dormant Mtb cells) results in resuscitation from dormancy. By creating a strain of E. coli cells which express the NamH gene (N-acetyl-muramic acid hydroxylase from Mtb responsible for peptidoglycan modification) a model Mtb cell wall will be generated. By examining the muralytic activity of purified RpfB on isolated muropeptide the fragments produced can be determined by HPLC, MALDI and LCMS so that it may be possible to identify the Rpf binding site. Ultimately the project aims include design and synthesis of a muropeptide analogue as an attempt to avert resuscitation of dormant cells by preventing the activation of S/T kinases believed to be responsible for the effects muropeptides released via Rpf.

Arine F. Ahmad

"Detection of pathogenic Balamuthia mandrillaris by culture-independent polymerase chain reaction (PCR)"

Balamuthia mandrillaris is a third genus of free-living amoeba that has recently been identified as causing fatal encephalitis in both immunocompromised and immunocompetent individuals. Limitations in the ability to isolate *B. mandrillaris* using conventional culture methods have hampered our understanding of the ecology of this amoeba. To date, only three environmental strains have been isolated from soil and dust samples. The objective of this study is to develop a simple and reliable method for extracting microbial DNA from environmental samples which is suitable for use in the detection of pathogenic *B. mandrillaris* by a nested PCR.

Soil and water samples were collected from various geographic locations. Following filtration or centrifugation, total DNA was extracted by lysis using UNSET buffer (urea-NaCl-sarkosyl-EDTA-tris), vortexing with glass beads, phenol-chloroform extraction and isopropanol precipitation. The DNA was further purified using a commercial kit (ZR soil microbe DNA kit (Zymo Research Corp., USA) prior to PCR amplification using *B. mandrillaris* specific primers.

Initial results showed that only water samples collected from a river downstream of a power station in France were PCR positive for *B. mandrillaris* (53%). In addition, positive soil samples were obtained from South California, South Aftrica and Portugal. Sequencing of representative PCR amplicons showed more than 99% homology with *B. mandrillaris* sequences deposited in GenBank. Attempts to culture *B. mandrillaris* from these samples have so far proved negative. None of the UK environmental samples were found to be positive by either cultivation or PCR. The association between the discharge of thermally polluted water from the power station and the presence of *B. mandrillaris* in the river is being investigated further as these preliminary results suggest thermally enriched habitats may be a source of the organism. The finding of positive soil samples from subtropical climate indicates that temperature may play a significant role in the localisation of this particular amoeba in these environments. The developed environmental DNA extraction

process and PCR methods will allow a greater understanding of the ecological diversity of *B. mandrillaris.*

Mohammed Almaghrabi

"The isolation of *Streptococcus pneumoniae* lytic phages and their in vivo and in vitro interactions"

To isolate pneumococcal lytic phages, different samples including sputum, throat swabs, pulmonary fluid and tissues from mice infected with invasive *Streptococcus pneumoniae* serotype (D39) were processed. Several phages with different morphologies were isolated and they were found to belong to Myoviridae and Siphoviridae families. The association between these phages and the observed clearance is under study. These samples were analysed by spot assays and plaque assays and phages were visualised using Electron microscopy. Isolated phages will be purified and their titre will be increased to make them more available for future processes. The overall aim of this project is to isolate pneumococcal phages that may be used as therapeutic agents in attempt to treat infections caused by *Streptococcus pneumoniae*. In addition, Phage therapy may be used against pneumococcal infection as the bacterium exhibits resistance to several antibiotics commonly used. Moreover, vaccines used to promote immunity against *Streptococcus pneumoniae* don not produce whole protection against all 91 pneumococcal serotypes. Alternative methods of combating pneumococcal disease are warranted as invasive pneumonia is a worldwide problem with a high mortality rate among children in developed countries.

Fathima Farveen Casim Sahib

"Environmental influences on bacterial biofilm formation"

Biofilms are microbial communities, which develop when microorganisms attach to surfaces. Biofilms are ubiquitous in natural and many industrial environments and are relevant in a variety of disciplines including medicine, dentistry, bioremediation, biofouling, water technology, engineering and food science. They are characterized by their structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. Biofilms have been found to be involved in a wide variety

of microbial infections in the body and have great significance for public health, because of their decreased susceptibility to antimicrobial agents. However, the primary problems are biofilms associated with indwelling devices such as intravenous catheters.

Stress and susceptibility to microbial infection have long been correlated. In recent years however, it has become clear that many bacteria are able to sense mediators of the host stress response and respond by activating growth and the expression of virulence factors. It has been shown that catecholamine stress hormones such as adrenaline and noradrenaline and structurally similar drugs are used to treat heart and kidney problems in seriously ill patients, can all promote the formation of staphylococcal and other bacterial biofilms.

A detailed study on the mechanisms of how catecholamines affect the formation of biofilms has not been conducted. Therefore, the primary goal of my study is to investigate how catecholamine stress hormones and inotropes may influence the virulence and ability of infectious bacteria to form biofilms.

Kate Hargreaves

"Isolation and characterisation of bacteriophages from environmental *Clostridium difficile* isolates"

Clostridium difficile is an important pathogen causing nosocomial diarrhoea with over 36,000 reported cases in UK trusts during 2008. Although the hospital environment is considered the ideal conditions for spread and occurrence of the disease, *C. difficile* has been isolated from a number of environmental sources such as soil and seawater, and from the guts of healthy people. *C.difficile* carriage rates is usually high in infants, up to 80%, when compared to the carriage rate of 3% in adults

Treatment of *C. difficile* is currently problematic and control of infections difficult due to its ease of transmission and survival through sporulation on surfaces outside the body. A number of novel strategies to treat and prevent infection spread are being explored, one of which is bacteriophage therapy. Bacteriophages that infect *C. difficile* could potentially be administered to patients or used prophylactically in cleaning treatments for surfaces. Alternatively phage-derived products such as endolysins could be used in place of whole phage.

C. difficile phages are relatively uncharacterized and largely unknown from environmental sources. This project aims to establish a collection of environmental strains, including from healthy infants, soil and animal dung in order to isolate both lytic and lysogenic phages associated with these strains. Phages will be morphologically and genetically characterised, with emphasis to investigate potential virulence factors within phage genomes. The collection will be tested against clinical isolates to determine host ranges and compared to clinical isolates' associated bacteriophages.

Janet Nale

"ISOLATION AND CHARACTERIZATION OF TEMPERATE BACTERIOPHAGES OF THE HYPERVIRULENT *Clostridium difficile* STRAIN R027"

Clostridium difficile is the aetiological agent of pseudomembranous colitis and nosocomial infections. The North American pulsed field gel electrophoresis type 1 and PCR ribotype 027 hypervirulent strain is responsible for increased mortality and morbidity due to the carriage of the binary toxin gene and mutations in the negative regulatory gene, which result in increase release of toxins. This ribotype has been reported to exist in 23 sub-clones identified by multi-locus variable tandem repeat analysis and found to vary in their severity. This study was designed to isolate and characterize temperate bacteriophages associated with these sub-clones of *Clostridium difficile* R027. Prophage induction was done using mitomycin C or norfloxacin at a final concentration of 3 µg/ml. Diverse phage morphologies belonging to the Caudovirales and phage tail-like particles were isolated from 50 out of the 51 samples induced so far. The genome size of the phages isolated was estimated to be around 30-40 kb. To gain an insight to the molecular diversity of these phages, the phage holin gene was amplified from 7 selected induced phages, sub-cloned and expressed in *E. coli*. The plasmids were isolated, purified and the inserts sequenced. Whole genome sequencing is also underway to identify potential novel toxins encoded by these phages. These findings will lead to greater understanding of the molecular biology of these phages and their role in the pathogenicity of *Clostridium difficile* R027.

Caroline Spink

"Analysis of the effects of plant-derived factors on the growth and physiology of lactic acid bacteria (LAB) and other microbial species"

The Bacteria responsible for the fermentation and preservation of milk products and other food stuffs are mainly lactic acid bacteria (LAB). LAB cultures are exposed to unfavourable conditions during processing and storage which decrease their survival and efficacy on revival and consumption. One problem with fermented foodstuffs is the action of unwanted spoilage LAB. Detecting the presence of these unwanted bacteria is currently a slow process, as most of them are fastidious and slow growing. There is considerable commercial interest in the development of compounds to improve the viability of LAB and to speed up their growth.

Dr Primrose Freestone and her colleagues have discovered that the addition of a crude extract of banana (LAB E) to both pathogenic bacteria and LAB has a marked effect on their growth. LAB E shortens the lag phase of healthy bacteria and markedly improves recovery of stressed LAB (Freestone Patent PCT/GB2006/050371).

The aims of my project are:

To find and describe the active ingredient of LAB E;

Do other fruits/vegetables also contain the same/similar compounds;

Establish what properties other than growth of the LAB are stimulated, e.g. production of biofilms;

Can new cheaper and more effective diagnostic media can be created utilising these compounds.

Asthma other Respiratory-related Studies

Latifa Chachi

"TNFα signaling in chemokine secretion in airway smooth muscle: Modulation by corticosteroids and IFNγ"

Asthma is a complex chronic inflammatory disease of the airways that is increasing in prevalence and morbidity world-wide. Understanding the mechanisms that drive the inflammation in the airways could lead to better therapeutic options. Many preclinical and clinical studies suggest that TNFα is an important player in the pathogenesis of asthma.

Interaction of TNF-α with airway structural cells such as airway smooth muscle (ASM) leads to the production of inflammatory chemokines that have been involve in promoting airway inflammation and corticosteroid resistance.

Objectives: The main goal of our studies is to characterize the key signaling events activated by TNFα that induce "pro-asthmatic" responses in human ASM cells.

Main findings: I first started by describing the expression and function of TRUSS (**T**NF **R**eceptor 1 –**U**biquitous **S**caffolding and **S**ignalling protein), a novel protein that was shown to interact with TNFR1 receptor in other cell types. Both RT-PCR and immunoblot assays showed that TRUSS is expressed in three human ASM cell lines. Co-immunoprecipitation shows that following TNFα stimulation for 30, 60, 120 min TRUSS is rapidly recruited to IKK complex by interacting with IKKα and IKKβ both NF-κB activating proteins. More importantly, TRUSS activation by TNFα was significantly increased in ASM cells derived from asthmatic patients when compared to normal subjects. The use of a soluble inhibitor BMS-345541 showed that TNFα-induced CCL-5 and CXCL10 chemokines by TNFα was dependent on IKK2 pathways. CCL5 induction by TNFα was significantly increased in asthmatic versus non-asthmatic cells.

Conclusion: TRUSS appears to represent an interesting target in the regulation of TNFα-inducible genes that are involved in asthmatic responses.

Future direction: We will test the hypothesis that an abnormal TRUSS-dependent NF-KB activation is involved in the exaggerated CCL5 secretion seen in asthmatic cells.

Abbie Fairs

"Fungal exposure, sensitisation and airways colonisation in asthma"

Background: Fungal spores are ubiquitous, and many have adverse effects on health. There is a distinct lack of indoor exposure thresholds, and quantification methods are not well standardised. Inhalation of fungal spores can lead to colonisation in damaged airways. Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to colonisation by *Aspergillus* species*;* predominantly *A. fumigatus.* Culture of *A. fumigatus* from sputum indicates airways colonisation; however, detection is rare via routine clinical laboratories. The incidence and clinical significance of other filamentous fungi from sputum of asthmatics is unclear.

Objectives:

- Establish baseline ranges of indoor airborne fungal spores.
- Optimise detection of *A. fumigatus* from sputum.
- Define the clinical and laboratory features of *Aspergillus*-associated asthma.
- Investigate the relationship between fungal exposure, sputum culture and sensitisation.
- Fully characterise fungi isolated from sputum of asthma patients.

Method: Sputum was induced in 79 asthma patients classified into 3 groups according to *A. fumigatus* sensitisation: (1) IgE-sensitised (2) IgG-only-sensitised; and (3) non-sensitised. 14 healthy controls were also studied. Sputum was streaked over fungal culture plates and incubated at 37°C. Fungi were examined by microscopy and *A. fumigatus* identified based on conidial head morphology. Other fungi were identified by sequencing.

Twenty-four hour indoor air samples were collected from 100 non-complaint Leicestershire properties and homes of 63 study participants (54 asthma patients and 9 healthy controls).

Result: *A. fumigatus* was cultured from 63% of IgE-sensitised asthmatics (n=40), 39% of IgG-only-sensitised asthmatics (n=13), 31% of non-sensitised asthmatics (n=26) and 7% of healthy controls (n=14). *A. fumigatus*-IgE sensitisation ($p = 0.016$), sputum neutrophil count ($p = 0.016$), sputum eosinophil count ($p = 0.024$) and positive sputum culture for *A. fumigatus* ($p = 0.046$), were important predictors of lung function ($R^2 = 0.493$).

Baseline data showed significantly higher levels of *Asp/Pen*-type spores in old, terraced properties. Culture positive had significantly higher *Asp/Pen*-type spore counts in their homes than culture negative asthmatics. There was no difference in home *Asp/Pen*-type spore concentrations between the 3 sensitisation groups.

Other filamentous fungi were cultured from sputum of patients from all three sensitisation groups, including patients who both did and did not culture *A. fumigatus*, resulting in 70%, 46% and 58% of group 1, 2 and 3 patients being culture positive for any filamentous fungi.

Conclusion: Approximately 60% of asthmatics culture fungi from their sputum. Whilst *A. fumigatus* is most commonly isolated, other fungi can also potentially colonise the airways; the clinical significance of this remains to be determined. Elevated exposure to *Asp/Pen*-type spores is correlated with detection in sputum.

Sumit Gupta

"Scanning the asthmatic airway: defining the relationship between physiology, inflammation and airway structure in asthma using computed tomography"

Asthma affects a population of 300 million worldwide with prevalence in UK being one of the highest in developed world. About half a million people in UK suffer from severe asthma and are at a particularly high risk of asthma attacks, hospitalization and death due to inadequately controlled disease, and often have severely impaired quality of life. Structural changes in the airways of asthmatic subjects, collectively termed as remodelling, are common. Computed tomography (CT) has emerged as a non-invasive research tool for quantitative assessment of proximal airway structure in patients with asthma. A critical gap in our understanding of severe asthma is the ability to relate airway structure to important clinical outcomes and identification of patients who will have recurrent asthma attacks and develop persistent airflow obstruction, features particularly pertinent to severe asthma.

We have conducted cross-sectional quantitative assessment of airway remodelling in severe asthma patients. A longitudinal study is underway to assess the airway structural changes in asthmatics. We are also investigating use of phantom models to standardise quantitative data for various factors including radiation dose, analysis software used or CT scanner manufacturer. The results presented (1) will compare CT derived dimensions of right apical upper lobe (RB1) bronchus between severe asthma sub-phenotypes determined by cluster analysis, (2) attempt to identify clinical features relating to patient's demographic profile, symptoms, pulmonary functions or airway inflammation that are best associated with geometry of RB1 bronchus and (3) Identify factors that influence CT derived quantitative measures and devise methods of standardisation.

Manjith Narayanan

"Lung alveoli continue to form till early adulthood in humans: new evidence from ^{3}He magnetic resonance"

Background:

The currently accepted view is that human pulmonary alveoli stop multiplying by the age of 2 – 3 years. This is based on older studies using outmoded histological techniques on

post-mortem human lungs. However, new evidence suggests that new alveoli form up to adulthood in other mammals. This has prompted us to re-examine the current paradigm of alveolization in humans. We have used helium-3(^{3}He) magnetic resonance (MR) to explore ongoing alveolization in childhood. Apparent Diffusion Coefficient(ADC) measured by ^{3}HeMR is a non-invasive measure of average alveolar size in the lung.

As resting lung volume increases almost fourfold between 7 to 21 years of age, we hypothesized (based on the current paradigm) that alveolar size would increase to the same extent.

Methods:

We measured the divisions of lung volume by spirometry and plethysmography in 102 healthy subjects aged 7-21 years. Functional residual capacity (FRC) determined by plethysmography was used as an measure of resting lung size. Using ^{3}HeMR, we determined the ADC of ^{3}He at FRC in these subjects. We computed the average linear dimensions of peripheral airspaces by q-space analysis of ^{3}HeMR data.

Results:

There was no evidence of an increase in ADC with age(r=-0.138, p=0.17) or FRC (r=-0.145, p=0.15). These relations did not change when controlled for potential confounding factors. Average linear dimensions of peripheral airspaces were negatively correlated with age (r = -0.361 p=0.04) but not with FRC (r= -0.281, p=0.12).

Conclusions:

The only plausible explanation for our observations is that new alveoli form continuously during childhood, which contradicts the current paradigm. This implies the potential for lungs to recover from early life insults and respond to emerging alveolar therapies. Conversely, it is possible that drugs, diseases or environmental exposures throughout childhood could affect adversely affect alveolisation.

Katy Roach

"The Role of the K+ channel Kca3.1 in Pulmonary Fibrosis"

Idiopathic pulmonary fibrosis is a common, progressive interstitial lung disease affecting more than 20,000 of the UK population, with 6,000 new cases diagnosed each year. Current treatments are ineffective. Ion channels are emerging as attractive therapeutic targets and in particular intermediate conductance Ca^{2+}-activated K^+ channel,

$K_{Ca}3.1$, has been shown to modulate the activity of several structural and inflammatory cells which play important roles in disease. We hypothesise that $K_{Ca}3.1$-dependant cell processes are a common denominator in lung fibrosis

The initial aim of the study was to determine the level of $K_{Ca}3.1$ channel expression in human lung fibroblasts.

Human lung fibroblasts derived from human lung were grown in vitro, and characterised by immunofluorescence. To determine the purity of fibroblast cultures, and therefore the optimum passage for future experiments, 2 donors were characterized by immunofluorescence at passage 2, 4 and 7. Western blot, RT-PCR and patch clamp electrophysiology were also performed to determine the level of $K_{Ca}3.1$channel expression at the different passages.

We have determined the optimum passage for future experiments and have shown that human lung fibroblasts can be cultured in vitro. We have also shown that human lung fibroblasts express $K_{Ca}3.1$ channel of both the protein and mRNA level, and whole cell patch clamping results confirmed fibroblasts express $K_{Ca}3.1$-like channel currents. Further characterization is underway and will be followed by further functional studies in the presence of $K_{Ca}3.1$ channel blockers.

Amanda Jane Sutcliffe

"Airway Smooth Muscle from Asthmatic subjects exhibit oxidative stress"

Oxidative stress is thought to play a significant role in airways diseases such as asthma. Airway smooth muscle cells (ASM) have been identified as playing a pivotal role in the pathophysiology of the asthmatic phenotype.

In response to various stimuli, activated inflammatory cells and environmental insults can generate oxidants, which lead to the formation of reactive oxygen species (ROS) and provoke amplification of the inflammatory processes in the airways, which can have detrimental effects, causing DNA and cellular damage.

Human airway smooth muscle cells from subjects with asthma (N=5) and healthy volunteers (N=4) were treated in the presence of absence of H_2O_2 and in the presence of absence of the DNA repair enzyme 8-oxoguanine DNA glycosylase (hOGG1). DNA strand breaks were assessed by tail moment using the comet assay.

Asthmatic ASM has an increased amount of 8-oxo-deoxyguanosine (8-oxodG), an established marker of oxidative DNA damage.

In the absence of H_2O_2 the ASM cells in asthmatics exhibited increased oxidative stress which was different compared to controls. Both increase in response to H2O2 and the change in oxidative damage was similar.

Therefore, background oxidative stress is increased in ASM cells derived from asthmatics compared to normal controls suggesting that asthmatic ASM cells are exposed to endogenous oxidative stress.

Heidi Wan

"Seminar title: Assessing the Role of Structural Cells in Asthma Progression/Exacerbation Using a 3-Dimentional Ex Vivo Cell Model"

Asthma is one of the major leading causes of death worldwide. Despite different therapies are currently being used; they are inadequate in accommodating the large asthmatic population due to the variations between individuals. Recent research has shown that airway cell contents and extracellular matrix (ECM) protein disposition are altered. For instance, the epithelium on asthmatic airways has previously been found disrupted (Cohen 2007) with abnormal cilial function *in vivo* (Thomas 2009, in submission). Asthmatic patients are more susceptible to micro-organism infection (Sukkar 2006). Furthermore, asthmatic airway ECM has got altered protein deposition, such as an increase in fibronectin disposition (Hastie 2002), and changes in cell contents such as smooth muscle hyperplasia (Benayoun 2003). This abnormal matrix environment may enhance the malfunction of the epithelium. Together with the role of infection, these may promote the exacerbation of the disease. Therefore, the hypothesis is that the malfunctions of epithelium in asthmatic airways are due to intrinsic abnormalities, which are revealed in an altered matrix environment, leading to chronic inflammation and predisposition of exacerbation.

To test this hypothesis, primary human bronchial epithelial basal cells were cultured and differentiated to ciliated cells *ex vivo*. An asthmatic micro-environment was then introduced to the ciliated cells to look at the cilial function of the cells by using high-power field imaging. The result showed that bronchial ciliated cells which were developed *ex vivo* have lost their abnormality in cilial function, but reappeared when fresh asthmatic sputum was introduced to the cells. This suggests that the inability in efficient mucociliary function

may be an intrinsic factor in asthmatic airway epithelium, which may involve the presence of microorganism(s).

The specie(s) of microorganism(s) in asthmatic sputum involved in this abnormality is yet to be confirmed. This may help with identifying new therapeutic targets for drug discovery. Furthermore, the possible role of an altered 3D matrix towards epithelial malfunction is yet to be identified. I shall examine it by introducing the differentiated epithelium atop a collagen matrix with cell contents such as primary human airway smooth muscle cells or human peripheral blood fibrocytes. These cells have been found associated with asthma progression (Johnson 2001, Quan 2004). With the development of the 3D model, I shall assess the interactions between the epithelium, the collagen matrix, and the cell embedded in the matrix. Changes in cell behaviours such as cell survival, proliferation, activation and possible migration within the matrix would be identified.

Renal Studies

Sarah Hosgood

"Ischaemia reperfusion injury in kidney transplantation; The role of preservation"

Introduction

Traditionally, organs are preserved under hypothermic conditions. This reduces the metabolism allowing the organ to be stored simply without the need for oxygen before transplantation. Nonetheless, the anaerobic environment causes substantial tissue injury which exacerbates the level of ischemia reperfusion (I/R) injury after transplantation. There is little experimental evidence on the effects of hypothermic injury within what is regarded the safe limit of kidney preservation. In addition, more evidence is needed to assess the effects of different preservation techniques on I/R injury so that methods can be improved. This study assessed the level of I/R injury in porcine kidneys after varying durations and different techniques of hypothermic preservation.

Methods

Porcine kidneys were preserved for periods of 2, 6, 18 or 24 hours (n = 6) to assess the effects of hypothermic preservation.

Three different solutions used for static storage; Hyperosomolar citrate (HOC), Histidine-Tryptophan-Ketoglutarate (HTK) and University of Wisconsin (UW) solutions were then compared to hypothermic machine perfusion (HMP) (n=6). Kidneys were preserved for 18 hours. Renal function, oxidative damage, inflammation and morphology were assessed after preservation by reperfusing the kidneys for 3 hours with autologous blood using an isolated organ perfusion system.

Results

During reperfusion levels of serum creatinine (Cr) levels were significantly higher in the 18 and 24h groups and creatinine clearance (CrCl) lower compared to the 2h group (P = 0.001, 0.003). Fractional excretion of sodium was significantly lower in the 2h group compared to the 18 and 24h [Area under the curve (AUC) 2h; 25.6 ± 27.0, 6h; 51.5 ± 38.2, 18h; 116.6 ± 37.5, 24h; 126.0 ± 34.1%:P= 0.003].

Intra-renal resistance was significantly lower in the HMP group compared to HOC and UW [AUC; HMP 3.8±1.7, HOC 9.1±4.3, UW 7.7±2.2, HTK 5.6±1.9, mmHg/min;P=0.006] and creatinine clearance (CrCl) was significantly higher compared to the UW group [AUC CrCl; HMP 9.8±7.3, HOC2.2±1.7, UW 1.8±1.0, HTK 2.1±1.8,ml/min/100g;P=0.004]. Tubular function was significantly improved in the HMP group (P=<0.05), however levels of lipid peroxidation were significantly higher (P=0.005).

Conclusion

This study demonstrated the progressive effects of hypothermic injury in porcine kidneys over a 24 hour period. The majority of injury appeared to occur over the first 18 hours with no further injury at 24 hours. HMP demonstrated a reduced level of preservation injury compared to the static techniques resulting in improved renal and tubular function and less tubular cell inflammation during reperfusion.

Eman Yousuf Abu-Rish

"Investigation of the intracellular pathways of Toll-like receptor signalling in human B cells"

Toll-like receptors are family of Pattern- recognition receptors which are structurally and functionally related to drosophilaToll receptors. In human, 10 members of TLRs family have currently been identified which play important roles in innate and adaptive immunity.

Recently, TLR9 targeting therapy has become a new approach to immunotherapy hence TLR9 will be the main interest of our study. In human immune cells, TLR9 is primarily expressed in resting B cells and plasmacytoid dendritic cells (pDC) where it is localized within intracellular endosomes.

Toll-like receptor 9 agonists, synthetic unmethylated CpG oligodeoxynucloetides (ODN), have demonstrated substantial potential as vaccine adjuvants, and as therapies for the treatment of cancer, infectious and allergic diseases.

Three major classes of CpG ODN were identified; class A which mainly induces pCD INFα secretion, Class B which mainly induces B-cell proliferation and cytokines production and class C that have combined intermediate effects of the former two classes.

CpG ODN 2006 which is a member of class B ODN is the agonist we are going to employ to investigate the intracellular signaling pathway of TLR9 in human B cells and several cell lines. B-cells activated with class B show increase in cellular proliferation, surface markers expression, cytokine secretion.

According to the results obtained by a previous PhD student, Areej Assaf, a non activating dose of this agonist might induce a tolerance to subsequent activating dose of CpG 2006, these results must be further verified through peer investigation of the tolerizing dose and the signaling pathway that initiates this effect as this could have important implications for the use of CpG-ODN in immunotherapy.

Furthermore, we are going to study the effect TLR9 activation and its agonist on the expression two B-cell stimulatory cytokines: B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) and their receptors in normal B-cells and cell lines. The clinical implication of this arises from the recent findings; that inappropriate activation of TLR7, TLR8, and TLR9 by stimulation with self motifs is involved in the pathogenesis of

autoimmune diseases such as systemic lupus erythematosus (SLE) which specifically we are going to study.

Pathogenesis of SLE involves the accumulation of self RNA and DNA containing apoptotic debris that is capable of activating TLR7, TLR8 and TLR9. In addition, BAFF and APRIL expression has been found to be elevated in patients with SLE which is shown to be augmented with CpG ODN treatment in mice giving rise to the potential benefit of the expected tolerizing dose of CpG ODN in the treatment of SLE.

For complete understanding of such effects we are going to explore the signaling pathways involved in TLR9-mediated BAFF expression, and the effects of TLR9 tolerance or inhibition on these.

Elvina Chrysanthou

"The lectin pathway of complement activation in cerebral ischaemia and reperfusion injury"

The complement system is a powerful defence mechanism of the innate immune system against pathogenic microorganisms. One of the activation pathways of the complement cascade, called the lectin pathway, is activated by the recognition of pathogen associated molecular patterns and the presence of oxygen deprived cells. Mannan binding lectin associated serine protease 2 (MASP-2) is an essential component of the lectin pathway and its absence renders this activation pathway dysfunctional. Brain ischaemia, generally seen during stroke, involves oxygen and nutrient deprivation, resulting in neuronal cell injury and cell death. The potential role of the lectin pathway in recognising oxygen-deprived cells and subsequent exacerbation of tissue damage has not, to date, been addressed in brain ischaemia and reperfusion injury and that is the aim of this project. The kainate acid injection model of stroke showed that the complement system is not activated by the excitotoxicity induced in the brain at least within 24 hours post injection. Furthermore, TTC staining of brain tissues after 30min of ischaemia and 24h of reperfusion using the 3 vessel occlusion stroke model indicated that MASP-2 deficient mice had a trend towards smaller infarct sizes compared to WT mice. Real time was also performed on sham operated mice giving the basal expression of markers of inflammation. Moreover, in vitro CNS ischaemia studies based on hypoxia and glucose deprivation on rat cortical neurons

were performed to characterise complement activation (following oxygen and glucose deprivation) due to structural changes of the cells leading to direct damage of the cells and secondly to test whether the activation is less in MASP-2 deficient serum. Finally, inhibitory peptides designed against human MASP-2 showed an inhibitory effect on the lectin pathway activation in mouse serum under in vitro conditions. Verification of the protective effects of the lectin pathway deficiency could provide a potential therapeutic window for the treatment of cerebral ischaemia and reperfusion injury using MASP-2 inhibitory antibodies administered to limit the inflammatory processes following ischaemic insults.

Chris Furze

"Engineering new biological activities into members of the collectin family of animal lectins"

The collectin family of animal lectins (carbohydrate-binding proteins) play key roles in the innate immune system. For example, mannose-binding lectin (MBL) is the initiating component of the lectin pathway of complement activation, which neutralises invading microorganism directly by targeting sugars on their surfaces and helps to direct and stimulate an effective adaptive immune response. This occurs as a consequence of the activation of MBL-associated serine protease-2 (MASP-2) zymogen. Another member of the collectin family, pulmonary surfactant protein-A (SP-A) also targets pathogens through their sugar epitopes and is an important regulator of the inflammatory state of the lungs.

SP-A has the same architecture as MBL however is unable to activate complement itself. One aim of the project was to introduce complement activity into SP-A by engineering in the MASP-2 binding site and altering the flexibility of the SP-A structure. We have demonstrated MASP-2 activation by the modified SP-A protein and building upon this result we would look to establish whether complement activation is happening.

In another project, the sugar specificity of MBL will be modified to target sugar epitopes commonly presented on the surface of tumour cells, but not normal cells. This activity could be used to specifically target and destroy abnormal cells by the host's immune response.

I will discuss the progress that has been made so far and also the questions our results have raised about how MASP-2 activation is controlled by MBL and the approach we shall take to find the answers.

Fatima Mohamed

"The Role of Complement Properdin in control and Virulence of Listeria monocytogenes"

Properdin or complement factor P is a normal blood protein secreted by white blood cells and endothelial cells, it is important in the immune defence and classically has a role in strengthening the activation of complement, a system of proteins essential in the first time defence against infection. Properdin acts by binding and stabilising two specific converting enzyme complexes, which are normally labile (C3bBb and C3bBbC3b).

Properdin has a particular role in fighting off meningococcal meningitis disease. Recent data document a role of properdin as pattern-recognition molecule that binds certain microbial surface, apoptotic cells and necrotic cells, and in maturation of dendritic cells and reactivity of macrophages.

Its role in infection with intracellular pathogens such as *Listeria monocytogenes* is not yet known. Therefore, my project so far has used cells specific immune from properdin-deficient and wild type mice to investigate the role of properdin in bacterial survival, cytokine release and phenotypic changes of the eukaryotic cells by electron microscopy study and proteomics.

I will report on first, definite results of my in vitro work, which for the first time point to a significant role of properdin in infection with the interacellular, Gram-positive pathogen *L. monocytogenes.* My planned in vivo experiments using properdin-deficient and wild type mice will demonstrate the relevance of my findings so far and further characterise the disease process.

Mahmoud Tawfick

"Using Recombinant DNA Technology in Vaccine Development"

Salmonella genus is one of the most common human enteric infections pathogens. These infections caused by Salmonella are associated with considerable morbidity and

mortality; especially in developing countries. Enteric fever is a worldwide problem and extensively widespread in the developing countries of the tropics. It is estimated about 21 million cases and 600 000 deaths caused by enteric fever occur throughout the world per annum. Salmonella enterica Serovar Paratyphi A (*S. paratyphi A*) is a member of Salmonella enteric subspecies enterica and is associated with paratyphoid fever.

It is usually thought that typhoid fever is more widespread and has a more severe clinical course than paratyphoid fever. However many recent studies propose that paratyphoid fever has become the most frequent cause of enteric fever in some areas in the world and that the clinical appearance of typhoid and paratyphoid fever are similar and impossible to distinguish. Current available vaccines provide protection only against typhoid fever. There are no modern licensed paratyphoid vaccines. Protection against *S. Paratyphi A* may have to await the development of a future paratyphoid A vaccine.

As the first and the most important step in colonization, the establishment of infection and starting the pathogenesis is the adhesion of bacteria to the human mucosal epithelial cells which mediated by adhesions. My project aims to isolation, expression of stkF gene which is (adhesion- like) putative fimbrial protein and using this recombinant protein in the production of polyclonal antibody in mice, diagnosis of *S. paratyphi A* infections by ELISA and then examining the protective effect of this antigen. Fimbriae are particularly attractive candidates for epitope display for several reasons: (1) they are present in extremely high numbers at the cell surface, (2) they are strong immunogens, and (3) they possess inherent adhesive properties. The majority of work dealing with fmbriae assisted peptide display has been focused on the development of recombinant vaccines.