University of Leicester

Department of Infection, Immunity & Inflammation

1ST ANNUAL POSTGRADUATE STUDENT CONFERENCE

15-17 April 2009

Bennett Building

Program & Abstracts

Wednesday, 15th April 2009

9.30-9.35	Conference Opening	
9.35-9.45	Head of Department Address	
	LT3 - Chair: Dr. Hasan Yesilkaya	LT5 - Chair: Dr. Catherine Pashley
9.45-10.15	Marialuisa Crosatti "Use of invertebrate models to study *Pseudomonas aeruginosa* virulence"	Fattah Sotoodehnejadnematalahi "Effect of hypoxia on Veriscan gene expression in human primary macrophages"
10.15-10.45	Yehia Mohamed "APC/Tumour cell hybrids as candidate tumour vaccines"	Sarah Hosgood "Ischaemia reperfusion injury in renal transplantation"
10.45-11.15	Manjith Narayanan "Pulmonary acinar structure and function after preterm birth"	Abbie Fairs "Utilising *Aspergillus fumigatus* culture as a means of aiding a diagnosis of ABPA, and assessing indoor fungal exposure in sensitised patients"
11.15-11.45	Tea/Coffee Break (in the foyer)	
	LT5 - Chair: Dr. Helen O'Hare	
11.45-12.15	Lorenza Francescut "Role of hypoxia in regulation of the matrix metalloproteinase 7 gene"	
12.15-12.45	Sarah Glenn "Genes involved in the attachment of *Listeria monocytogenes* to abiotic surfaces"	
12.45-13.15	Neil Martin "Defining phenotypes in exercise asthma"	
13.15-14.15	Lunch (in the foyer)	
	LT3 - Chair: Dr. Anthony Keeble	
14.15-14.45	Rasheedah Ahmad "investigating steroid resistance mechanisms in asthma in particular airways smooth muscle cells"	
14.45-15.15	Cristina Pollard "Studies on the assessment and outcome of human islet autotransplantation"	
15.15-15.45	Kate Hargreaves "Isolation and characterisation of bacteriophages from environmental *Clostridium difficile* isolates"	
15.45-16.15	Tea/Coffee Break (in the foyer)	

Thursday, 16th April 2009

	LT3 - Chair: Dr. Ruth Saunders	LT5 - Chair: Dr. Izabella Pawluczyk
9.30-10.00	Salwa Abdalla "Mechanisms of biofilm formation by *Listeria monocytogenes*"	Clement Nyame "Activation of peroxisome proliferator-activated receptors in proximal tubular epithelial cells by albumin-bound fatty acids and GW501516; an *in-vitro* study"
10.00-10.30	Heidi Wan "The development and validation of *ex-vivo* models to study the interactions between structural cells and inflammatory cells in asthma"	Chris Furze "Engineering new biological activities into members of the collectin family of animal lectins"
10.30-11.00	Kayleigh Martin "Neutrophils utilise different signalling pathway in response to chemokines and growth factors in a 3D microenvironment"	Abdulkareem Alherz "Elucidating genes up-regulated by hypoxia and LPS in human macrophages"
11.00-11.30	Tea/Coffee Break (in the foyer)	
	LT5	
11.30-12.30	Post-doc Seminar: Dr. Natalie Garton "Do mycobacterial lipid bodies have a role in the chemosensitivity and transmission of TB?"	
12.30-13.30	Lunch (in the foyer)	
	LT5 - Chair: Dr. Russell Wallis	
13.30-14.00	Mona Bafadhel "Biomarkers in chronic obstructive pulmonary disease"	
14.00-14.30	Jon van Aartsen "Characterisation of a novel genomic island-borne *Klebsiella pneumoniae* fimbrial operon"	
14.30-15.00	Azam Hayat "*Neisseria meningitidis* infection study in MASP-2 deficient mice"	
15.00-15.30	Tea/Coffee Break (in the foyer)	
	LT3 - Chair: Dr. Umakhanth Venkatraman Girija	
15.30-16.00	Sumit Gupta "Computed tomography analysis of severe asthma"	
16.00-16.30	Manoj Menon "The use of ultrasound to measure quadriceps size in COPD"	

Friday, 17th April 2009

	LT3 - Chair: Dr. Obolbek Turapov	LT5 - Chair: Dr. Nick Lynch
9.30-10.00	Hemant Kulkarni "Aerosol spread of pathogens"	Amanda Sutcliffe "Synthetic function of airway smooth muscle is altered in asthma"
10.00-10.30	Anita Raj "Do patients with asthma and oesophageal dysfunction have eosinophilic oesophagitis?"	Sarah Smeaton "The nature of protection by pneumococcal proteins pneumolysin and neuraminidase"
10.30-11.00	Andrew Bell "Do fat and lazy tubercle make TB hard to treat?"	Arine Ahmad "Molecular and immunological studies of pathogenic free-living amoebae"
11.00-11.30	Tea/Coffee Break (in the foyer)	
	LT5	
11.30-12.30	Invited Speaker: Dr. Karl Wooldridge "Vaccine against bacteria and why we don't have a universal meningococcal vaccine yet"	
12.30-13.30	Lunch (in the foyer)	
	LT5 - Chair: Dr. Linda Franklin	
13.30-14.00	Mohamed Alblihed "Immunomodulatory role of (R) and (S) enatiomers of β2 agonists in airway smooth muscle: implications in asthma pathogenesis"	
14.00-14.30	Syed Kashif Haleem "Role of lectin pathway recognition molecule ficolin-A In pneumococcal infection"	
15.00-15.30	Tea/Coffee Break (in the foyer)	
	LT3 - Chair: Dr. Barbara Rieck	
15.30-16.00	Brenda Kwambana "Molecular characterization of the development and composition of the nasopharyngeal microbiome: impact of PCV7 vaccination"	
16.00-16.30	Nadia Yousaf "Normal range for 24-hour cough frequency"	
16.30-17.00	Concluding Remarks: Dr. Caroline Beardsmore	

Name:	Sarah Glenn (5th year part-time)
Supervisor:	Prof. Peter Andrew
Seminar title:	Genes involved in the attachment of *Listeria Monocytogenes* to abiotic surfaces

Listeria monocytogenes is a gram-positive bacillus, which can cause a variety of infections in human collectively termed Listeriosis. *L. monocytogenes* has previously been shown to attach to a variety of different surfaces. Attachment to abiotic surfaces allows these organisms to prolong their survival. Due to this ability, high numbers of bacteria are often isolated from food processing/packaging factories. Strains isolated from these factories are associated with outbreaks of Listeriosis. The molecular mechanisms behind the process of attachment of *Listeria monocytogenes* are still poorly understood. Using transposon mutants from a previously made library an existing attachment assay was modified to allow screening of strains for reduced attachment in comparison to the wild type at a variety of temperatures. Two isolates (M237 and B380) were found to show significantly reduced attachment in comparison to the parental strain 10403s at temperatures of 10ºC, 20ºC and 30ºC but not at 37ºC. Nested PCR of the transposon identified mutations either in or just upstream of a BglG transcriptional antiterminator. Subsequently deletion mutants are being made in these genes. The gene *relA* had previously been shown to have reduced surface attached growth in comparison to the parent. Using the modified assay a strain with a deletion mutation in this gene was shown to have reduced attachment when effectively starved of fresh nutrients, confirming previously studies suggesting that initial attachment and subsequent biofilm formation is essential in response to starvation. A preliminary PCR of wild type strains isolated from dairies would indicate however that the *relA* gene might not be present in all environmental isolates. This work so far suggests three possible genes that may be essential for initial attachment to abiotic surfaces.

Name:	Arine Ahmad (1st year/APG)
Supervisor:	Dr. Simon Kilvington & Prof. Peter Andrew
Seminar title:	Molecular and immunological studies of pathogenic free-living amoebae

Balamuthia mandrillaris and *Naegleria fowleri* are free-living amoebae that are able to cause fatal encephalitis both in humans and animals. Limitation in culturing the *B. mandrillaris* using the conventional culture plates has hampered the study of the amoebae. To date, only two environmental strains have been isolated from soil samples. In addition, morphological similarities between the pathogenic and non-pathogenic *Naegleria* has highlighted the need for a better diagnostic technique. The aims of this study are 1) to develop a culture-independent polymerase chain reaction (PCR) technique for rapid detection of both free-living amoebae, particularly from environmental samples and 2) to develop specific antibodies (monoclonal and by phage-display technology) against *B. mandrillaris* for used as diagnostic tools when combined with flow cytometry and immuno-magnetic separation.

A suitable DNA extraction method was developed using a combination of UNSET buffer and glass beads. Subsequently, the DNA was further purified using a commercial kit prior PCR amplification using specific primers. Preliminary results show that out of 89 water sample collected from a power station in France, 7 were positive for *B. mandrillaris*. Confirmation by DNA sequencing showed more than 99% homology with *B. mandrillaris* sequences deposited in GenBank. These findings indicate that water with high temperatures could also be a potential reservoir for *B. mandrillaris*. None of the 18 soil samples tested have given positive result. Currently, assay for the detection of *N. fowleri* is being done using nested PCR in order to compare the results with those obtained using the cultivation technique.

Name:	Yehia Mohamed (2nd year)
Supervisor:	Dr. Mike Browning
Seminar title:	APC/Tumour cell hybrids as candidate tumour vaccines

Cancer is the second main leading cause of death worldwide after CVDs. Treatment strategies being used in cancer treatment are surgical excision, chemotherapy, and radiotherapy which have a lot of side effects on the normal tissues. Tumour immunotherapy is a good strategy for treatment of most malignant diseases. In our laboratory, my supervisor produced new hybrid cell lines

by fusion of professional antigen presenting cells with parent tumour cells, these hybrid cell lines have the ability to present most tumour associated antigens of their parent tumour cells and in the same time have the T-cell stimulating ability through expression of HLA class I, HLA class II, and co-stimulatory molecules like CD80, CD86, and CD40. My project aims to investigate the ability of some of these hybrids to induce a specific T-cell response using PMBC from normal healthy allogenic donors and tumour bearing patients.

Name:	Fattah Sotoodehnejadnematalahi (2nd year)
Supervisor:	Dr. Bernie Burke
Seminar title:	Effect of hypoxia on Veriscan gene expression in human primary macrophages

Hypoxia or low oxygen tension is a hallmark of many pathological tissues such as solid tumours and found at ischemic and wound sites which are basically characterised by high infection and inflammation. Macrophages are a type of white blood cell which originates from monocytes. They are phagocytic cells which are capable of pathogen up-take and direct destruction. Macrophages have been shown to accumulate in large numbers in hypoxia sites such as pathological tissues including solid tumours, and also non pathological tissues such as spleen.

Previous studies have shown that certain hypoxia inducible genes are up-regulated by macrophages under hypoxic conditions; an extra cellular matrix (ECM) proteoglycan versican is one of the hypoxia inducible genes.
Versican is a member of the large aggregating chondroitin sulphate proteoglycans and expressed in various adult tissues such as blood vessels, skin, and developing heart. Increased versican expression is often observed in tumour growth in tissues such as breast, brain, ovary, gastrointestinal tract, prostate, and melanoma.

Recent work has investigated the effect of hypoxia on the mRNA of versican on monocyte derived macrophages under hypoxia and comparing them with cells under normoxic condition. It has been shown that monocyte derived macrophages under hypoxia produced 700-fold more versican mRNA than normoxic cells.

To further elucidate the mechanism responsible for the up-regulation of versican mRNA by hypoxia, this work will attempt to define the DNA sequence within the versican promoter which up-regulates mRNA levels in hypoxic human macrophages and then identify the hypoxia inducible transcription factors, which bind to the versican promoter and up-regulate it.

Name:	Neil Martin (1st year/APG)
Supervisor:	Prof. Ian Pavord
Seminar title:	Defining phenotypes in exercise asthma

Asthma is a significant and growing problem in the UK. Exercise related symptoms occur in up to 50% of asthmatic patients and are often the most incapacitating aspect of the disease in young people. Evaluation of exercise-induced asthma is not straightforward as exercise related respiratory symptoms are common in all adolescents. To complicate matters further, endurance athletes, particularly those habitually exercising in physically challenging environments, commonly report exercise related respiratory symptoms and have a high incidence of airway hyperresponsiveness. Anecdotal reports have suggested that exercise-related symptoms in athletes are associated with different patterns of airway inflammation and a diminished response to corticosteroids. Thus, within the population of young adults with exercise-related respiratory symptoms, there may be multiple phenotypes of disease, differing in their pathophysiology and treatment response. Against this background there are controversies in how to effectively diagnose and manage patients with exercise related symptoms. Straightforward pulmonary function testing in fit young people is not sufficient and exercise testing itself is expensive, time consuming and impractical in most settings. This has led to the substitution of specialised airway challenge tests in the diagnosis of exercise-induced bronchoconstriction, working either directly on the airway or indirectly via inflammatory mediators. Varying responses to these challenge tests by individuals with the same symptom complex have made the diagnostic challenge even more complex. More information is required before we can conclude that these tests are a suitable surrogate for investigating the heterogeneity of exercise-induced airway symptoms and determining who is going to respond well to corticosteroid treatment.

We plan to characterise the bronchoconstrictor and airway inflammatory response to exercise in a large and heterogeneous population of young adults who report exercise-related symptoms, compare this to the response seen in asymptomatic controls and determine whether differences in response are related to diagnosis and baseline demographic characteristics including habitual exercise, the exercise environment, airway inflammation and airway responsiveness. We will compare the response to the proposed substitute challenge tests (eucapnic voluntary hyperventilation, mannitol challenge, methacholine challenge) relating this to phenotype and the response to inhaled corticosteroids, aiming to determine the most effective testing regime for identifying clinically important, treatable pathology. The large amount of data generated will be subjected to cluster analysis in the hope that novel phenotypes might be identified. The results of this study will enable larger more focused projects to be carried out in the near future.

Name:	Manjith Narayanan (1st year/APG)
Supervisors:	Dr. Caroline Beardsmore & Prof. Mike Silverman
Seminar title:	Pulmonary acinar structure and function after preterm birth

Background: In fatal cases, extreme preterm birth has been associated with simpler acinar structure and larger and fewer alveoli. There are no equivalent data on survivors. We measured acinar structure and function in long-term survivors of preterm chronic lung disease.

Methods: We compared 11 ex-preterm (24-32 weeks gestation) children with chronic neonatal lung disease to 20 term-born healthy children of similar age (11 years), height and weight. Children performed spirometry, plethysmography and multi-breath nitrogen washout (MBNW) and underwent hyperpolarised He-3 magnetic resonance scanning (HHe3MR). The contribution of acinar airways (Sacin) and conductive airways (Scond) to ventilation inhomogeneity was calculated from MBNW. We derived the apparent diffusion coefficient (ADC), a measure of restriction of diffusion of helium at acinar level and a surrogate for alveolar size, from HHe3MR data.

Results: Spirometric and plethysmographic indices that differed between ex-preterms and controls are shown in the table. Sacin was significantly higher (ie more acinar inhomogeneity) in preterms, but not Scond and ADC.

Table: Indices of lung function and acinar structure in preterms and terms. Values are mean(standard deviation)

	Preterm	Control	p
FEV1/FVC (z-score)	-0.79(1.1)	0.13(0.69)	0.02
FRC (z-score)	-0.14(0.65)	-1.08(0.95)	0.003
S_{acin} (l^{-1})	0.186(0.088)	0.095(0.045)	0.007
S_{cond} (l^{-1})	0.056(0.049)	0.036(0.027)	0.24
ADC (cm^2s^{-1})	0.117(0.008)	0.123(0.017)	0.23

Conclusions: Children born preterm had increased ventilatory inhomogeneity at acinar level (Sacin) when compared to controls, suggesting persistence of acinar airway disease. However, mean ADC and thus alveolar size did not differ, implying that catch up in alveolization may be possible.

Name:	Sarah Hosgood (1st year/APG part-time)
Supervisor:	Prof. Michael Nicholson
Seminar title:	Ischaemia reperfusion injury in renal transplantation

Introduction: Ischaemia reperfusion (I/R) injury is a serious complication in renal transplantation. The re-introduction of oxygenated blood after a period of ischaemia initiates a cascade of complex actions that involves the generation of reactive oxygen species (ROS), inflammatory process and activation of

the complement system. The long term consequences of I/R injury in clinical transplantation results in reduced graft survival. It is therefore important to investigate the mechanisms and therapies including organ preservation techniques that may ameliorate this injury process to improve graft survival.

Methods: Reperfusion model: Porcine kidneys were subjected to a significant level of warm ischaemia and cold ischaemic injury. They were then reperfused with oxygenated autologous blood on an isolated organ perfusion circuit. Kidneys were treated with the membrane gaseous mediator, Hydrogen Sulphide (H_2S). Three doses of H_2S were infused into the arterial arm of the isolated circuit during reperfusion; Control, 1mM, 0.5mM and 0.1mM H_2S (n = 4). To investigate the mechanisms of ischaemic injury and assess renal function, tissue, blood and urine samples were analysed.

Results: Renal function: 1mM and 0.5mM hydrogen sulphide significantly improved the renal blood flow and lowered intra-renal resistance (P =< 0.05). Serum creatinine (Cr) fall and Creatinine clearance (CrCL) were significantly improved with the treatment of 0.5mM and 1mM H_2S. (Area under the curve (AUC) Cr µmol/L.h; control 2257 ± 152.1, 1mM 1549 ± 286.6, 0.5mM 1647 ± 310.1, 0.1mM 1812 ± 328.7; P = 0.013) (AUC CrCl ml/min/100g.h; control 1.5 ± 1.5 1mM 5.2 ± 2.35, 0.5mM 7.57 ± 6.33, 0.1mM 5.57 ± 2.87; P =0.04). Doses of 1mM and 0.1mM H_2S reduced renal tubular damage with significantly lower levels of total nitric oxide in the urine compared to the control (1mM; 25.45 ± 4.02, 0.1mM; 13.58 ± 10.02, Control; 45.42 ± 10.71µmol/L: P = 0.002).

Conclusion: This study provides new evidence of the physiological role of hydrogen sulphide in ischaemically damaged porcine kidneys. Hydrogen sulphide improved renal blood flow and ameliorated the renal dysfunction associated with ischaemic damage. It therefore has potential as a new therapy against I/R injury in kidney transplantation.

Name:	Clement Nyame (2nd year)
Supervisor:	Prof. Nigel Brunskill
Seminar title:	Activation of peroxisome proliferator-activated receptors in proximal tubular epithelial cells by albumin-bound fatty acids and GW501516; an in-vitro study

Peroxisome Proliferator-Activated Receptors, PPARs, members of nuclear hormone receptor superfamily are ligand-dependent transcription factors. They play crucial role in regulating genes that are involved in lipid metabolism, glucose homeostasis, cell growth and differentiation. Also implicated in immune and inflammatory responses.

The three isotypes identified in the human kidneys to date are PPAR-α, β/δ and γ. Previous study from Prof. Brunskill's laboratory has demonstrated that albumin-bound fatty acids induce human proximal tubule cell apoptosis through PPARγ activation.

The aims of this study were to; assess expression of PPARs, establish PPAR response element (PPRE) assay, and investigate the effects of various fatty acids associated with albumin, and other ligands on PPAR activity in HK2 cells.

Western blot and different levels of plasmid DNA transient transfections using Lipofectamine™ 2000 and Fugene-6 were employed to assess protein expression. Luciferase gene reporter assay was established and activation of PPARs determined. Cytotoxic effects of different ligand concentrations studied using MTT assay.

The results have shown native expression of PPARβ and γ, but not α in HK2 cells. Using plasmid DNA concentration of 0.5µg per well resulted in the over-expression of PPARs- β and γ by approximately 50% compared to control.
With luciferase studies, 15-deoxy-$\Delta^{12,\ 14}$-prostaglandin J_2 ($15dPGJ_2$) activated PPRE in a dose-dependent manner with a maximum of about 3.5 fold change over control at 5µM. This response was augmented to 5.5 fold change of control when PPARγ was over-expressed. Also, 5µM GW501516 activated PPRE with enhanced response of approximately 4 fold increase over control with PPARβ over-expression ($P<0.05$, n=3). Again, 5mg/ml of albumin-bound fatty acid but not albumin without fatty acid gave similar results of PPRE activation. These concentrations of ligands used were non-toxic to cells.

In conclusion, this study has shown that HK2 cells constitutively express PPARβ and γ but not α, and that these factors are activated by various ligands including fatty acid bound to albumin. This

interaction may provide an intervention in proteinuric renal diseases where proximal tubular cells are overloaded with lipids.

Name:	Cristina Pollard (1st year/APG)
Supervisor:	Mr. Ashley Dennison
Seminar title:	Studies on the assessment and outcome of human islet autotransplantation

Total pancreatectomy and islet autotransplantation represents an unusual but important salvage procedure for patients with intractable abdominal pain. In these patients where all other approaches have failed islet transplantation potentially obviates or reduces the need for exogenous insulin administration. Unfortunately few units have the combination of an adequate referral base, surgical expertise and the technical ability and experience to reliably and safely perform islet isolation and transplantation to justify the performance of this procedure. As there are only a small number of units that do have the necessary resources and can therefore safely offer this clinically, there is a paucity of reliable data.

In Leicester this procedure has been performed since 1994 and over 50 patients have been treated representing one of the three largest series in the world and comfortably the largest in Europe. The Leicester experience in all aspects of the procedure represents a unique opportunity to study the patients already transplanted, and those who will receive transplants in the next 2-3 years. Potentially this may answer some important questions about the procedure and methods of optimizing it to improve the short and long-term outcome after the transplantion which could lead to improvements in the future performance of autotransplants and allografts.

This research project will investigate aspects of the procedure covering the preoperative phase, technical aspects of the surgery and islet infusion, and results of the treatment in the longer term.

Name:	Marialuisa Crosatti (2nd year/APG)
Supervisor:	Dr. Kumar Rajakumar
Seminar title:	Use of invertebrate models to study *Pseudomonas aeruginosa* (*P. aeruginosa*) virulence

P. aeruginosa is an opportunistic pathogen heavily implicated in hospital-acquired diseases. It possesses a core genome and mobile elements (for example genomic islands, plasmids and phages) which acquisition or loss is affected by various circumstances and, among other reasons, they allow it to survive and prosper in many type of environments. However, ORFs annotation of mobile elements is often inconclusive making it difficult to assign specific phenotypes. We propose to allot a function or functions to genomic island by comparing isogenic strains in several models of infection.

We focused our effort on tRNA-harbored genomic islands by creating deletion-mutants lacking of them and by capturing them in a plasmid: we propose to compare mutants (with and without islands) with wild-type strains in several models of infections and growth conditions to understand phenotype/phenotypes associated with the mobile elements. Although mice are commonly used to study P. aeruginosa virulence factors, we are utilizing alternative organisms that raise less ethical issues and are easier to manage such as *Caenorhabditis elegans* (*C. elegans*) and *Acanthamoeba* spp (*A. castellanii* and *A. polyphagia*).

C. elegans is a nematode (multicellular) while *Acanthamoeba* is an amoeba (unicellular) but they are both free-living organisms that feed on bacteria. Because P. aeruginosa may be found in the same environment, it is plausible it evolved defense mechanism to protect against them, some of which are used during human infections.

There was not difference in the survival of *C. elegans* (strain CF512) on isogenic P. aeruginosa PA14 strains during a slow-killing assay. PA14 WT has been recognized as a particularly virulent strain whereas the isogenic mutant used was lacking of two genomic islands previously implicated in virulence (*Pseudomonas aeruginosa* pathogenicity island [PAPI]-1 and PAPI-2). However, a significant difference was observed when PA14 WT was compared with PA01, a less virulent strain. C. elegans sensitivity to *P. aeruginosa* clinical isolates proved to be rather diverse in this type of assay and further experiments are needed to underpin the reasons.

Assays with *A. polyphagia* on Non-nutrient agar (NNA) confirmed the difference seen between PA14 WT and PA01 and the lack of difference between PA14 WT and his isogenic double mutant lacking both PAPI-1 and PAPI-2. The same assay with *A. castellanii* uncovered a reduction in virulence with the double mutant showing a phenotype intermediate between PA14 WT and PA01.