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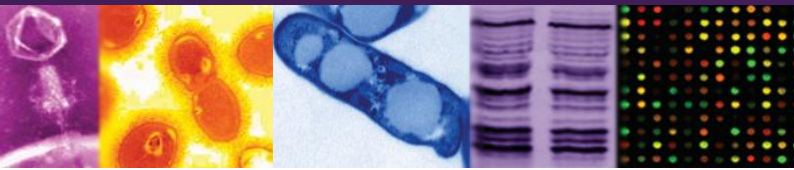

Department of Infection, Immunity & Inflammation

5th Annual Postgraduate Student Conference

10th- 12th April 2013

Programme and Abstracts

Preface

Welcome to the Fifth Annual Postgraduate Departmental Conference. Research in the Department of Infection, Immunity and Inflammation is quite diverse and the Student Conference is the one time in the year when students can get exposure to the work of the many different projects being undertaken.

PhD students from across the department are able to use this opportunity to provide information about their journey through their projects in a conference-style format, pitched to a group of colleagues. This experience is intended to offer students a chance to improve their presentation skills, exchange ideas and become familiar with the interdisciplinary life of the department.

We would like to thank both Professor Peter Bradding from the Lung Institute section of the Department of Infection, Immunity and Inflammation and Dr Chris Bayliss, Department of Genetics, University of Leicester for agreeing to give the keynote addresses. Where possible we have tried to theme the sessions and you will see that the Wednesday presentations are themed around 'bacterial pathogenicity', while the presentations on Thursday are related to 'lung disease'.

Feedback from the presentations plays a vital part in the student experience of the conference and we would encourage you all to provide constructive criticism through comments and questions.

Thank you for attending and participating in this Conference, and we hope you enjoy the presentations from both students and invited speakers.

Organisers: Sadiyo Siad, Greer Arthur, Nima Abbasian, Nawal Helmi, Asel Sarybaeva and Adelina Gavrila (Student Representatives of PGSSC 2013).

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Keynote Speakers

Dr Chris Bayliss

"Genetic variation during persistent carriage of Meningococci"

Wednesday 10th April 14.05-14.50

Prof Peter Bradding

"Interactions between mast cells and structural airway cells in the pathogenesis of asthma"

Thursday 12th April 12.00-12.45

Programme

Wednesday 10th April 2013, MSB LT2	
10.30-11.00	Arrival
11.00-11.25	Welcome Professor Nigel Brunskill
	Session 1 (Bacterial pathogenicity)
	Chair: TBS
11.25-11.50	Jamie Marshall Activation of the Complement system by the *Streptococcus pneumoniae* toxin, pneumolysin.
11.50-12.15	Firas Younis "Transcriptional regulation of pneumococcal pyruvate formate lyase"
12.15-12.40	Vitor Fernandes "Genetic basis of susceptibility to invasive pneumococcal disease (IPD)."
12.40-13.05	Bayad Saeed Assessment of the therapeutic utility of complement regulators in models of haemolysis and *Streptococcus pneumoniae* infection.
13.05-14.05	Lunch
	Session 2 (Bacterial pathogenicity)
	Chair: TBS
14.05-14.50	**Keynote Speaker: Dr Christopher Bayliss "Genetic Variation During Persistent Carriage of Meningococci"**
14.50-15.15	Tolis Panayi "The therapeutic applications of *Clostridium difficile* bacteriophages"
15.15-15.40	Eva Horvath-Papp "Understanding the contribution of Integrons to Multi-Drug Resistance in *Acinetobacter baumannii"*
15.40-16.05	Nutan Prasai Sapkota "Contribution of Tn*AbaR*23 on the phenotype of *Acinetobacter baumannii*"
16.05-16.25	Depesh Pankhania "Serine/Threonine protein kinases of *Burkholderia pseudomallei"*
	Closing

Thursday 11th April 2013, MSB LT2	
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	Session 3 (Lung disease)
	Chair: TBS
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10.10-10.35	Noor Al-Khathlan " The clinical value of the lung clearance index (LCI) in monitoring cystic fibrosis (CF) lung disease"
10.35-11.05	Greer Arthur " Investigating the role of ion channels in airway mucus hypersecretion in asthma"
11.05-11.35	Refreshments
	Session 4 (Lung disease)
	Chair: TBS
11.35-12.00	Jaspreet Sahota "The characterisation of phages developed for the treatment of *Pseudomonas aeruginosa* infection in Cystic Fibrosis (CF) patients"
12.00-12.45	**Keynote Speaker: Professor Peter Bradding** **"Interactions between mast cells and structural airway cells in the pathogenesis of asthma"**
12.45-13.45	Lunch
	Session 5 (Lung disease)
	Chair: TBS
13.45-14.10	Dhan Desai "Elevated sputum interleukin-5 and submucosal eosinophilia in obese severe asthmatics"
14.10-14.35	Rebecca Jayne Fowkes "β 2-Agonist Responses in Human Lung Mast Cells and Airway Smooth Muscle."
14.35-15.00	Sally Stinson "Exploring the expression and function of human CRTh2 in asthma"
15.00-15.25	Joe Morley "Aspergillus fumigatus exposure and health"
15.25-15.50	Leonarda Di Candia "The role of HMGB1 in the regulation of airway smooth muscle cell function"
	Closing

Friday 12th April 2013, MSB LT2	
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	Session 7
	Chair: TBS
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10.10-10.35	Nima Abbasian "Micoparticle Formation And Hyperphosphataemia: A Novel Mechanism For Cardiovascular Risk In Chronic Kidney Disease"
10.35-11.00	Amin Bakir "The Effect of Phagocytosis by Neutrophils on Mycobacterial Gene Expression"
11.00-11.30	Refreshments
	Session 8
	Chair: TBS
11.30-11.55	Ros Azeana Abdul Aziz "Molecular typing of *Klebsiella pneumonia* by Multilocus Sequence Typing (MLST)"
11.40-12.05	Toyosi Obasanjo "Investigation of fimbrial systems in *Klebsiella*"
12.05-12.30	Nino Iakobachvili "Resuscitation Promoting Factors: Roles and Mechanisms in Infected Macrophages"
12.30-12.45	Closing
	Parallel Session 7a (In room 208A)
	Chair: TBS
09.45-10.10	Asel Sarybaeva "Characterization of *M.tuberculosis* Rv 3489 as a "player" in nonreplicating persistence."
10.10-10.35	Sadiyo Siad "Importance of mast cells in infection with mycobacteria"
10.35-11.00	Raghad Hassan Sanyi "Characterization of mycobacterial proteins essential for replication in macrophages"
11.00-11.30	Refreshments

	Parallel Session 8a (In room 208A)
	Chair: TBS
11.30-11.55	Ameen Alwashmi "Recombinant human Thrombospondin1 and Properdin are both capable of triggering platelet activation in vitro"
11.40-12.05	Emma Comber "Spectroscopic studies of the interactions between lipid membranes and a pore forming toxin"

Abstracts

Day 1, 10th April, MSB LT2

Jamie Marshall

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

Supervisor(s): Dr Russell Wallis, Prof. Peter W. 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.

Firas Younis

Transcriptional regulation of pneumococcal pyruvate formate lyase

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

Streptococcus pneumoniae depends on carbohydrates to produce its energy through fermentation. Galactose is the main source of sugar in respiratory tract, and can be found within the structure of mucin. The pneumococcus can cleave and utilise galactose from complex glycoproteins. Pyruvate formate lyase (PFL) is a key enzyme in galactose metabolism. The activity of PFL leads to generation of mixed acids, such as formate, lactate, acetate, and ethanol, with ATP release.

pfl is regulated allosterically, posttranslationally by the activity of PFL-activating enzyme, and transcriptionally. Information on how *pfl* is regulated transcriptionally is very limited. Hence, this study aims to determine the regulation of this gene at genetic level. Through analysis of a strain mutated in *pfl* by microarray, we identified differential regulation of seven transcriptional regulators relative to the wild type. We hypothesise that these genes regulate *pfl* transcriptionally.

To begin, the microarray results were confirmed by qRT-PCR. To demonstrate the direct interaction of these transcriptional regulators with the promoter region of *pfl*, the genes were cloned and expressed to produce recombinant proteins. *Pfl* promoter region was mapped, amplified and cloned. Following sequencing, the cloned fragment was cleaved by restriction endonuclease and was used as a target in electrophoretic mobility shift assay (EMSA). The results showed that three of the seven transcriptional regulators were able to bind to *pfl* promoter region. One of these proteins was CcpA (catabolite control protein A), which is known to repress the expression of pfl. The binding of CcpA to *pfl* promoter was specific. On the other hand, the transcriptional regulators X and Y bound to both *pfl* and a different probe used as a negative control, indicating non-specific interaction of these proteins. Currently, its being investigated if the proteins X and Y have any role in indirect regulation of *pfl.*

Vitor Silva Entrudo Fernandes

Genetic basis of susceptibility to invasive pneumococcal disease (IPD).

Supervisor: Prof. Peter W. Andrew

Understanding of host genetic factors that contribute to susceptibility/resistance to pneumococcal disease may pave the way for targeting therapy or prophylaxis to high-risk individuals as well as helping to unravel the complexities of the immune response to *S. pneumoniae*-infection.

Inbred mouse strains allow exploration of the influence of genetic elements on disease resistance in an otherwise uniform background. Previous work identified two mouse strains with differing responses to type 2 pneumococcal pneumonia and bacteraemia: the highly resistant BALB/c and the susceptible CBA/Ca mouse strain. Genomic analysis of F_2 BALB/c x CBA mice revealed a single major linkage with survival and bacteraemia on proximal chromosome 7, within a region of approximately 8cM near to *D7Mit77*. This quantitative trait locus (QTL) is associated to susceptibility to IPD and was named *Spir1* (*Streptococcus pneumoniae* infection resistance 1).

Congenic mice with a 99.9% BALB/c genome into which the CBA/Ca *Spir1* locus was inserted show susceptibility to IPD, confirming that genetic elements within *Spir1* influence disease-resistance. The presentation will show genetic and susceptibility data collected to narrow the QTL region to less than 0.5 cM (fewer than 5 genes) in order to identify the causative gene(s) of susceptibility and subsequently to investigate how the identified gene(s) influences the pathology of pneumococcal disease.

Bayad Saeed

Assessment of the therapeutic utility of complement regulators in models of haemolysis and *Streptococcus pneumoniae* infection.

Supervisor: Prof. Wilhelm Schwaeble

Complement is a part of our innate immune defence system and plays an important role in fighting infection and maintaining the integrity of our body. More than 30 plasma components form a highly regulated cascade of activation events. My study involves the functional characterisation of regulators working across the three different activation routes of complement, the classical pathway, the alternative pathway and the lectin pathway activation route. I have analysed the biological effects of pathway specific activators of complement and their impact on the crosstalk between these separate activation pathways. The biological read-out for most of my experiments is either the haemolytic and bacteriolytic activity of complement or the clearance of defined pathogens through complement opsonisation mediated phagocytosis. My results clearly indicate a closer cooperation between each of the three pathways than anticipated.

Tolis Panayi

The therapeutic applications of *Clostridium difficile* bacteriophages.

Supervisor: Dr Martha Clokie

Clostridium difficile is a major nosocomial pathogen that has been identified as a major causative agent of antibiotic-mediated diarrhoea. In recent years there were outbreaks in Europe, Canada and the USA associated with a high mortality. In 2011 alone there were 2053 deaths involving *C.difficile* in England and Wales. Pathogenic strains are characterised by multiple resistances to some of the most commonly used antibiotics, which allows it to establish an infection after the disruption of the colonic microbiota. As a result alternative methods of treatment are being investigated.

Bacteriophages, viruses that replicate on bacteria, are one such alternative. They have several traits that make them suitable for therapy, such as a high degree of specificity that allows them to kill their target while not harming the normal flora in

the process. However, before any application of phage therapy, understanding the basic biology of phage-*C. difficile* relationship is necessary.

Currently, there is very limited information regarding these, largely due to the difficulties in *C. difficile* isolation and maintenance. Dr Clokie's research group has successfully established a large collection of *C .difficile* bacteriophages that can infect clinically important *C. difficile* strains.

My work involves the continued isolation and characterisation of phages to evaluate their potential in treating *C. difficile* infections. Their ability to effectively infect and kill *C .difficile* will then be tested both *in vitro* and *in vivo*.

Eva Horvath-Papp

Understanding the contribution of Integrons to Multi-Drug Resistance in *Acinetobacter baumannii*.

Supervisor: Dr. Kumar Rajakumar

Introduction: Class 1 integrons are semi-mobile genetic structures that harbour a cassette array of variable antibiotic resistance conferring genes and are widespread in hospital pathogens. This study looks at the types of genes contained and their frequency in *A. baumannii*

Methods: *A. baumannii* isolates obtained from the University Hospitals of Leicester (UHL; n = 12) and a range of other European sources (EUR; n = 50) were PCR-screened for class 1 integrons using primers in regions conserved to class 1 integrons. Structures were then established using restriction typing and PCR mapping with cassette-specific primers and compared with those represented within freely available *A. baumannii* DNA sequence data.

Results: 47 of the 62 isolates possessed intact and/or partial class 1 integron structures that could be classified into 11 class 1 integron types, based on distinct cassette array conformations. Two of these types, both of which have previously been identified in *A. baumannii*, were highly dominant in both the UHL and EUR collections. Two other types had only previously been identified in other species. A further three types were novel. Five of the class 1 integron structures detected

(10.6%; 3 types) were disrupted by IS elements, including three that lacked the *intI1* gene itself.

Conclusion: Despite the diversity of class 1 integron types identified, two gene cassette array conformations appeared to be common within both sets of isolates studied. Three of the 11 types identified exhibited IS-based restructuring, suggesting that this may be a relatively common feature in *A. baumannii*. Additionally, three isolates possessed variants lacking *intI1* suggestive of the idea that carriage of this gene and/or increased gene-dosage is disadvantageous in *A. baumannii*.

Nutan Prasai Sapkota

Contribution of Tn*Aba*R23 on the phenotype of *Acinetobacter baumannii*

Supervisor: Dr. Kumar Rajakumar

Acinetobacter is a gram-negative bacterium and is ubiquitous in nature. To date, 29 species of *Acinetobacter* are named with several unnamed species. Among various species, *Acinetobacter baumannii* has been considered as clinically important because of its frequent association with the nosocomial and community acquired infections and its remarkable ability to acquire, accumulate and disseminate resistant determinant leading to the widespread emergence of multi drug resistance. The plasticity of the genome of *Acinetobacter baumannii* allows acquisition, accumulation and rearrangement of the genetic determinants which helps the bacteria to thrive and prosper on biotic/abiotic surfaces for a long stretch of time and under harsh conditions. This study aims to investigate the phenotypic contribution of the resistant island Tn*AbaR*23 in *Acinetobacter baumannii* strain A424. The Tn*AbaR*23 island deleted mutants were generated by allelic exchange. The loss of the island was confirmed by PCR and pulsed field gel electrophoresis. Antibiotic susceptibility profile of the mutants was compared to the parent wild type. Relative fitness of the mutants was compared with the parent wild type in *invitro* and *invivo* head-to-head competition assay. Spontaneous deletion of 25 kb multiple antibiotic resistant regions was detected in a sub population of A424WT. These spontaneous mutants were found susceptible to exhibit similar antibiotic susceptibility profile as the island deleted mutants.

Depesh Pankhania

Serine/Threonine protein kinases of *Burkholderia pseudomallei*

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

Burkholderia pseudomallei is the causative agent of Melioidosis, a potentially fatal invasive infection in both animals and humans. Despite a substantial research effort since its initial discovery in 1911, relatively little is known about the pathogenicity and metabolism of *B. pseudomallei.*

The aim of this study is to understand the roles of four genes encoding putative serine/threonine protein kinases (STKs): *BPSL0220*, *BPSL0597*, *BPSL1828*, *BPSS2102.* Phosphorylation by STKs coordinates a vast array of signal pathways in both eukaryotic and prokaryotic organisms.

To date, we have constructed recombinant STK proteins, which have been expressed in *Escherichia coli* and purified to test for protein kinase activity. BPSL0220, BPSL0597 and BPSS2102 have been shown to autophosphorylate and phosphorylate a general kinase substrate confirming the activity.

Furthermore, we have constructed specific mutants in each of the four genes deleting the STKs domain. These mutants are currently being accessed against wild type to determine their roles in *B. pseudomallei* virulence and regulation of cellular processes.

This study should provide valuable information on the role of these putative STKs in *B. pseudomallei* virulence and regulation of cellular processes.

Bethan Barker

Is there a relationship between airway inflammation, systemic inflammation and skeletal muscle dysfunction in COPD?

Supervisor(s): Prof. Chris Brightling, Dr. Michael Steiner

Background: COPD is characterised by chronic airway inflammation and fixed airflow obstruction, but the clinical significance of systemic manifestations such as elevated systemic inflammatory markers, weight loss and skeletal muscle dysfunction is increasingly recognised. The aetiology of these systemic features remains unclear, with pulmonary overspill of inflammatory mediators or independent inflammation both suggested as mechanisms. Although associations have been observed between bacterial colonisation and airway inflammation, as well as between systemic inflammation and skeletal muscle depletion, the interplay between bacterial colonisation, airway inflammation, systemic inflammation and skeletal muscle dysfunction remains unclear.

Aim: To explore how clinical and muscle phenotypes inter-relate in COPD and how these change over time.

Progress: We have established an observational COPD cohort with patients extensively characterised with regards to; (a) airway bacterial load and inflammation, (b) lung disease nature and extent, (c) systemic inflammatory biomarkers, and (d) skeletal muscle bulk, strength and exercise capacity. Quadriceps muscle micro-biopsies have also been obtained from a sub-group. Initial cross-sectional analyses have shown skeletal muscle depletion is associated with severe airways obstruction and elevated blood neutrophil count, but not with airways inflammation or bacterial load.

Plans: We plan to examine the repeatability of muscle bulk and strength measures over time, to relate these measures to airway and systemic inflammation, and to explore whether dynamic changes in these measures occur during exacerbation episodes. We will also quantify inflammatory cell infiltration in quadriceps biopsies

and explore associations between muscle, systemic and airways inflammation both in stable state and during exacerbation episodes.

Noor AL-Khathlan

The clinical value of the lung clearance index (LCI) in monitoring cystic fibrosis (CF) lung disease

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

Pathological, imaging and physiological studies have suggested that CF lung disease affects primarily small airways and at an early stage.[1, 2] Conventionally, FEV_1 obtained from spirometry is regarded as the gold standard to monitor the disease progression in CF. However; it lack sensitivity to small airways disease. Alternatively, LCI, a measure of ventilation inhomogeneity (VI), derived from multiple-breath nitrogen washout (MBNW) has been recognized as a more sensitive marker of small airways dysfunction in early CF lung disease, but its exact clinical significance is unknown.

Therefore, we aimed to obtain longitudinal lung function measurements and clinical data from school-age children with mild to moderate CF lung disease in order to understand the role of LCI and whether it can track early changes in lung pathology. We also aimed to track changes in VI at convective (S_{cond}) and acinar (S_{acin}) airways as disease progressed. In addition to assess the lung peripheral microstructure using a relatively new technique of hyperpolarised helium magnetic resonance scanning (^{3}HeMR) that measures the apparent diffusion coefficient (ADC), a surrogate measure of alveolar size.

To date we have obtained data from a total of 36 children, of whom 33 have had two measurements and 11 have completed testing on 3 occasions. Our finding shows no statistical significant changes in VI indices obtained from 11 children on 3 occasions (a year apart). On the contrary, FEV_1 z-score does change statistically significant. The direction of the change in FEV_1 suggests gradual decline in lung function as disease progressed over the three years of the study period. Data from helium study shows that ADC values obtained from sub-group of children with CF were not different from that obtained from normal children but values tended to fall within the lower range.

Greer Arthur

Investigating the role of ion channels in airway mucus hypersecretion in asthma

Supervisor(s): Dr Erol Gaillard, Prof. Peter Bradding

Airway mucus hypersecretion is a chronic feature of asthmatic airways and is thought to be caused by increased airway epithelial mucin production and secretion. Mucus-secreting airway epithelial cells such as goblet cells are thought to synthesise and secrete these mucins via a Ca^{2+}-dependent exocytic pathway. The exocytic pathway of mucus-secreting cells is hypothesised to be comparable to that of mast cells, which have been shown to release their intracellular vesicles via a mechanism that can be enhanced by the activity of two ion channels: $K_{Ca}3.1$ and CRACM. The expression and roles of $K_{Ca}3.1$ and CRACM in mucus-secreting airway epithelial cells requires further investigation, with particular focus on the role of these two ion channels in exocytic release of mucin glycoproteins. Initial investigations include examining the expression of the two ion channels in primary human bronchial epithelial cells (HBECs) cultured in undifferentiated and differentiated states; examining the expression of the predominant secreted respiratory mucins, MUC5AC and MUC5B, in undifferentiated and differentiated primary HBECs; and investigating the electrophysiological properties of $K_{Ca}3.1$ and CRACM channels expressed by primary HBECs.

Jaspreet Sahota

The Characterisation and Exploitation of Phages in the Treatment of *Pseudomonas aeruginosa* Infections of Cystic Fibrosis Patients

Supervisor(s): Dr. Martha Clokie and Prof. Aras Kadioglu

Pseudomonas aeruginosa is the 4th most prevalent hospital acquired infection and the predominant infectious agent of cystic fibrosis (CF) sufferers, with 80% of CF morbidity caused by respiratory infections. The typical antibiotics treatment has faced increasing multi-drug resistance over the last 2 decades, with *P. aeruginosa* developing a range of resistance mechanisms. So much so that in cystic

fibrosis sufferers long term infections are essentially impossible to remove via antibiotics. As an alternative, bacteriophages (phages) are viruses that specifically infect bacteria and have great therapeutic potential.

Phages have been used in Eastern Europe and Former USSR countries alongside antibiotics for almost a century. They have accumulated extensive knowledge in phage isolation and have large collections of phages. By collaborating with the Eliava Institute, Georgia, several *P. aeruginosa* phages have been isolated and purified from two of their original 'phage products'. They were tested against the dominant UK *P. aeruginosa* strains, of which 98% were susceptible to the phages. Including all those defined as multi drug resistant. We also investigated the potential use of these phages as prophylactic agents and their ability in treating biofilms. On-going research focuses on optimising the delivery methods of the phages and their effectiveness in tissue culture models.

This research will eventually provide an antibiotic alternative to help both reduce the respiratory associated fatalities caused in cystic fibrosis sufferers as well as being a potential prophylactic for all hospital patients.

Dhan Desai

Elevated sputum interleukin-5 and submucosal eosinophilia in obese severe asthmatics

Supervisor: Prof. Chris Brightling

Rationale: The relationship between airway inflammation and obesity in severe asthma is poorly understood.

Objectives: We sought to determine the relationship between sputum mediator profiles and the distribution of eosinophilic inflammation and obesity in severe asthmatics.

Methods: Clinical parameters and 8 mediators in sputum were assessed in 131 severe asthmatic subjects from a single centre categorised into lean, overweight and obese groups defined by their body mass index. In an independent group of severe asthmatics (n=45) and healthy controls (n=19) eosinophilic inflammation was

enumerated in bronchial submucosa, blood and sputum and related to their body mass index.

Measurements and Main Results: Sputum interleukin (IL)-5 geometric mean [95% confidence interval] (pg/ml) was elevated in the obese (1.5 [1.0-2.3]) compared to overweight (0.9 [0.7-1.1]; p=0.02) and lean (0.7 [0.5-1.0]; p=0.01) asthmatics (p=0.007) and was correlated with the body mass index (r=0.29, p<0.001). There was no relationship between body mass index, the sputum cell count or other sputum mediators. In the bronchoscopy group the submucosal eosinophil number in the asthmatic subjects was correlated with body mass index (Spearman's rank correlation rs=0.38, p=0.013) and the median (interquartile range) number of submucosal eosinophils was increased in obese (19.4 [11.8-31.2]) cells/mm2 versus lean subjects (8.2 [5.4-14.6]) (p=0.006). There was no significant association between sputum or peripheral blood eosinophil counts and body mass index.

Conclusion: Sputum IL-5 and submucosal eosinophils, but not sputum eosinophils are elevated in obese severe asthmatics. Whether specific anti-eosinophilic therapy is beneficial, or improved diet and lifestyle in obese asthma has anti-inflammatory effects beyond weight reduction, requires further study.

Rebecca Jayne Fowkes

β_2-Agonist responses in human lung mast cells and airway smooth muscle.

Supervisor: Prof. Peter Bradding

Evidence supports a key role for the human lung mast cell (HLMC) in asthma pathogenesis. HLMCs localise within the airway smooth muscle (ASM) of asthmatic individuals of all types and severity, where they remain chronically activated. At present there are no good inhibitors of HLMC activation available for clinical use and current mast cell stabilisers are of poor efficacy when given regularly *in vitro* and *in vivo*.

β_2-adrenoceptor agonists primarily target the ASM to induce bronchodilatation whilst conferring protection against bronchoconstrictor stimuli. Additional anti - inflammatory effects of β_2-agonists have been proposed and β_2-agonists have been shown to be potent, efficacious inhibitors of FcεRI-dependent

HLMC release *in vivo* and *in vitro*. However, an anti-inflammatory role of β_2-agonists remains controversial as the protective effects of β_2-agonists are lost upon chronic administration or acute exposure in the presence of the cytokine, stem cell factor (SCF).

SCF is critical for HLMC growth, differentiation and survival. Soluble and membrane - bound forms of SCF are expressed by ASM and expression is enhanced within the asthmatic airway. The SCF receptor, c-KIT is a receptor tyrosine kinase known to modulate the function of G protein-coupled receptors such as the β_2-adrenoceptor (β_2-AR). SCF has been implicated in the loss of beneficial effects of β_2-agonists on HLMCs.

Aims: To compare effects of HLMC/ASM co-culture and HLMC mono-culture on HLMC number, constitutive and FcεRI - dependent HLMC mediator release and responsiveness to β_2-agonists.

Experimental Approach: HLMC histamine and tryptase release were measured using radioenzymatic and colorimetric assays, respectively. SCF neutralisation experiments were performed in order to determine the effect of ASM - derived SCF on HMLC responsiveness to β_2-agonists. Immunofluorescence was used to assess β2-AR phosphorylation at tyrosine 350.

Sally Stinson

Exploring the expression and function of human CRTh2 in asthma.

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

CRTh2 (Chemoattractant Receptor-Homologous molecule expressed on Th2 cells) is a G-protein coupled receptor that has been implicated in the pathogenesis of allergic diseases. Its major endogenous ligand prostaglandin D2 (PGD2) is released in large amounts by mast cells during allergic reactions and is found at high levels in the bronchoalveolar lavage fluid of asthmatics. Most of the literature for CRTh2 describes its expression and function on peripheral blood derived eosinophils, Th2 cells and basophils. To date there is no literature that describes CRTh2 expression on asthmatic tissue.

This project has been funded by AstraZeneca to investigate the expression and function of human CRTh2 in asthma. Immunohistochemistry has been used to demonstrate that CRTh2 is expressed on a sub-set of inflammatory cells and epithelial cells on normal and asthmatic biopsies. Quantitation of the immunohistochemical staining revealed that there was significantly more CRTh2 positive inflammatory cells in severe asthmatic biopsies compared to normal biopsies. Conversely, significantly less CRTh2 positive epithelial cells were found in the severe asthmatic biopsies compared to the normal biopsies. In vitro expression work on isolated mast cells, normal and asthmatic epithelial cells has confirmed CRTh2 expression on these cell types. Further work aims to elucidate a clinically relevant function of CRTh2 on epithelial cells and currently an Airway Liquid Interface (ALI) system is being investigated as a model to develop this area of work.

Joe Morley

***Aspergillus fumigatus* exposure and health.**

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

An important part of the waste management process is the composting of organic waste and the activity of microorganisms is central to this process. Industrial scale composting sites release large amounts of microbial matter into the air, and some components of this bioaerosol release are able to cause health problems. *Aspergillus fumigatus*, one of the predominant compost related fungi, is a well-studied allergen and opportunistic pathogen. People who work on compost sites potentially have long term exposure to high levels of *A. fumigatus* spores and their health may be adversely affected by this exposure. This study aims to ascertain what level of *A. fumigatus* spores compost workers are routinely exposed to and to see if their health is affected.

A key consideration in studying airborne microbes is the sampling procedure used. There is a wide range of different sampling devices and sample analysis methods which could potentially impact on the data produced. Examples of both personal and static samplers have been compared using a combination of

culture-based, microscopic and molecular techniques. Both controlled and natural environments have been utilised with the aim of identifying which sampling procedure(s) are most suitable for analysis of the air at and around composting facilities.

The recruitment of participants who work on compost sites is crucial for this project and informing the workers about the aims of the project without causing unnecessary concern about their health is important. Working with a composting company, a strategy for enrolling compost workers has been developed to achieve this.

Leonarda Di Candia

The role of HMGB1 in the regulation of airway smooth muscle cell function.

Supervisor(s): Prof Christopher Brightling, Prof John Challiss

Introduction: High mobility group box 1 (HMGB1) is a damage-associated molecular pattern (DAMP) that can be released by stressed/injured cells and signals through pattern recognition receptors (PRRs) including the receptor for advanced glycosylation end products (RAGE) and Toll-like receptor 4 to promote inflammation and tissue repair. We hypothesised that activation of the HMGB1/RAGE axis contributes to airway smooth muscle (ASM) dysfunction, which is a feature of asthma.

Results: HMGB1 levels were significantly higher in sputum samples from severe asthmatics vs healthy controls using ELISA (815.8±163.9ng/ml vs 345.8±136.1ng/ml, p=0.038). Previously we found reduced intracellular HMGB1 expression in cultured primary ASM cells isolated from asthmatics vs non-asthmatics, suggesting possible release into the extracellular milieu; however HMGB1 was undetectable in cell supernatants by ELISA. ASM cells responded to rhHMGB1 with a small but significant reduction in reactive oxygen species production (300 and 1000ng/ml, DCFDA assay), which is increased in asthma and has been linked to enhanced ASM contractility and airway hyper-responsiveness , a trend towards increased NF-κB subunit p65 nuclear translocation (300ng/ml) and a

trend for increased wound healing after 24h following incubation with 10 and 30ng/ml rhHMGB1 and following RAGE blockade.

Conclusions: These data suggest that HMGB1 levels may be elevated in the airways in asthma and may be able to influence ASM behaviour. Future work will clarify the source of elevated HMGB1 found in the asthmatic airways by staining of bronchial biopsies, and will further elucidate the role of rhHMGB1 in the regulation of ASM cell function and the receptor/signalling pathways involved.

Day 3, 12th April, MSB LT2

Sumia Essid

The role of C- peptide in muscle metabolism in diabetic nephropathy.

Supervisor(s): Prof Nigel Brunskill, Dr Alan Bevington

Background: Considerable evidence indicates that C-peptide exerts a crucial role in slowing the progression of type1 diabetic complications and activates the Erk and Akt signaling pathways in a number of different cell types. However, the role of C-peptide in skeletal muscle is poorly understood. The aim of this study was to investigate whether C-peptide could activate these signaling pathways in rat skeletal muscle cell line L6, and whether C-peptide could exert functional effects on proliferation, differentiation or survival of these cells, or modulate the toxic effect of Simvastatin – a drug commonly used in diabetics which exerts toxic effects on muscle.

Method: L6 rat skeletal muscle cells at the myoblast stage were cultured and treated with doses of rat C-peptide up to 3 nM at different time points from 5 min to 72h. Extracellular-signal-regulated kinase 1/2 (ERK1/2) and protein kinase B (Akt) activation were determined by immunoblotting with phospho-specific antibodies. Cell viability was analysed by methylthiazoletetrazolium assay (MTT) and cell mass and proliferation were determined by measuring total protein and total DNA.

Result: Rat C -peptide induced the phosphorylation of ERK1/2 and Akt in L6 myoblasts within 5 minutes in a concentration-dependent manner. Simvastatin exerted a potent toxic effect on the cells (judged by MTT assay) at doses as low as 10uM. While C-peptide had little effect on cell growth or viability by itself, initial results suggest that 3nM C-peptide strongly protects myoblasts from the myotoxicity effect of Simvastatin.

Conclusion: It is concluded that C-peptide is bioactive in this cell line and (at least in the presence of Simvastatin) may exert functionally important survival effects, possibly by activating survival pathways through Akt.

Nima Abbasian

Microparticle formation and hyperphosphataemia: a novel mechanism for cardiolovascular risk in chronic kidney disease.

Supervisor(s): Dr Alan Bevington, Dr Karl Herbert and Dr James Burton

Background: Increased plasma inorganic phosphate (hyperphosphataemia) is common in Chronic Kidney Disease (CKD) and associated with increased cardiovascular (CV) risk. Microparticles (MP) are submicron-sized membrane-derived vesicles released from cells following activation and/or apoptosis. MPs are elevated in CKD and are possible mediators/markers of CV risk. We proposed that MP formation is driven partly by hyperphosphataemia, however the mechanism is unclear. Here we investigated the relationship between raised extracellular inorganic phosphate (Pi), endothelial dysfunction and MP formation.

Methods: EA.hy926 endothelial cells were exposed to elevated Pi. Intracellular Pi (iPi) was measured based on phosphomolybdate formation. Phosphate-induced apoptosis, ROS generation, and MP release were investigated using Flow Cytometry (FACS) and NanoSight nanoparticle tracking analysis (NTA). The effect of Pi on cell viability and VE-Cadherin (CD144) expression was measured by MTT and FACS respectively. Sedimentable protein particles in culture media were measured by Lowry assay.

Results: Higher extracellular Pi was accompanied by a) significant increase in iPi with maximal response after 48h ($P<0.0001$), b) acute (90 min) MP release from cells detected by NTA ($P<0.04$) c) increased sedimentable protein particles after 24h ($P=<0.03$), d) decreased/increased expression of CD144 on cell monolayers and sedimentable particles respectively $(P<0.05)$. MTT assay indicated significantly reduced cell viability in Pi-treated cells $(P<0.05)$. No apparent effect of high Pi on apoptosis and ROS was observed ($n=14$ *and 6 respectively*) indicating the contribution of other phosphate-induced signalling pathways in this mechanism.

Conclusion: The study demonstrates that hyperphosphataemia increases iPi concentration, modulates cell function and increases MP release from human endothelial cells, and suggests a novel mechanism for increased CV risk in CKD.

Amin Bakir

The effect of phagocytosis by neutrophils on Mycobacterium tuberculosis gene expression.

Supervisor: Dr Bernard Burke

My work is a transcriptomic study to investigate the possible effects of phagocytosis by neutrophils on gene expression profile of *M. tuberculosis* (Mtb). The idea of this work comes from previous studies indicating that in the sputum of TB patients Mtb shows a distinctive gene expression profile akin to the transcriptome of non-replicating persistent Mtb *in vitro*. Since neutrophils are the predominant cells with the highest number of associated Mtb compared to other phagocytic cells in sputum and bronchoalveolar lavage from TB patients, my hypothesis is that the neutrophils may be responsible for this distinctive gene expression profile of Mtb in human sputum.

Therefore, neutrophils were infected with Mtb and RNA was extracted from intracellular bacilli. The results from spectrophotometric measurement and qRT PCR indicate for successful removal of host nucleic acid and mycobacterial genomic DNA from isolated RNA sample. However, the source of extracted RNA is the main concern now. Our approach to find whether the extracted RNA is intracellular or extracellular is to use mycobacterial genes that become exclusively up-regulated inside phagocytes.

The future plan is to do microarray to get the global gene expression of intracellular Mtb in infected neutrophils then compare it to the transcriptome of Mtb growing *in vitro* and in human sputum. This is to understand the role of neutrophils in pathogenesis and transmission of tuberculosis.

Ros Azeana Abdul Aziz

Molecular typing of *Klebsiella pneumonia* by Multilocus Sequence Typing (MLST)

Supervisor: Dr.Kumar Rajakumar

Introduction:*Klebsiella pneumonia* is one of the most common Gram-negative bacteria showing increasing resistance to multiple antibiotics and second most important cause of Gram negative bloodstream infection (BSI) worldwide. The genomes of *Klebsiella sp* remain poorly studied with a limited data on genome plasticity, virulence-associated genes and genes that contribute indirectly towards antibiotic resistance phenotypes.

Aims: This study aims to reveal the specific associations between information gathered on clinical and socio-demographic risk factors for *K. pneumoniae* BSI in adults with molecular epidemiology characteristics of *K. pneumoniae*.

Methods: The genetic relationship between *K. pneumoniae* isolates were established by using previously described and validated Multi Locus Sequence Typing (MLST). Twenty-two *K. pneumoniae* clinical isolates and two reference strain were included for comparison. The identification of all clinical strains was confirmed by BioMerieux® Vitek II Compact & API20E®. PCR amplifications were carried out to amplify the internal fragments of seven housekeeping genes and each amplicon were sent for sequencing. Allelic profile and ST analysis were performed at public MLST website and eBURST v3 for clonal relation.

Results: Nucleotide variation was observed at all genes and revealed four new alelles and five new ST. Even no clonal complex observed among clinical isolates, two of the isolates shown to be the founder in comparison to whole MLST dataset. This data will soon be correlated to the clinical and socio-demographic risk factors.

Conclusion:MLST will discriminate most epidemiologically unrelated strains and analysis of larger sample sets needed in order to provide a much improved understanding of the evolutionary origin and dissemination of *K. pneumonia* strains.

Toyosi Obasanjo

Investigation of fimbrial systems in *Klebsiella*

Supervisor: Dr.Kumar Rajakumar

Klebsiella spp, gram negative bacteria of *the Enterobacteriaecae* family, is a common pathogen that causes hospital-acquired urinary tract infections, septicemia, and pneumonia, as well as community acquired pneumonia. The ability of these organisms to adhere to tissue surfaces in host is an important step to the development of infection. Hence, the expressions of fimbrial adhesins found in type I pili play an important role in its adherence to biotic and abiotic surfaces as well as in the formation of biofilms. These fimbrial protein adhesins in *Klebsiella* are encoded by the *fim* operon, which is homologous to the *E.coli fim* operon.

Following the discovery of *Klebsiella* pneumonia strains containing a novel *fim2* encoding genomic island, several strains tested for typical *fim* operon were negative. Instead of harbouring the *fim* operon, these strains were discovered to contain a *fim* variant at the same locus dubbed *fim3*. Mapping and bioinformatic analysis show high degree of similarity between these two operons except for *fimK* gene. Within the *fim3* operon, over 50% of the *fim3K* gene has been truncated at its 3' end.

Although the general function of *fimK* gene in *Klebsiella pneumoniae* has not been described, we employ several methods to understand and characterise the basis and function of this recent evolution in *Klebsiella*. We also use several methods to understand the relationship between *fim*/*fim3* operon and *fim2* which may provide better insight into the function of *fim2* operon**.**

Nino Iakobachvili

Resuscitation Promoting Factors: Roles and Mechanisms in Infected Macrophages

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

Members of the *Mycobacterium tuberculosis* complex are the causative agents of tuberculosis, a major global health threat. The majority of individuals are thought to be infected latently whereby the bacteria are in a non-replicating dormant state, and the individual is asymptomatic. It is clear that *M. tuberculosis* is a highly adapted, very successful pathogen and this is in part due to the large number and variety of proteins that it is able to secrete via multiple different pathways. Many of these secreted proteins have been shown to be essential for the virulence, survival and dormancy of this organism, and function in a variety of different roles including inhibiting phagosomal acidification, promoting survival within macrophages and resuscitating dormant bacteria. Others have been implicated in immunogenicity and inducing the host immune response such as those belonging to the CFP-10/ESAT-6 family and the resuscitation promoting factors family, as well as immune evasion.

Although in recent years such a vast number of important proteins have been identified, their precise roles during tuberculosis pathogenesis and their mechanisms of action remain poorly understood. Our work reports the use of *M. marinum* infected macrophages to model tuberculosis infection. This organism is very closely related to *M. tuberculosis* and is known to cause a pathology similar to human tuberculosis when infecting zebrafish, its natural host, or when superficially and opportunistically infecting humans. We have used immunofluorescence and confocal microscopy to address the expression and localisation patterns of a number of important secreted mycobacterial proteins during different stages of infection.

Asel Sarybaeva

Characterization of *M.tuberculosis* Rv 3489 as a "player" in non-replicating persistence.

Supervisor(s): Dr. Galina Mukamolova, Prof. Mike Barer

Latency and drug tolerance are associated with a dormant subpopulation of *Mycobacterium tuberculosis* (*Mtb*). In experimental dormancy models, the transition to non-culturability or a non-replicating state is followed by the upregulation of genes encoding proteins involved in stress resistance and alternative metabolic pathways**.** The molecular mechanisms of many proteins that are involved in non-replicating persistence are awaiting characterization.

Rv3489 is a small protein of unknown function that is essential for *Mtb* replication *in vivo*. It was found to be upregulated in several dormancy models and is thus likely to be important for mycobacterial persistence. Rv3489 is co-transcribed with OtsA, one of the key enzymes involved in trehalose biosynthesis that is also essential for Mtb growth *in vivo*. Together with OtsB it produces trehalose from glucose and glucose-6-phosphate. Multiple pathways for trehalose synthesis exist in *Mtb*, however the OtsA-OtsB pathway was found to be essential for *Mtb* growth and virulence in a mouse model.

A large body of evidence suggests that accumulation of trehalose facilitates microbial survival under stressful conditions. Moreover, in mycobacterial, trehalose is also involved in cell wall biogenesis and serves as a structural component of important cell wall constituents. We found Rv3489 to be an attractive candidate for inactivation with subsequent investigation of its function. It represents an ideal drug target due to its small size, specificity to mycobacterial species and its predicted transmembrane localization.

Sadiyo Siad

Importance of mast cells in infection with mycobacteria

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

Mycobacterium marinum (*M. mar*) is a commonly used model organism to understand the pathogenicity of *Mycobacterium tuberculosis (M. tb). Mycobacterium tuberculosis* associates with mast cells, which are important effectors in the innate immune defence against pathogens and respond by degranulating.

Using transmission electron microscopy (TEM), we found that unlike macrophages and dendritic cells, which phagocytose mycobacteria, mast cells internalise the bacilli, sub-localising them to their cytoplasm. Both, intracellular and membrane bound *Mycobacterium marinum* increased significantly in number for up to 120 hours of incubation at an MOI of 0.5. FACS analyses showed 5-8% of bacterial uptake by mast cells.

To investigate inflammatory response of the host cell during mycobacterial infection, real-time polymerase chain reaction (qPCR) was used.

We show that the infected mast cells react acutely in a pro-inflammatory manner by up-regulating nucleotide-binding oligomerization domain-containing protein 2 (NOD2), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX2), matrix metalloproteinase 9 (MMP9) and cathelicidin peptide (LL-37), albeit it to varying extents depending on MOI and time point.

In conclusion, our study shows that mast cells may be co-determinants in the outcome of mycobacterial infection.

Raghad Sanyi

Characterization of mycobacterial proteins essential for replication in macrophages.

Supervisor(s): Dr Bernard Burke, Dr Galina Mukamolova

Despite many years of research there is still much to know about the mechanisms employed by *Mycobacterium tuberculosis (Mtb)* in order to survive and

resist being killed by the host's immune system. The success of this pathogen lies in its ability to stay alive and persist in a potentially hostile environment represented by the macrophage phagosome. *Mtb* can actually persist and replicate inside macrophages instead of being killed by them. Hence there is a desperate need to identify the molecular mechanisms and associated proteins enabling mycobacterial survival and replication inside macrophages. In the case of *Mtb* 40% of the predicted genes encode proteins of unknown function. Recent studies have shown that several mycobacterial proteins may play distinct roles during different stages of infection. In particular, Rv1219c and its Ortholog in BCG, BCG1279c are important for the long term survival inside macrophages. While a gene, encoding PPE51 protein was highly up-regulated in *Mtb* after 2 hours of macrophage infection. The main focus of my project is to investigate the role of these two proteins for late and early stages of infection. A PPE51 deletion mutant is being constructed in Mtb H37Rv using the flexible cassette method and macrophages will be infected to investigate if this protein has a role in replication inside macrophages.

Ameen Alwashmi

Recombinant human Thrombospondin1 and Properdin are both capable of triggering platelet activation in vitro

Supervisor: Prof. W. Schwaeble

Thrombospondin1 (TSP1) is one of five distinct members of the thrombospondin family. It is secreted from cells such as endothelial cells and platelets (i.e. Thrombocytes). A plasma protein called Properdin, which is known to act as a complement regulatory protein, is nearly entirely composed of Thrombospondin repeat-motifs (TSR) that show a high degree of homology to the TSR of TSP1. TSP1 is known to play a crucial function in thrombus formation at sites of injury. Recombinant human TSP1 was expressed in a eukaryotic system using the cell line HEK 293 (Human embryonic kidney). The expression rhTSP1 to encode full length was designed the full open reading frame of TSP1 was cloned into the expression vector pCEP4 for stable transfection into HEK293 cells. TSP1 is

known to be involved in cell adhesion and platelets aggregation (Thrombus formation). Most of the adhesion and aggregation functions of TSP1 have been located to the N-terminal TSR domains of TSP1. The high degree of homology between the TSRs in TSP1 and Properdin implied that not only TSP1, but also Properdin could act on platelets directly. My results demonstrated that in fact platelets activation and aggregation can occur within 10-20 minutes after adding either rhTSP1 or rhProperdin concentration (15µg/ml) both at time as monitored by expression of P-selectin receptor (CD62P) on activated platelets. P-selectin expression is considered to be a guidance marker for platelet activation. In my assay, intact platelets were fixed in formal saline and used to coat a 96 maxi-sorb ELISA plate in order to quantifying the binding of rhProperdin in comparison to rhTSP1. Surprisingly, both show nearly similar binding to intact platelets.

The functional activities of rhTSP1 and rhProperdin were also tested by ELISA to monitor recombinant integrin alpha 2b/beta3 (GPIIb/IIIa complex) (CD41/CD61) a hallmark of platelet activation. This ligand recognises the sequence motif RGD an adhesion motif found in such as fibrinogen, fibronectin and thrombospondin. CD41 mediates platelet/platelet interaction via fibrinogen binding. rhProperdin was quantified by ELISA and showed detectable but lesser binding with CD41 in comparison to TSP1. In addition, my analysis demonstrates also binding of rhProperdin to other extracellular matrix proteins such as; collagen I, IV and fibronectin, and human fibrinogen and thrombin. Comparative analysis between Properdin and rhTSP1 binding was established using ELISA. Surprisingly, rhProperdin shows obviously binding to those extracellular matrix proteins except collagen IV. Like TSP1, Properdin appears to cross-link between platelet/platelet activation by mediating binding interactions with GPIIb/IIIa and fibrinogen. Our results show that not only TSP1 but also Properdin can bind to fibrinogen and can also mediate platelet activation by inducing expression of the platelet activation marker CD62P. My results indicate that Properdin can be considered as an enhancer (cross-link) for platelet/platelet activation through its ability to bind to fibrinogen

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Emma Comber

Spectroscopic studies of the interactions between lipid membranes and a pore forming toxin.

Supervisor(s): Prof. Peter W. Andrew, Dr Rana Lonnen, Dr Andrew Hudson

Proteins that interact with lipids in cell membranes are widespread in biology and the actions of these proteins can be important to human health. Many proteins can insert across the lipid bilayer and cause major structural changes in the membrane. In the case of pore forming proteins, this leads to a breach in the membrane which results in the leakage of cell components.

The bacteria, *streptococcus pneumoniae*, is the main cause of pneumonia world-wide, as well as many other infections, and still causes 1.5 million deaths a year. The pore forming toxin pneumolysin is essential for infection with *streptococcus pneumoniae*. Pneumolysin forms pores in cholesterol containing membranes, leading to cell death. Unfortunately, there is very limited information on the detailed mechanism of lipid-protein and protein-protein interaction that results in the structural changes taking place in the membrane. The aim of the research is to develop methods to investigate the pore forming mechanism of pneumolysin. In the future, these methods can then be applied to the study of any pore forming protein.

The mechanism of pore formation by pneumolysin is being studied in unilamellar lipid vesicles. Optical tweezing is used to isolate a single vesicle from an ensemble, and the interaction of pneumolysin with the lipid membrane is revealed by microspectroscopy of the trapped particle. The onset of pore formation has been determined by fluorescence measurements of the translocation of molecular dyes across the lipid membrane. Raman measurements are used to investigate the composition and structural changes occurring in the membrane.

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