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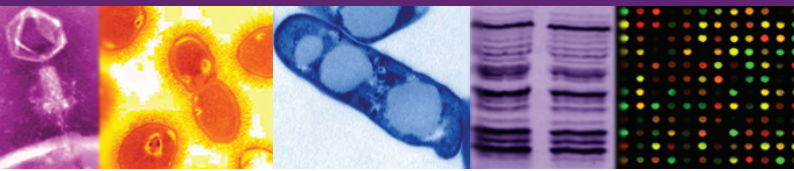

Department of Infection, Immunity & Inflammation

3rd Annual Postgraduate Student Conference

4th- 6th April 2011

Programme and Abstracts

Preface

Welcome from Department of Infection, Immunity and Inflammation, Postgraduate Student Staff Committee (PGSSC).

Striving to become better scientists, presenting our research professionally is a fundamental skill for the challenges that lie ahead of us. This conference provides a great opportunity for us to learn about the broad range of research topics that are being pursued within our department.

With the continuous support from the department, the Postgraduate Easter Conference has come to its 3rd year. This year, the conference is even more student-driven, with the student committee members taking a lead role in organising the event. The conference primarily targets 1st and 2nd year postgraduate students who present their year of research in a conference-styled format. Talks are categorized into themed sessions: Immunology, Respiratory diseases, Infection, and Others. Our 3rd and 4th year postgraduate students also take part by chairing sessions which is an essential skill to have when it comes to more senior posts. The refreshment and printing costs are generously being covered by our department. Furthermore, it is our honour to have the keynote speakers, Prof Mike Silverman (emeritus professor) to be sharing with us his experience and view on doing research as a career, and Prof Mark Jobling (Department of Genetics) to be talking to us the relationship between infectious disease and human genome.

When possible, feedback is provided from our experienced members of staff to the speakers to develop their presentation skills. We also strongly encourage you all to provide constructive criticism through comments and also questions. Above all, we would like to say thank you for your participation. We sincerely hope you enjoy the conference and the networking it provides.

Heidi Wan, Depesh Pankhania, Nawal Helmi and Sadiyo Said
Student Representatives of PGSSC 2011

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Abstracts

Day 1, 4th April, MSB LT2

Muhammad Adnan

"Structure function studies of pneumolysin"

Supervisor(s): Prof. Peter Andrew

The pneumococcus is one of the most important human pathogens, causing life threatening invasive diseases such as pneumonia and septicaemia, 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 53KD 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 plans are 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, express d4 with 11 extra amino acids at the N-terminus and express d4 fused to MBP. The purified d4 will be used in structural studies (NMR), to study interaction with the complement system and to test if it is a suitable vaccine.

Joshua Agbetile

"Isolation of filamentous fungi from sputum in asthma is associated with reduced post-bronchodilator FEV_1"

Supervisor(s): Prof. Andrew Wardlaw

Fungal sensitisation is common in severe asthma, but its clinical relevance, and the relationship to airway colonisation with fungi, is unclear. The range of filamentous fungi that may colonise the airways in asthma is not known.

Objective: To provide a comprehensive analysis on the range of filamentous fungi isolated from sputum in asthmatics and report the relationship between fungal sputum culture and the clinico-immunological features of their disease.

Methods: Patients with moderate-severe asthma were recruited and at a single stable visit underwent: spirometry; sputum fungal culture and a sputum cell differential count; skin prick testing to both common aeroallergens and an extended fungal panel (positive ≥ 3mm); specific IgE to *Aspergillus fumigatus* by CAP (positive >0.35 kU/L). Fungi were identified by morphology & species identity confirmed by DNA sequencing.

Results: 28 different species of filamentous fungi were isolated from sputa of 54% asthmatics, >1 species detected in 17%. This compared with 3 (17%) healthy subjects culturing any fungus ($p<0.01$). *Aspergillus* species were most frequently cultured followed by *Penicillium* species. Post bronchodilator FEV_1 % predicted in the subjects with asthma was 71% (+/-25) in those with a positive fungal culture vs 83% (+/-25) in those who were culture negative, ($p<0.01$). There were no differences in sputum cell differential counts between culture positive and negative patients.

Conclusions: A large number of thermotolerant fungi other than *Aspergillus fumigatus* can be cultured in sputum from moderate to severe asthmatics and a positive culture is associated with an impaired post-bronchodilator FEV_1. Sensitisation to these fungi is also common.

Noor Ali AL-Khathlan

"Abnormalities in the Lung Periphery in Cystic Fibrosis"

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

Cystic fibrosis (CF) is the most prevalent hereditary disease in the Caucasian population.(1) Conventionally, the progression of CF is monitored with spirometry, which is insensitive for detecting the involvement of peripheral airways until the disease is well-advanced.(2) There is growing evidence about the effectiveness of ventilation distribution tests such as multiple-breath nitrogen washout (MBNW) in detecting early changes in peripheral airways.(3,4) Analysis of the washout provides various indices of lung function including lung clearance index (LCI), and measures of ventilatory inhomogenity in the conducting and acinar airways (S_{cond} and S_{acin}). (5) The latter two indices can detect abnormalities in the airways at an earlier stage than spirometry.(3,5) My overall aim is to collect longitudinal lung function data from children with CF to identify the location of ventilation inhomogenity. Children have a wide range of tidal volumes, and their MBNW measurements are limited by the lack of standardised data collection and analysis. Therefore, the initial work has focused on standardising the methodology. Starting with adult healthy volunteers, I am examining the impact of rate and depth of breathing on MBNW indices. Separately, the impact on S_{cond} of excluding extreme breaths (i.e. $\pm$ 10% of the mean exhaled tidal

volume) from MBNW analysis has been examined and changes in excess of 25% have been found in 5/16 children.

To date, the first set of lung function tests (i.e. routine spirometry, plethysmography and MBNW) has been performed for 32 CF children. Preliminary data indicated an elevation in LCI and S_{acin} in almost all children. However, S_{cond} remains normal.

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Aarti Parmar

"Increased expression of immunoreactive Thymic Stromal Lymphopoetin in Severe Asthma"

Supervisor(s): Prof. Peter Bradding

Background: Thymic stromal lymphopoietin (TSLP) is a cytokine implicated in the pathophysiology of asthma through two pathways: a TSLP-OX40L-T cell axis and a TSLP-mast cell axis. Whether these pathways operate in human asthma is unknown.

Aims: To investigate whether mucosal TSLP protein expression relates to asthma severity and distinct immunological pathways.

Methods: GMA-embedded bronchial biopsies from healthy subjects (n=12) and patients with asthma (n=36) were immunostained for TSLP, OX40, OX40L, CD83, IL-13, and inflammatory cell markers. Extent of immunostaining was correlated with clinical data.

Results: Specific TSLP immunoreactivity was evident in both the airway epithelium and lamina propria of both healthy and asthmatic subjects. TSLP expression was significantly elevated in asthma as a whole compared to healthy controls. TSLP + cells also co localised with mast cells. Immunostaining for OX40, OX40L and CD83 in the airways was sparse, with no difference between asthmatic patients and normal control subjects. IL-13 staining was increased in non-epithelial cells within the airway epithelium in severe asthma.

Conclusions: TSLP expression is elevated in severe asthma despite high dose corticosteroid therapy. Although no activity of the TSLP-OX40L-T cell pathway was detected within asthmatic bronchial mucosa, it is possible that this pathway operates in secondary lymphoid organs such as draining lymph nodes. Mast cells and TSLP+ cells are in close approximation, suggesting that the

TSLP-mast cell axis is active in asthmatic bronchial mucosa and may be important in contributing to the chronicity and severity of disease.

Leonarda Di-Candia

"Expression of the Receptor for Advanced Glycation End products and High Mobility Group Box 1 in human airway smooth muscle cells"

Supervisor(s): Prof. Chris Brightling, Prof. John Challiss

Tissue injury and cellular stress (e.g. following pathogen invasion or trauma) can lead to the release of intracellular molecules known as damage-associated molecular patterns (DAMPs), which leak out of necrotic cells or are actively secreted by immune and non-immune cells (Kono 2008, Gardella 2002, Porto 2006). DAMPs signal through pattern recognition receptors (PRRs), such as the receptor for advanced glycation end-products (RAGE). RAGE has been implicated in several pathophysiological conditions, including diabetes, vascular disease, Alzheimer's disease, inflammation, and cancer (Schmidt 1999, Deane 2007, Kostova 2010). RAGE is abundantly expressed in the lung; however the expression of RAGE and DAMPs in specific airway cell types is poorly understood (Gefter 2009, Schlueter 2003).

It is hypothesised that mesenchymal airway cells, which are involved in respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD), express RAGE and DAMPs and that their expression/function is changed in airway disease.

The expression of RAGE and high mobility group box 1 (HMGB1), a RAGE ligand that functions as a DAMP, are being characterized *in vitro* in primary human airway smooth muscle (ASM) cells using RT-PCR, flow cytometry, immunofluorescence, and western blotting and *ex vivo* in bronchial biopsies using immunohistochemistry.

Preliminary results showed RAGE and HMGB1 mRNA and protein expression in cultured human ASM cells. Moreover flow cytometry data suggest an increase in cell surface RAGE expression in asthmatic compared with non-asthmatic ASM cells.

Future work will examine the signalling pathways activated following binding of HMGB1 to RAGE and the functional consequences of this activation.

Rececca J Fowkes

"β2-Agonist Responses in Human Lung Mast Cells and Airway Smooth Muscle"

Supervisor(s): Prof. Peter Bradding

Asthma is a major burden for patients and healthcare systems around the world. Currently, asthma is managed using inhaled corticosteroids which reduce airway inflammation and ease symptoms in around 90% patients. However, approximately 10% of asthmatics are poorly responsive. Such resistant patients have increased morbidity and over-utilise healthcare resources. In addition to inhaled steroids, asthmatics use β2-adrenoceptor (β2-AR) agonists which relieve and prevent bronchoconstriction by relaxing airway smooth muscle. However, these treatments may also work poorly and in some cases may reduce asthma control when administered regularly. Hence, there is an unmet need for novel compounds that control the inflammatory and/or tissue remodelling aspects of asthma.

Both human lung mast cells (HLMCs) and airway smooth muscle (ASM) cells express β2-AR and co-localise within the asthmatic airway, forming an interaction that is important in disease pathology. *In vitro* and *in vivo* studies show that acute exposure to β2-agonists limits release of mediators such as histamine from immunologically-activated HLMCs, protecting against allergen-induced bronchoconstriction. However, chronic HLMC exposure to β2-agonists causes rapid β2-receptor desensitisation and tolerance to treatment, removing a potentially important therapeutic benefit.

This research will test the hypothesis that the novel long-acting β2-agonists BI-1744 and BI-11054 induce less β2-AR desensitisation in HLMC than existing drugs. In addition we will study their effect on HLMC migration, adhesion, mediator release and their ability to synergise with glucocorticoids. A further aim is to examine the mechanism by which ASM induces HLMC mediator release in co-culture, and whether these compounds can inhibit this process.

Katy Roach

"Increased expression of the K^+ channel $K_{Ca}3.1$ in myofibroblasts from patients with idiopathic pulmonary fibrosis"

Supervisor(s): Prof. Peter Bradding

Idiopathic pulmonary fibrosis (IPF) is a common, progressive interstitial lung disease. Current therapies are ineffective: in consequence novel approaches to treatment are required urgently. Ion channels are emerging as attractive therapeutic targets and in particular, the Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ modulates the activity of several structural and inflammatory cells which play important roles in model diseases characterized by tissue remodelling and fibrosis. We hypothesise that $K_{Ca}3.1$-dependant cell processes are a common denominator in IPF.

Aim: To determine whether the $K_{Ca}3.1$ channel is expressed in human lung myofibroblasts, key effector cells in IPF.

Lung myofibroblasts were derived from healthy lung removed for carcinoma surgery and from lung biopsy tissue obtained from patients with IPF. Cells were grown in vitro, and characterised by immunofluorescence. Western blot, immunofluorescence and RT-PCR were used to determine the level of $K_{Ca}3.1$ channel expression in healthy and IPF cells. Patch clamp electrophysiology was performed to show the presence of functional $K_{Ca}3.1$ channels.

Both healthy and IPF myofibroblasts expressed $K_{Ca}3.1$ channel mRNA and expression increased significantly following 24 hours stimulation with TGF-β (10 ng/ml). $K_{Ca}3.1$ protein was also present in myofibroblasts from both groups. $K_{Ca}3.1$ ion currents were elicited in 63% of healthy and 77% of IPF myofibroblasts tested ($p=0.0127$) at passage 4, using the $K_{Ca}3.1$ channel opener 1-EBIO. These currents were blocked by TRAM-34 (200 nM), a highly selective $K_{Ca}3.1$ blocker. The currents induced by 1-EBIO were significantly larger in IPF cells compared to healthy cells ($P<0.0001$)

We show for the first time that human lung myofibroblasts express the $K_{Ca}3.1$ K+ channel, $K_{Ca}3.1$ currents are larger in cells from patients with IPF. These findings raise the possibility that blocking the $K_{Ca}3.1$ channel will inhibit pathological myofibroblast function in IPF, and thus offer a novel approach to IPF therapy.

Dhananjay Desai

"Cytokine profiling using cluster and factor analysis in sub phenotypes of severe asthma"

Supervisor(s): Prof. Chris Brightling

Rationale: Severe asthma is a heterogeneous disease. Defining its phenotypic heterogeneity is likely to shed light upon its immunopathogenesis and direct therapy.

Objectives: To determine the clinical and biological sub-phenotypes of severe asthma.

Methods: Subjects were recruited from a Difficult Asthma Clinic at a single centre (n=203) and assessments of lung function, allergen testing, asthma control and sputum induction were undertaken. We performed multivariate analysis on baseline clinical parameters to determine factors associated with frequent exacerbations (≥3 severe exacerbations/year) and airflow obstruction. Sputum supernatants from 164 of these subjects were analysed for 23 mediators using the Meso-Scale Discovery platform. We performed k-means cluster analysis using the baseline clinical parameters and sputum cell differentials to determine clinical clusters and principal component analysis to determine latent patterns of mediator expression, which were mapped onto the clinical

sub-groups. The repeatability of the biological latent factors was assessed in paired samples in 106 subjects and in three samples in 66 subjects.

Measurements and Main Results: Airflow obstruction was significantly associated with male gender and airway inflammation. We did not find predictors for frequent exacerbators. We identified 4 clinical clusters and 5 latent biological factors. The biological latent factors were differentially expressed in subjects stratified by sputum cell counts, asthma control and exacerbation frequency, but were not significantly different across the clinical clusters. The within subject repeatability of mediators was moderate; biological factors were consistent and tracked with sputum cell counts.

Conclusions: Cytokine profiling in severe asthma revealed repeatable latent biological factors that are associated with cellular profiles and inform the asthma phenotype.

Abdulwahab Zaid Binjomah

"Mycobacterial heterogeneity in sputum and pure culture"

Supervisor(s): Prof. Mike Barer

Tuberculosis (TB) is a worldwide health problem caused by *Mycobacterium tuberculosis* (Mtb). Only pulmonary TB infections transmit the disease and more than eight million new cases occur annually. It has been proposed that the bacilli in sputum discharged from pulmonary TB patients express properties necessary for their transmission to the new host.

Most pulmonary TB worldwide is diagnosed by examining sputum samples with a stain (Auramine O) that differentiates Mtb from other bacteria on the basis of its acid fastness. Acid fast (AF) staining of Mtb is used as a gold standard for TB diagnosis, despite the current advances in molecular biology. It has been reported that AF staining declines as Mtb cells persist in animal infection and non-replicating cultures. Moreover, there have been some reports of non AF cells occurring in human infections. Therefore, the presence of non-AF Mtb in sputum is an important consideration.

The aim of this study is to optimise the detection of tubercle bacilli in sputum and pure culture by immunofluorescence using an anti-Mtb antibody raised against a standard protein preparation (PPD) in combination with Auramine O AF staining to characterise the various Mtb sub-populations more accurately. Our primary target is to identify a population of non-acid fast Mtb cells in sputum, thus an immunofluorescence method was developed; this has been applied in combination with Auramine O labelling to tuberculous sputum samples.

The initial results demonstrate two apparently mutually exclusive populations (AF positive antibody negative and the converses). In contrast with Mtb from pure culture, a low number of bacilli interacting with the antibody were observed, compared with high numbers of Auramine O-positive cells.

This surprising result raises the possibility of relating these newly recognised subpopulations of Mtb in sputum to the clinical status of patients, their response to treatment and their infectiousness.

Latifa Chachi

"KCa3.1 ion channel blockers restore corticosteroid sensitivity in cytokine-treated airway smooth muscle (ASM) cells from both COPD and asthmatic patients"

Supervisor(s): Dr. Amrani Yassine

Background: The K+ channel KCa3.1 is expressed by several inflammatory and structural airway cells including mast cells and airway smooth muscle (ASM). We have proposed that this channel may play roles in the development of both airway inflammation and remodelling in asthma and COPD. The role of KCa3.1 channels in chemokine secretion by ASM is not known.

Aims: To investigate the expression of KCa3.1 in ASM in the airways of healthy and asthmatic subjects, and its function in ex vivo cultured primary human ASM cells.

Methods: Tissue was collected at bronchoscopy from subjects with asthma and healthy controls, and either processed into GMA for immunohistochemistry, or dissected for the culture of ASM. Further ASM samples were cultured from patients with COPD undergoing lung resection for carcinoma. To examine ASM chemokine production, we used our well-established cellular model of corticosteroid resistance (TNF□/IFN□-treated ASM cells).

Results: KCa3.1 immunostaining was evident in the ASM in healthy subjects and patients with asthma. There was no difference in the level of expression between healthy subjects (n=7), and those with moderate (n=5) and severe (n=6) asthma. In cultured ASM cells exposed to TNF□/IFN□, both ELISA and RT-PCR demonstrated expression of CX3CL1, CCL5 and CCL11 which were (1) synergistically produced at 24 h and (2) completely resistant to fluticasone pre-treatment (100 nM). We found that KCa3.1 block alone did inhibit the secretion of CX3CL1 but not CCL5 or CCL11. Interestingly, the failure of fluticasone to suppress CX3CL1, CCL5 and CCL11 expression in response to TNF□/IFN□ combination was reversed by TRAM-34 or ICA , a selective inhibitor of KCa3.1 channels. The increased anti-inflammatory action induced by the TRAM-34-fluticasone combination or ICA-fluticasone combination were observed in cells derived from healthy (n=3), asthmatic (n=3) and COPD (n=4) patients. In addition, restoration of corticosteroid sensitivity by KCa3.1 blockers was associated with an increased GR phosphorylation on serine 211 residues.

Conclusions: Together, these data suggest that targeting KCa3.1 channels could serve as a novel approach to enhancing/restoring steroid sensitivity in pulmonary disease.

Osama Eltboli

"Anti-IL-5 in patients with moderate to severe COPD patients and sputum eosinophilia"

Supervisor(s): Prof. Chris Brightling

Background: COPD is defined by the presence of fixed airflow obstruction with limited reversibility but its airway inflammatory and remodelling profiles are heterogeneous. About 20 to 40% of COPD patients have sputum eosinophilia. IL-5 is critical in survival of eosinophils and sputum IL-5 is associated with a sputum eosinophilia and can be reduced by oral corticosteroids.

Study Hypothesis: MEDI- 563 is a monoclonal antibody that binds to IL-5 receptors (IL-5Rα). Blocking these receptors will result in destruction of eosinophils in COPD subjects who have sputum eosinophilia, which in turn will reduce the exacerbation rate.

Primary objective: A Phase 2a, double-blind, placebo-controlled study to evaluate the efficacy of multiple subcutaneous doses of MEDI-563, on the rate of moderate-to-severe acute exacerbations of COPD (AECOPD) in adult subjects with moderate-to-severe COPD who exhibited sputum eosinophilia (≥ 3.0% sputum eosinophilia in the previous 12 months or at screening) compared to placebo.

Secondary Objectives:

1) To evaluate the safety and tolerability of MEDI-563 in this subject population.
2) To evaluate the effect of MEDI-563 on reducing hospitalizations due to AECOPD.
3) To assess the effect of MEDI-563 on health-related quality of life measurements using the COPD St. George's Respiratory Questionnaire (SGRQ-C) and the Chronic Respiratory Questionnaire self-administered standardized (CRQ-SAS).
4) To describe the effect of MEDI-563 on the BODE (**B**ody Mass Index, Airflow **O**bstruction, **D**yspnea, and **E**xercise Capacity) index.

ENDPOINTS ASSESSMENT: The primary endpoint is the **number of moderate-to-severe AECOPD** after their first dose of MEDI-563 injection to Day 393/ Early Discontinuation visit.

Louise Haste

"How Exacerbating! The development of a murine model of Pulmonary infection to better understand the causes of COPD exacerbations"

Supervisor(s): Prof. Peter Andrew and Dr. Aras Kadioglu

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of morbidity and mortality in adults in developed countries. COPD is characterized by long periods of stable symptoms, but exacerbations frequently occur which result in a permanent worsening of symptoms. Studies have shown a link between bacterial colonization of the lower airways of COPD sufferers and an increase

in exacerbation frequency. Common bacterial colonisers include *Haemophilus influenzae* and *Streptococcus pneumoniae*. Consequently, this project aims to develop a murine model of long-term pulmonary infection with *S. pneumoniae.* This model could then be used to test hypotheses of how exacerbations occur and also give a better understanding of the role that bacterial infections play in the progression of COPD. To develop this model various approaches were taken. Initially a range of doses of a serotype 2 pneumococcus, in varying dose volumes were administered intranasally to MF1 outbred mice. It was seen that at lower dose volumes, nasopharyngeal colonisation could be achieved, however it was not possible to achieve lung colonisation at 7 days. This experimental approach was repeated with other serotypes of *S. pneumoniae,* in a variety of murine strains. The desired model has recently been achieved. Colonisation of the lungs was observed at 14 days in 80 % of inbred CBA/Ca mice dosed with a serotype 19F pneumococcus. This serotype was also able to colonise the lower airways of MF1 mice, with 40 % of mice colonised at 7 days.

Vijay Mistry

"The relationship between PBMC secretion of IFN-alpha following TLR7 and TLR9 activation and lung function in COPD"

Supervisor(s): Prof. Chris Brightling

Introduction: COPD is a major health burden and is associated with airway inflammation. Viral and bacterial infections play a key role in exacerbations in COPD and have been implicated in the persistence of symptoms. Whether there are abnormalities in the innate immune response in airway disease increasing their susceptibility to infection is uncertain. The relationship of the inflammatory response to these airway pathogens at stable state and exacerbations is also poorly understood and how this may lead on to structural airway remodelling is poorly defined. We sought to investigate the relationship between clinical parameters in COPD and the *in vitro* response to TLR 7 and 9 activation using imidazoquinline R848 (TLR7 agonist) and CpG (TLR9 agonist).

Method: PBMCs were isolated from 20ml of heparinised blood from healthy and COPD subjects. The cells were stimulated, with 10μM R848 (TLR7 agonist) and 1μM CpG (TLR9 agonist), for 24 hours. IFN-α secretion was measured in cell free supernatant using commercially available ELISA kits.

Results: 37 COPD and 8 healthy control subjects were recruited. 27 of the COPD subjects and 3 controls were male. The mean (SD) FEV_1 (L) and FEV_1/FVC of the COPD subjects was 1.32 (0.58) and 0.5 (0.13) respectively. There was no significant difference in IFN-α (pg/ml) release when comparing health 74 (30) to disease 59 (42) (p=0.33) when stimulating TLR 7. However, TLR 9 stimulation gave a significant increase in IFN-α production in the COPD group 204 (106) vs the healthy subjects 114 (61) (p=0.003). TLR9 stimulation was also significantly increased

compared to TLR7 in disease ($p<0.0001$). There was a significant negative correlation with lung function and TLR 7 stimulation in the COPD group, FEV_1 ($r=-0.46$, $p=0.005$); FEV_1 % predicted ($r=-0.37$, $p=0.024$); FEV_1/FVC ($r=-0.39$, $p=0.002$). TLR 9 stimulation had a significant negative correlation with gas exchange, KCO % predicted ($r=-0.55$, $p<0.001$) and TLCO % predicted ($r=-0.43$, $p=0.01$).

Conclusion: The release of IFN-α by PBMCs was increased in COPD compared to healthy controls following TLR 9, but not TLR 7 stimulation. The IFN-α release after TLR7 and 9 activation was related to lung function.

Eva Horvath-Papp

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

Supervisor(s): Dr. Kumar Rajakumar

Acinetobacter baumannii is a non-fermentative Gram-negative bacillus, normally found in water reservoirs and soil. However, it has become a major nosocomial infection, causing, amongst others, ventilator-associated pneumonia, septicaemia, skin-, wound- and urinary-tract infections. *A. baumannii* emerged as a major problem during the 1970s, as its unique ability to gain resistance to antibiotics resulted in a significant proportion of strains (up to 30% in the U.K.) being classed as multi-drug resistant (MDR). Known resistance determinants include multidrug efflux pumps, target modification, and antibiotic-modification enzymes.

Aminoglycosides are a class of antibiotics that were until recently, used successfully against *A. baumannii*. However, within the last 25 years, their widespread use has resulted in increased resistance. Yet, in many cases, it has been observed that genes for resistance against now redundant aminoglycosides have persisted in the *A. baumannii* gene pool, despite the removal of selection forces.

The aim of this project is to understand why these genes have been preserved, specifically focusing on the incorporation and excision of gene cassettes within the semi-mobile structures known as integrons.

Work is in progress to recover aminoglycoside resistance genes through marker rescue techniques, and analysing the structure of integrons in wild-type clinical strains of *A. baumannii*.

Future work will be centred on assessing the impact of integrase activity on rearrangement within these integrons, and analysing the difference of antimicrobial susceptibility between integrons possessing only one, or two independent aminoglycoside resistance genes. Comparison of the differences may shed light on the persistence of resistance genes no longer actively selected for in clinical environments.

Nino Iakobachvili

"Mycobacterial resuscitation promoting factors: roles and mechanisms in infected macrophages"

Supervisor(s): Dr. Galina Mukamolova and Prof. Mark Carr

Tuberculosis remains a global health problem; however, the molecular mechanisms underlying tuberculosis pathogenesis are only slowly starting to emerge. *M. tuberculosis* complex cells produce a large and diverse range of secreted proteins, including members of the ESAT-6/CFP-10 (esx) and resuscitation promoting factor (Rpf) families. It is now apparent that many of these secreted proteins play critical but as yet poorly defined roles in pathogenesis, such as the escape of *M. tuberculosis* complex cells from arrested phagosomes and the formation of actin tails to facilitate direct cell to cell infection. Rpfs possess muralytic activity and have been implicated in many processes, including resuscitation of dormant cells, intracellular growth and modulation of the cytokine response of infected macrophages, but precise roles and mechanisms remain unclear. We have recently identified that *M. tuberculosis* complex RpfA shows over 50% sequence homology to the *Listeria* protein responsible for actin tail formation, which suggests a specific and key role for RpfA in pathogenesis. The proposed project is aimed to investigate the roles and mechanisms of action of individual *M. marinum* Rpf proteins in infected macrophages, including the potential role of RpfA in bacteria-mediated actin fibre assembly. The work will exploit the potential of confocal microscopy studies of *M. marinum* infected macrophages, which is a proven model for infection by *M. tuberculosis* complex cells.

Jessica Loraine

"The role of RpfB released muropeptides in resuscitation of dormant *Mycobacterium tuberculosis* cells"

Supervisor(s): Dr. Galina Mukamolova

Mycobacterium tuberculosis, the causative agent of the deadly human disease tuberculosis, has long been known for its unique ability to generate dormant forms and latent infections (LTBI) in human. This latent infection can reactivate resulting in active tuberculosis and resuscitation of dormant tuberculosis bacilli has been considered as a critical factor. Resuscitation-promoting factors (Rpfs), muralytic enzymes produced by actively growing mycobacteria, stimulate resuscitation of dormant bacteria and reactivation of chronic tuberculosis. The enzymatic activity of Rpfs has been proven to be essential for their resuscitation and growth stimulatory effects. One of the current hypotheses suggests that the muropeptide fragments, released by Rpfs from peptidoglycan (PGN), play an important signalling role and activate serine/threonine protein kinase mediated initiation of growth. Mycobacteria possess a complex cell wall and purification of

peptidoglycan is a laborious and time consuming process. Therefore the methodology for production and purification of muropeptides from *E. coli* has been established.

Mycobacterial murein is notoriously resistant to digestion with different muralytic enzymes due to the glycolylation of muramic acid (a major component of PGN). We hypothesize that mycobacterial Rpfs are specifically adapted for digestion of glycolylated murein and glycolylated muropeptides are important for resuscitation. We generated an E. coli strain over-expressing NamH enzyme, responsible for the glycolylation of muramic acid. Using mass spectroscopy, rp-HPLC, NMR, chemical modification and biochemical assays we have confirmed the presence of both glycolylated and wild type muramic acid residues in the namH over-expressing strain. Initial RpfB released muropeptide data corroborates the hypothesis that this modification is essential for RpfB activity.

Jamie Marshall

"Activation of the Complement system by the *Streptococcus pneumoniae* toxin, pneumolysin"

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

The common respiratory pathogen *Streptococcus pneumoniae* is a major cause of human disease worldwide. It is a principal agent for bacterial pneumonia, septicaemia, and meningitis, causing >1.2 million infant deaths per year. Pneumolysin (PLY) is a toxin and important virulence factor produced by *Streptococcus pneumonia.* It is a 53 kD protein with four domains, which is produced by virtually all clinical isolates of pneumococcus. In animal studies, isogenic mutant pneumococci lacking pneumolysin are able to be cleared from the lungs of immune-competent mice.

PLY lacks an N-terminal secretion sequence and requires lysis of the pneumococci to be released into the host organism. It has two main effects on the host organism; firstly it is able to oligomerise and insert itself into cholesterol-containing host membranes to form pores; resulting in widespread lysis of host cells and cell death. The second, less well understood function is to activate the host complement system; the current thoughts are that widespread, rapid activation of complement uses up all the available complement proteins, leaving the host without a first-line of defence.

This project aims to understand, at a molecular level, how the pore forming toxin PLY activates the complement system. This is an important question, because complement activation is vital for virulence. For example, *in vivo* studies have shown that mice infected with pneumococci expressing a mutant form of PLY, deficient in complement activation, have less severe pneumonia and bacteraemia compared with mice infected with wild type bacteria. Thus, targeting the complement activation function of PLY could lead to new therapeutics.

Fatima Mohamed

"Complement Properdin- Key Player in Survival of Murine Listeriosis"

Supervisor(s): Dr. Cordula Stover

Complement factor P or properdin is a glycoprotein of the immune defence and is secreted by leukocytes and endothelial cells. It is the only positive regulator and plays a major role in regulating the alternative pathway of the complement system by binding and stabilising two specific converting enzyme complexes, which are normally labile (C3bBb and C3bBbC3b).

So far, mouse models have shown a contribution of complement, in particular complement receptor 3 (CR3) and C5 to survival from infection with *Listeria monocytogenes* a Gram-positive bacterium intracellular pathogen. The aim of this study is to analyse by in vitro and in vivo experiments the importance of properdin in the control of *L. monocytogenes* numbers and the host response.

For the in vitro assay, dendritic cells and macrophages were derived from bone marrows of properdin-deficient and wild type mice and showed greater intracellular load of *Listeria* in cells from wild type mice, which also produced more IFN-gamma and nitric oxide compared to cells from properdin-deficient mice for these time points. Surface expression for CD40 was increased in infected dendritic cells from wild type compared to properdin-deficient mice.

Next the in vivo experiment, after intravenous infection with *L.monocytogenes* (EGD-e), properdin-deficient mice died significantly more by 48 hours than wild type mice. So far we conclude that properdin contributes significantly to host survival of septicemia with *L. monocytogenes*. Future planned in vivo experiments will demonstrate the relevance of the findings so far and further characterise the disease process.

Janet Nale

"Isolation and characterisation of temperate bacteriophages of the hyper-virulent *Clostridium difficile* 027 strain"

Supervisor(s): Dr. Martha Clokie

Recently, it has been shown that the *Clostridium difficile* 027 can be divided into several sub-clades. These sub-clades were also found to vary in the severity of *C. difficile* infection. The presence of prophages in genomes of *C. difficile* could influence their pathogenicity. Little is known about bacteriophage carriage in the *C. difficile* 027 clinical isolates. Therefore this study was designed to characterise temperate bacteriophages of *C. difficile* 027 sub-clades from clinical isolates as a first step to understanding their potential role in disease.

Ninety-one *Clostridium difficile* 027 clinical isolates (characterised into 23 sub-clades) were induced using mitomycin C or norfloxacin. Myoviruses and siphoviruses were identified in 60 and 4, of the strains, respectively. There were also phage tail-like particles in the other 26 strains. Whole genome size of the phages was ~ 15-50 kb. There was a strong correlation between phage carriage and the sub-clades.

To gain insight to the molecular diversity of these phages, primers targeting the phage holin, capsid and portal protein genes were designed. Primers were used to amplify these genes from six different ribotypes of *C. difficile*. Phylogenetic analysis showed there is a relationship between ribotype and phage sequences, implying that phage carriage is relatively stable. The *C. difficile* phage clade is distinct from other members of the Clostridial family, and other bacterial species, which suggests these phages have a narrow host range.

These findings show that phage carriage within *C. difficile* 027 is highly variable and genes of these phages could provide insight to *C. difficile* strain and phage evolution.

Depesh Pankhania

"Serine/Threonine protein kinases of *Burkholderia pseudomallei*"

Supervisor(s): Dr. Ed Galyov and Dr. Helen O'Hare

Burkholderia pseudomallei is a Gram-negative saprophytic bacillus, which is endemic to tropical and sub-tropical regions, predominantly South East Asia and Northern Australia. This highly motile facultative intracellular pathogen is the causative agent of Melioidosis, a potential fatal invasive infection in animals and humans. Despite being initially identified in 1911, relatively little is known about the pathogenicity and metabolism of *B. pseudomallei.*

The *B. pseudomallei* genome sequence has become a vital resource allowing *in-silco* analysis to focus experimental studies. My research is focused on four genes encoding putative Serine/Threonine Protein Kinases (STKs): *BPSL0220*, *BPSL0597*, *BPSL1828*, *BPSS2102*. STKs are responsible for the phosphorylation of proteins; this is a highly regulated mechanism of post-translational modification, which coordinates a vast array of signal pathways in both eukaryotic and prokaryotic organisms. In bacteria these pathways include: biofilm formation, cell wall biosynthesis, metabolism, sporulation, stress response and virulence.

The aim of this study is to understand the roles of the four putative STKs in *B. pseudomallei* virulence and the regulation of cellular processes. Currently, I am constructing single and double crossover mutant plasmids to be conjugated into *B. pseudomallei.* Once constructed, these specific mutants in each of the four genes will be used to elucidate the role of the putative STKs in *B. pseudomallei* virulence and/or metabolism. Also, to perform biochemical analysis of the four putative STKs, I have expressed BPSL0220 and BPSL1828 proteins in *E. coli* and confirmed that the recombinant proteins are soluble. Further work is required to express soluble BPSL0597 and BPSS2102 proteins.

Fathima Farveen Sharaff

"Analysis of the effects of ICU medications on bacterial biofilm formation"

Supervisor(s): Dr. Primrose Freestone

Bacteria in most environments are not found in a single cell, planktonic (free-living) form but exist in huge communities composed of bacteria and bacterial slime, collectively called a biofilm. Bacterial biofilms may form on a wide variety of surfaces, and are very relevant to human life, and health disciplines, including medicine, dentistry, bioremediation, water treatment, and engineering. In the medical context, it is estimated that biofilms are involved in up to 80% of human infections. A biofilm mode of life makes single celled microbes behave almost as multicellular organisms. Living in the protective coating of a biofilm slime layer defends against attack from cells and proteins of the immune system, and antibiotics.

Infections of indwelling medical devices, such as intravenous catheters, by bacterial biofilms are particularly difficult to treat due to their high antibiotic resistance. I am interested in what influences biofilm production in the clinical setting. It has been shown that catecholamine inotropes, which are medications used to treat critically ill patients, can stimulate bacterial pathogen biofilm formation. My project is about investigating the mechanisms by which this occurs. This research could help to create a better understanding of medically related factors involved in biofilm related infections.

Sadiyo Siad

"Role of human mast cell in host-pathogen interactions during mycobacterial infection"

Supervisor(s): Dr. Cordula Stover and Dr. Galina Mukamolova

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis is responsible for the death of over two million people each year. *M. tuberculosis* is a non-motile, rod-shaped, obligatory aerobic, intracellular human pathogen with a complex cell wall. Its cell wall structure gives Mtb, its privilege to survive and replicate within phagosomes of macrophages where it arrests phagosome maturation and macrophage response to tuberculosis infection.

This presentation will show the central role of human mast cell in host-pathogen interactions during mycobacterial infection. Human mast cells are widely distributed tissues throughout the body where they rapidly produce immune mediators, such as cytokines and chemokines to elicit protective innate immune responses and physiological responses, to enhance other effector cell recruitment and to modify responses to infection through antibody dependent activation and effects on adaptive immunity. More fascinating, they exhibit to have a role in innate immune response, maintaining tissue homeostasis, wound or tissue repair and angiogenesis. Little is known regarding the role of mast cells in interaction with mycobacterial infection.

I will present the knowledge gained so far from my infection studies using human mast cell line (HMC-1), Bacilli Calmette-Guerin (BCG) and *Mycobacterium marinum* (a facultative pathogen) as a model system.

Uzal Umar

"Staphylococcus Chromosome Cassette *mec* (SCC*mec*) in *Staphylococcus aureus*: impact on phenotype and virulence"

Supervisor(s): Dr. Kumar Rajakumar and Dr. Julie Morrisey

Staphylococcus aureus is an opportunistic pathogen which causes mile to life-threatening infections in humans and its treatment has been problematic on account of resistence to all classes of antimicrobials. The Staphylococcus Chromosome Cassette (SCC*mec*), the mobile genetic element, encodes *mec*A the central determinant for meticillin resistance and all other β-lactams in staphylococci. The impact of distinct SCC*mec* elements present in the clinically-derived strains (BH1 CC [SCC*mec* type II] and ME2 [SCC*mec* type IV]) and the fully sequenced MRSA strain (PM64) was investigated. SCC*mec*-minus derivatives of these strains were obtained by introduction of the temperature-sensitive pSR_2 to over-express *ccr*AB and consequently up-regulate spontaneous excision of SCC*mec*. Excision of SCC*mec* was confirmed by PCR and determination of susceptibility to oxacillin. BH1 CC SCC*mec*-minus reverted to a borderline oxacillin resistance

phenotype (MIC 2.0 μg/ml). Protein profiles of the three pairs of isogenic strains were preliminary investigated with no obvious impact of the SCC*mec* on non-covalently bound surface, cell wall and supernatant proteins in exponential and stationary phases of growth in low nutrient medium $\pm Fe^{2+}$ supplementation. Examination of the biofilm-forming phenotype of the isogenic strains suggested that SCC*mec* type II impact positively on biofilm-forming phenotype of BH1 CC in tryptic soy broth supplemented with 1% glucose. SCC*mec* type II element in BH1 CC appeared to impact negatively on β-haemolytic activity while the SCC*mec* type IV in ME2 did not impact on β-haemolysis. Preliminary investigation of the isogenic strains for the impact of the SCC*mec* on global virulence did not yield statistically significant data. The results imply that by some unknown mechanism(s), SCC*mec* type II affect core genome in BH1 CC.

Eman Abu-rish

"**Investigation of the intracellular pathways of Toll-Like Receptor 9 signaling in human B-cells**"

Supervisor(s): Dr. Mike Browning

TLR9 targeting therapy has become a new approach to immunotherapy through the introduction of synthetic CpG-ODN (TLR9 agonists) and inhibitory-ODN (INH-ODN). Inappropriate activation of TLR9 by self CpG motifs is thought to be involved in the pathogenesis of the autoimmune disease, systemic lupus erythematosus (SLE), resulting in the production of B-cell activating factor (BAFF).

In human, TLR9 is expressed mainly by B-cells and plasmacytoid dendritic cells. However, the effect of CpG-ODN on the expression of BAFF and its receptors (BAFF-R and TACI) by B-cells, and the mechanisms underlying such effects, have not yet been studied. Moreover, the effect of TLR9 inhibitors, such as INH-ODN, has not yet been elucidated.

CpG-2006 (3μg/ml) was used to stimulate normal human B-cells and BJAB, RPMI and RAMOS cell lines. BAFF mRNA expression was measured by qPCR and was significantly increased in B-cells and RAMOS (BJAB and RPMI cells constitutively expressed BAFF, which was not significantly increased by CpG-2006 stimulation). In addition, intracellular BAFF expression was measured by flow cytometry and was found to be significantly increased in RAMOS cells and normal B-cells. Screening of BAFF receptor expression after stimulation showed that TACI, but not BAFF-R, was significantly up-regulated in B-cells. INH-ODN treatment (15μg/ml) did not result in a significant suppression of CpG-induced BAFF expression by RAMOS. Signaling experiments revealed that NF-κB pathways might be involved in CpG-induced BAFF expression in RAMOS cell line.

Further studies are needed to investigate the signaling pathways, and the effect of INH-ODN on CpG-induced BAFF expression.

Ahmed Alzaraa

"Ultrasound contrast agents detect perfusion defects in an *ex-vivo* autologous perfused porcine liver: A useful tool for the study of hepatic ischaemia-reperfusion injury"

Supervisor(s): Mr. David Lloyd

Introduction: An *ex-vivo* autologous perfused porcine liver model is used in our laboratory to investigate liver physiology. Previous studies revealed macroscopic areas of hypoperfusion with consequent metabolic changes but these changes were also observed in apparently well perfused organs. To determine whether this represented undetected perfusion defects we employed an ultrasound contrast agent.

Methods: Five pigs were sacrificed at the local abbatoir and autologous blood immediately collected into a pre-heparinised container. For each pig, the liver was harvested, flushed with 1L cold Soltran solution (Baxters, UK) and transported in an ice container. An extracorporeal circuit consisting of a centrifugal pump, oxygenator, heat exchanger and a blood reservoir was connected to the liver and the perfusion started. After one-hour 2.2ml of contrast agent (Sonovue, Bracco, Italy) was injected in the portal vein and cine loops recorded on a GE logiq E9 ultrasound machine (GE Healthcare, USA) for 220 seconds. The whole sequence was repeated after 4 hours.

Results: After one hour the entire parenchyma enhanced strongly with and without the contrast agent. However after 4 hours multiple perfusion defects appeared which could only be identified following the use of a contrast agent.

Conclusions: After four hours the addition of a contrast agent revealed perfusion defects that were not detected in non-enhanced images. This is an important addition to *ex-vivo* models and will facilitate detailed studies of ischemia-reperfusion in explanted livers.

Nawal Helmi

"The effect of perfluorocarbon therapy on *Streptococcal Pneumoniae*-induced Vaso-occlusive crises in sickle cell mouse lung"

Supervisor(s): Dr. Hitesh Pandya and Prof. Peter Andrew

Sickle Cell Anaemia (SCA) is a chronic haemoglobinopathy. It results from a genetic defect in the globin gene, which leads to a haemoglobin S (HbS). SCA is characterised by a chronic haemolytic anaemia and 'vaso-occlusive crises'. Both result from distorted (sickle) red blood cells (RBCs).

Individuals with SCA also have a significantly greater risk of overwhelming pneumococcal infection, which occurs at a much higher rate than for other encapsulated bacteria, suggesting that individuals with SCA are uniquely vulnerable to pneumococci. *Streptococcus pneumoniae* is a common cause of pneumonia (an inflammatory condition of the lung), which can cause vaso-occlusion in SCA patients and this is due to asplinea. In severe cases RBC transfusion is necessary. Blood transfusion reduces the risk of further vaso-occclusion but recurrent transfusions risks iron overload. An alternative to RBC transfusion is artificial blood. For example Perfluorocarbons (PFC) have an O_2 carrying capacity which is 40 to 50 times higher than haemoglobin. As observed in *ex vivo* and *in vivo* models sickle red blood cell -induced vaso-occlusion is often partial, allowing for decreased remnant flow. Hence, if oxygen is delivered to these areas decreased obstruction might be achieved. The hypothesis of this study that treatment with PFC will reverse sickle-cell induced pulmonary vascular-occlusion in SCA mice with pneumococcal lung infection.

The effect of the perfluorocarbon emulsions were tested *in vitro* and *in vivo* on *S.pneumoniae* to determine whether it encouraged the pneumonia-causing bacteria to grow. This was done using two different strains of the pneumoniae D39 serotype 2 and TIGR 4 serotype 4 and growth was assessed in Chemical Defined Medium (CDM).

Initial results showed that contrary to the expectations PFCE increases the growth of the *S.pneumoniae in vitro* and resulted in rate of death in infected mice.

Keynote Speaker Bibliography

Prof. Mike Silverman

Mike Silverman started his career as a House Physician at St George's Hospital in London in 1967, and was an actively Physician and Surgeon at Hillingdon Hospital in Uxbridge, Southampton General Hospital, and Royal Brompton Hospital in London between 1967 and 1972. In 1973 he went to Ahmadu Bello University in Nigeria as a lecturer and a Senior Registrar. Two years later, Prof Silverman came back to this country, carrying out lecturing and research activities in University of Bristol and at Hammersmith Hospital in London. In 1994, he was honoured with the professorship in Paediatric Respiratory Medicine by Royal Postgraduate Medical School in London. Then he moved to the University of Leicester in 1995 as a Professor in Child Health.

Prof. Silverman is now an honour emeritus professor of child health. Based at the Leicester Royal Infirmary, his research focused on looking at asthma and the different phenotypes of airway disease in young children, including natural history and response to interventions; techniques include analysis of inflammatory processes through to epidemiology and clinical trials. For many years Professor Silverman and his colleagues have been following a group of white and South Asian children born in Leicestershire, to hope to discover how a child's genetic, ethic and environmental background affect their risk of wheeze and asthma. This wide ranging information could help doctors treat wheezy children more effectively and consistently, ensure that they give each child the most appropriate treatment.

Selected Publications:

Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, Von Mutius E, Farrall M, Lathrop M, Cookson WO: GABRIEL Consortium. Large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363(13):1211-21

Spycher BD, Silverman M, Kuehni CE. Phenotypes of childhood asthma: are they real? Clin Exp All 2010;40(8):1130-41

Staley KG, Kuehni CE, Strippoli MP, McNally T, Silverman M, Stover C. Properdin in childhood and its association with wheezing and atopy. Pediatr Allergy Immunol 2010;21(4 pt 2):e787-91

Prof. Mark Jobling

Mark Jobling was brought up and educated in Durham, and went to Oxford to study Biochemistry in 1981. He then did a DPhil at the Genetics Laboratory there, where he developed an interest in human Y chromosomes. In 1992 he moved to the Genetics Department in Leicester to study human Y chromosome diversity, under an MRC Training Fellowship, where he has remained until today, currently under the second renewal of a Wellcome Trust Senior Fellowship.

One of the recent projects Professor Jobling currently working on is "Sex, genomes, history: molecular, evolutionary and cultural effects on human genetic diversity" funded by Wellcome Trust Senior Research Fellowship. This project addresses two major questions: How has residence on the sex chromosomes affected the long- and short-term evolution of genic and non-genic sequences in primates? And how do the molecular evolutionary forces acting on these sequences interact with general and sec-specific population processes, and how can knowledge of molecular and population-level influences illuminate the histories of human population themselves?

Selected publications:

Rosser, Z.H., Balaresque, P. and JOBLING, M.A. (2009) Gene conversion between the X chromosome and the male-specific region of the Y chromosome at a translocation hotspot. Am. J. Hum. Genet., 85, 130-134

King, T.E. and JOBLING, M.A. (2009) Founders, drift and infidelity: the relationship between Y chromosome diversity and patrilineal surnames. Mol. Biol. Evol., 26, 1093-1102

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