Preface

Welcome to the Sixth Annual Postgraduate Departmental Conference. Research in the Department of Infection, Immunity and Inflammation is quite diverse and the Postgraduate 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 Professor Nigel Brunskill for opening the conference, and Professor Andrew Wardlaw, Dr Alan Bevington, Dr Yassine Amrani and Dr Martha Clokie for kindly agreeing to give the keynote lectures.

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

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

Organisers:
Chee Kay Cheung, Jonathan Decker & Tariq Daud.

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Dr Alan Bevington –Tuesday 1st April 2014: 09.40 - 10.20

"Inorganic phosphate toxicity and cardiovascular death in chronic kidney disease: More than just rubble in the arteries"

Professor Andrew Wardlaw - Tuesday 1st April 2014: 13.30 – 14.15:

"The role of eosinophils in asthma"

Dr Yassine Amrani – Wednesday 2nd April: 09.30 – 10.20

"Novel insight into the molecular mechanisms of corticosteroid insensitivity in severe asthma"

Dr Martha Clokie - Wednesday 2nd April: 13.30 – 14.15:

"Bacteriophage-bacterial relationships: who wears the trousers?"

Programme

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Day 1, 1st April, MSB LT2

Dalia Alammar

Proteinuriamediated damage of Proximal Tubular Epithelial Cells (PTECs) – a mechanism of renal pathology in multiple myeloma involving Megalin phosphorylation

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

Introduction:

Multiple Myeloma is a blood cancer arising from antibody producing plasma cells. The developing increase in protein concentration leads to proteinuria, a pathological excess of plasma proteins in the urine (> 0.01g/100ml) and a hallmark of progressive renal dysfunction. The reason for this is that the normal reabsorption capacity of the kidney is exceeded. Reabsorption of proteins utilises specialised uptake receptors. Megalin is one such PTEC receptor. In proteinuric nephropathy the cytoplasmic tail of megalin (MegCT) is phosphorylated.

Purpose:

The project pursues the hypothesis that protein overload mediated renal toxicity comes from the interaction between proteins in the urine and megalin on the PTECs. This is tested by studying intracellular signalling through phosphorylation of MegCT and the pro-inflammatory response.

Methods:

Establishing a proteinuric model in *vitro* using **HK-2** cells (a proximal tubular **cell** line from normal kidney) and treating with high concentrations of HSA-FFA (human serum albumin-free fatty acid) to induce cellular damage. This is measured by MTT and LDH assays. Detecting the phosphorylation signalling of cytoplasmic tail of megalin by utilising antibodies directed against specific activation sites of the intracellular portion of megalin (Western blot). Measuring the inflammatory response by PCR.

Results: Exposing the HK2 cells to a relevant pathological concentration of HSA-FFA for prolonged time has a toxic effect and leads to damage of the cells. In addition, phosphorylation of the cytoplasmic tail of megalin is activated; complementC3and pro inflammatory cytokines are increased.

Discussion: This study describes the effect of protein overload on the phosphorylation of MegCT in PTEC. Potentially this uncovered mechanism may be attractive for drug development to benefit patients with kidney failure and help to inhibit the progression of proteinuric nephropathy.

Chee Kay Cheung

The role of filtered IgA in the progression of IgA nephropathy

Supervisor: Dr Jonathan Barratt, Dr Karen Molyneux & Professor Nigel Brunskill

Introduction:

Immunoglobulin A nephropathy (IgAN) is the commonest cause of glomerulonephritis worldwide, and is characterised by mesangial IgA deposition. However, severity of IgA deposition does not correlate with disease progression. In IgAN, higher urinary levels of IgA are observed compared to healthy subjects, yet the direct effect of IgA on the proximal tubule is unclear. These studies aim to examine the effect of IgA on the proximal tubule.

Methods:

IgA1 was purified from healthy subjects and patients with IgAN by Jacalin-affinity chromatography, and separated into monomeric (mIgA) and polymeric (pIgA) fractions by fast protein liquid chromatography (FPLC). Human proximal tubular epithelial cells (PTEC) were incubated with mIgA and pIgA for 24 hours before measurement of interleukin-6 (IL-6) release. PTEC were also incubated with samples prepared in an identical process from a patient with IgA deficiency for comparison. Finally an in vivo model of IgA deposition was established in C57BL/6 mice by oral and parenteral administration of bovine gamma globulin (BGG).

Results:

mIgA and pIgA caused a dose-dependent increase in IL-6 release from PTEC which was not replicated by the IgA-deficient samples. pIgA isolated from the patient with IgAN had the greatest effect. Oral and parenteral immunisation of BGG in C57BL/6 mice led to mesangial IgA, IgG and complement component C3 deposition.

Discussion:

pIgA has the greatest effect on PTEC stimulation and warrants further study into its molecular composition, PTEC receptor involvement and intracellular signalling pathways. The in vivo model will allow study into important events in the pathogenesis of IgAN, including IgA deposition and the effect of filtration of IgA into the proximal tubule.

Sumia Essid

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

Supervisor: 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.

Zinah Dheyaa Razzaq Zwaini

The inflammatory response of renal proximal tubular epithelial cells in ischemia reperfusion injury

Supervisor: Dr Cordula Stover & Dr Bin Yang

Introduction:

Renal transplantation transforms the lives of patients with end stage renal disease. Unfortunately, different complications such as ischemia reprfusion injury (IRI) rejection cause failure of allograft. Nevertheless, the exact mechanism of IRI-

mediated damage is still unclear but the activation of the complement systems has pleotropic roles in pathogenesis of renal IRI. Proximal tubular epithelial cells (PTEC) are most vulnerable to IRI. The aim of this study is to establish a reproducible *in vitro* model of IRI and to investigate the inflammatory reaction of renal PTEC and the complement system involvement in particular proprdin in IRI.

Methods:
PTEC of normal human kidney (HK-2), human podocytes and renal tubular cells (RTC) of properdin KO/ WT mice are exposed to hypoxic chamber and/ or Locke's buffer before reperfusing for 48hrs. On other line, the above cells are exposed to endotoxin (LPS) and/or hypoxic chamber.

Results and discussion:
More cell damage is observed when using both Locke's buffer and hypoxic chamber simultaneously than using each separately. The same is noticed with LPS. TNF-α is released more from ischemic cells than control and this is abrogated in the presence of erythropoietin (EPO). Factor B, CD36, PCNA and iC3b are expressed from HK-2 cells exposed to IRI. Regarding the properdin KO mouse, there are no significant differences in the LDH release among the studied groups. However, further experiment to confirm the results and to fulfil whole the aims from this project are mandatory.

Marwh Gassim Aldriwesh

Developing a prognostic test for risk of infectious peritonitis in peritoneal dialysis patients

Supervisor: Dr Primrose Freestone & Dr Jonathan Barratt

Introduction:
Peritoneal dialysis (PD) is the simplest and most economical technique in use as a treatment for kidney failure patients. However, since PD involves repeated administration of large volumes of fluid with the peritoneal cavity, this form of renal dialysis has two major clinical complications: protein loss and infection leading to peritonitis. While there has been intensive aseptic technique education of medical staff and patients, the factors that predispose PD patients to the development of peritonitis are still not fully understood. It is hypothesised that there is a correlation between levels of certain protein(s) present in the peritoneal dialysate (HPD) and the stimulation of microbial growth, virulence and toxicity.

Methods:
The protein profiles of different HPDs derived from different PD patients were investigated using proteomic techniques. In addition, microbiological studies were conducted to investigate if the growth, virulence and toxicity characteristics of bacteria in HPD differed between patients.

Results:
Initial studies performed on the first dwell of six HPDs derived from six PD patients have shown similar protein profiles, but variability in the total protein levels was found. It has also been discovered that the ability of HPD to modulate bacterial growth and virulence varied among PD patients.

Discussion:
The similarity in protein profiles among different patients suggests that there are major proteins lost by all PD patients, although further sequencing and protein identification work is required to identify those proteins. Furthermore, differences in total protein levels/proteins present among the HPD samples could be the reason behind the different bacterial growth profiles.
A major priority for future work is the collection of additional HPD samples from new PD patients, as well as follow-ups on the HPD samples from existing patients, in order to study how the HPD protein profiles change over time. Collectively, this data will be used to identify the factor(s) that might be putting the PD patient at risk of infection.

Michael Ghebre

Biological clustering supports both "Dutch" and "British" hypotheses of Asthma and Chronic Obstructive Pulmonary Disease

Supervisor: Prof Chris Brightling; Prof John Thompson & Dr Chris Newby

Background:
Asthma and chronic obstructive pulmonary disease (COPD) are heterogeneous diseases. We sought to determine, in terms of their sputum cellular and mediator profiles, the extent to which they represent distinct or overlapping conditions supporting either the 'British' or 'Dutch' hypotheses of airway disease pathogenesis.

Methods:
We compared the clinical, physiological characteristics and sputum mediators between 86 subjects with severe asthma and 75 with moderate-to-severe COPD. Biological subgroups were determined using factor and cluster analyses on 18 sputum cytokines. The subgroups were validated on independent severe asthma (n=166) and COPD (n=58) cohorts. Two techniques were used to assign the validation subjects to subgroups; linear discriminant analysis, or the best identified discriminator (single cytokine) in combination with subject disease status (asthma or COPD).

Results:

Discriminant analysis distinguished severe asthma from COPD completely using a combination of clinical and biological variables. Factor and cluster analyses of the sputum cytokine profiles revealed three biological clusters; Cluster 1: asthma predominant, eosinophilic, high Th2 cytokines; Cluster 2: asthma and COPD overlap, neutrophilic; Cluster 3: COPD predominant, mixed eosinophilic and neutrophilic. Validation subjects were classified into three subgroups using discriminant analysis, or disease status with a binary assessment of sputum IL-1β expression. Sputum cellular and cytokine profiles of the validation subgroups were similar to the subgroups from the test study.

Conclusion:
Sputum cytokine profiling can determine distinct and overlapping groups of subjects with asthma and COPD, supporting both the 'British' and 'Dutch' hypotheses. These findings may contribute to improved patient classification to enable stratified medicine.

Adelina Gavrila

Dissociated steroids as novel alternatives for the treatment of corticosteroid resistance in asthma

Supervisor(s): Dr. Yassine Amrani & Prof. Christopher Brightling

Introduction:
Compound A (CpdA), a synthetic phenyl azirdine precursor isolated from the Namibian shrub (Salsola tuberculatiformis Botschantzev), was shown to have potent dissociative and anti-inflammatory properties *in vitro*, with reduced side effects *in vivo*. Whether such dissociated compounds could be used in steroid resistant conditions has not been investigated.

Methods and Results:
Here we used CpdA in a cellular model of cytokine-induced corticosteroid resistance in human airway smooth muscle (ASM) cells to determine the anti-inflammatory mechanisms involved. ELISA assays showed that CpdA differentially inhibited production of steroid-resistant chemokines (CCL5, CX3CL1, CXCL10) induced by TNFα/IFNγ from both healthy (n=4) and asthmatic subjects (n=7). To investigate whether CpdA acts via a GR-dependent pathway, we assessed the ability of CpdA to induce well-known GR-inducible genes. Firstly, RT-PCR assays showed that CpdA did not induce the expression of Glucocorticoid-induced leucine zipper (GILZ) (n=3). In addition, in contrast to Fluticasone (FP), CpdA treatment did not induce GRα nuclear translocation by Immunofluorescence (2-6hr). Furthermore, using the known steroid antagonist RU486 (1μM) had no effect on the inhibitory action of CpdA on the induction of FP-resistant CCL5 induced by TNFα/IFNγ (n=4). We then investigated the effect of CpdA on the kinetic activation of IRF-1 by

Western Blot experiments. The TNFα/IFNγ combination induced an increase of IRF-1 over basal and CpdA inhibited cytokine-induced IRF-1 activation (n=6). The inhibitory effect of CpdA was confirmed by immunofluorescence experiments (n=3).

Conclusion:
These data suggest that the dissociated compound CpdA suppressed expression of different corticosteroid-resistant chemokines in human ASM cells possibly via GR-independent pathways, including the inhibition of IRF-1.

Ros Azeana Abdul Aziz

Epidemiology and molecular characterization of *Klebsiella pneumoniae* clinical isolates

Supervisor(s): Dr Kumar Rajakumar

Background:
Klebsiella pneumoniae 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:
The genetic diversity and the clinical relevance of the drug-resistant Klebsiella pneumoniae isolates from hospital settings are largely unknown. We thus conducted this prospective study to analyze the molecular epidemiology of **K. pneumoniae** isolates from patients being treated in the University Hospitals of Leicester NHS Trust (UHL) for the period of July 2011 to October 2012.

Method:
Antibiotic susceptibility testing, PCR amplification and sequencing of the drug resistance-associated genes, and multilocus sequence typing (MLST) were conducted. The program eBURST v 3.0 was used to identify the different clonal complexes. Every patient was counted only once during his hospital stay regardless of the number of positive cultures and a total of 63 isolates of consecutive non-repetitive *K. pneumoniae* strains were obtained from patients suffered with blood stream infection (BSI) within Leicestershire. The allocations of clonal lineage were established by using previously validated MLST approach. Selected isolates were tested in phage therapy and survival assays were performed in *Galleria mellonella* infection model.

Result: Nucleotide variation was observed at all seven genes and revealed more than 20 distinct allelic profiles with several novel sequence types (ST) and allele

sequences demonstrating that the UHL-collected *K. pneumoniae* were genetically diverse and heterogenous further proving that the MLST database is still novel and continuously growing. Demographic analysis revealed that 72% of the infection risk factors were healthcare-related.

Conclusion: Combination of several genotyping and phenotyping analyses create a very useful tool that will discriminate most epidemiologically unrelated strains, however analysis of larger sample sets needed in order to provide a much improved understanding of the evolutionary origin and clonal dissemination of *K. pneumoniae* strains.

Mutaib Mashraqi

Assessing the role of Haptoglobin (Hp) as a protein serves as a critical major opsonin in the innate immune defence against *Staphylococcus aureus.*

Supervisor: Prof Wilhelm Schwaeble and Dr Russell Wallis

Haptoglobin is an acute-phase plasma protein. It is a marker for many inflammatory-related diseases. Its best known function is to eliminate haemoglobin from plasma and to prevent loss of iron through the kidneys, thereby protecting the kidneys from damage and sequestering iron. It was also shown in an *in vitro* study that haptoglobin binds lipoteichoic acid (LTA) of *Staphylococcus aureus*.

Ahmed Abdullah Ahmed

Assessment of the role of the lectin pathway of complement activation in the pathophysiology of thrombosis using experimental models of inflammatory diseases.

Supervisor(s): Professor Wilhelm Schwaeble

Introduction:

DIC, septic shock and haemolytic uremic syndrome (HUS) all have in common that they cause serious diseases due to inflammation related coagulopathy.

Complement activation via the lectin path way has proved has a vital role during thrombosis complicating inflammation and /or infection. Therapeutic blockade of the complement system by monoclonal anti- C5 antibodies (Eculizumab) has recently been shown to provide an effective therapeutic approach in the treatment of HUS. This encouraged testing of more complement blocking antibodies to assess their ability to treat such thrombotic events.

Methods:

- **Model of HUS:**

Wild type mice C57BL/6 were used in all experiments. All mice were injected with shigatoxin2 and Lipopolysaccharide with anti-MASP-2 antibodies or anti-properdin antibodies or control non-blocking antibodies.

- **Model of thrombosis using Intravital microscopy and FITC photo-illumination:**

The blood vessels of the Cremaster muscle of C57BL wild type mice male were subjected to FITC photo- illumination injuries under Intravital microscope to induce thrombosis. The mice were injected with anti-MASP-2 monoclonal antibodies (provided by our commercial partner OMEROS corporation, Seattle, USA) or with control antibodies and they were compared with MASP-2 -/- mice.

Results:

- **HUS model**: There is no significant difference in the survival among all tested mouse groups used in this model. Blocking of complement activation by using monoclonal anti-MASP-2 or monoclonal inhibitory anti-properdin antibodies respectively had no effect on the course of the disease.
- **Photo illumination induced thrombosis model**: treatment of mice with monoclonal anti-MASP-2 antibodies prolonged the time required for blood vessel to be completely blocked and shows that MASP-2 inhibition provides a significant therapeutic degree of MASP-2 dependent protection from thrombotic pathologies.

Discussion:
Absence of Shigatoxin receptors in murine endothelial cells makes the phenotype of HUS in rodents different from human. Blocking of complements was proved to be in effective because of the absence of thrombotic microangiopathy and mice are likely to die from tubular necrosis or neurotoxicity.

Direct photo illumination induction of thrombosis in Cremaster blood vessels induces endothelial cell death leading to liberation of intracellular components as nucleic acids RNA. Extra cellular RNA was shown to be risk factor of thrombosis and my work has shown that RNA is able to activate lectin pathway via MASP-2. Blocking of lectin pathway may be of benefit in decreasing the effect of RNA release by distresses cells triggering coagulation on coagulation system.

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

The complement system represents a major role in innate immunity. It can be activated by three different main ways: classical, alternative and lectin pathways. The classical activation is initiated by the binding of C1q to targets. Under uncontrolled complement activation different types of soluble and membrane-bound inhibitors act as controllers to prevent tissue damages as FH. FH can compete and inhibit C1q binding to some targets , so it can act as regulator for the classical pathway. Moreover, the increasing of FH : C1q molar ratio can affect complement activation by diminishing C4b fixation. Therefore, the aim of this project is to find new aspects of control of complement activation. ELISA is a major technique has used in this project and the first step is the optimization for this technique. Different buffers have been used for C1q and FH binding test. However, FH binding was high in both physiological and low salt concentration buffer, while C1q bind better in low salt concentration. Different ligand were tested for C1q and FH binding and it showed variation in its binding with both proteins. Some of these ligand have been selected to be tested on complement activation.

Saleh Ali Alshamrani
Assessment of the roles of the classical, the alternative and the lectin activation pathways of complement in experimental mouse models of Neisseria meningitides infection
Supervisor(s): Prof. Wilhelm Schwaeble & Prof. Peter Andrew

Introduction:
Neisseria meningitidis is a gram negative, bean-shaped diplococcus that causes bacterial meningitis and septicemia worldwide, with mortality rates as high as up to 10%. The complement system is a vital part of the immune system, and plays a

crucial role in fighting against invading *Neisseria meningitidis*. It is composed of many proteins that interact with each other to form a network of protection against *Neisseria meningitidis*. However the independent role of each complement pathway needs to be clarified. This project aims to illustrate the relation between the different complement system activation pathways and *Neisseria meningitidis* infection by using experimental murine models.

Methods:
ELISA, FACS, SBA, phagocytosis assay and infection studies.

Results:
The results from current study revealed that both MBL-A and MBL-C were able to bind to different strains of *N.meningitidis* whereas ficolin-A showed no binding to any of the *N.meningitidis* strains tested. A limited binding of CL-11 has been observed to different strains of *N.meningitidis*.
C3 deposition on the surface of different meningococcal strains was studied in MASP-$2^{-/-}$ serum at high serum concentrations and under buffer conditions allowing complement activation through all three pathways. Interestingly, a very high level of C3 deposition was observed in MASP-$2^{-/-}$ serum.

Discussion:
Complement pathway specific *in vitro* assays showed that all of the tested *N. meningitidis* strains used throughout this study induced complement activation in mouse serum, mainly through the lectin and the alternative pathway. Both pathways appeared to be necessary for complement deposition. Substantial levels of C3 deposition were observed on different strains belonging to different serogroups of *N. meningitidis*
To analyse this further, C3 deposition on the surface of different meningococcal strains was studied in MASP-$2^{-/-}$ serum at high serum concentrations under buffer conditions allowing complement activation through all three pathways. Interestingly, a very high level of C3 deposition was observed in MASP-$2^{-/-}$ serum suggesting that the alternative pathway either provides compensation to the absence of the lectin pathway functional activity in MASP-$2^{-/-}$ mice sera or that the remaining lectin pathway enzymes MASP-1 and MASP-3 play a role in driving the alternative pathway on *N.meningitidis*.

Izzat Abdulsatar Mezher Al-Rayahi
Role of Complement Protein Properdin in Tumour Growth and Metastasis
Supervisor(s): Dr Cordula Stover; Dr Mike Browning and Dr Lee Machado

Much cancer research has focused on understanding the development of tumour cells. The tumour microenvironment plays a significant role in the establishment and

progression of malignant cells. In recent years, the role of some components of the immune system in the establishment and progression of malignant lesion is being analysed. It has been shown that complement is one of the factors which contribute in determining the outcome of cancer progression.Properdin, as the only positive regulator, amplifies complement activation. Therefore, this project investigates the role of complement protein properdin in the control of tumour growth and metastasis. This is done using properdin deficient mouse lines, and subcutaneous and intravenous syngeneic tumour models. The project measures myeloid derived suppressor cells in the response to syringenic tumours (FACS) and characterises markers (PCR, western blot) in gene deficient compared with wildtype mice. Recent results will be present.

Sadam Yaseen

Revealing the molecular basis of the C4-byapass mechanisms: lectin pathway provision of C3b in absence of C4

Supervisor: Prof. Wilhelm Schwaeble & Prof. Peter Andrew

Introduction:
Lectin pathway of complement activation is an important mechanism of innate immune defence against invading microorganisms. The pathway consists of five different pattern recognition molecules i.e. Ficolins (M, L & H), Mannose-Binding Lectin (MBL) and Collectin 11 (CL-11), as well as three MBL-associated serine proteases (i.e. MASP-1, MASP-2 and MASP-3). Extensive studies have shown that MASP-2 cleaves C4 and C4b-bound C2, thereby mediating lectin pathway activation. The mechanism of complement activation by the lectin pathway in C4 and C2 deficient subjects is stilled obscure. Here, we explain the mechanism of lectin pathway activation in the absence of C4.

Methods:
C3 activation by MASP-2 was determined in serum free-conditions and in C4 KO, MASP-1/3 KO and MASP-2 KO mouse sera. The mechanism of that activation was confirmed by using specific inhibitor for MASP-2, SGMI-II, beside C1INH.

Results:
The molecular bases of complement lectin pathway activation in C4 deficient or C2 deficient subjects were studied. Recombinant human MASP-2 was able to cleave purified human C3 in serum-free conditions and this activation could be inhibited using a MASP-2-specific inhibitor (SGMI-II) or by C1 INH. Endogenous mouse serum MASP-2 showed same activity towards purified C3. Furthermore, MBL/MASP-2K complex activated C3 on mannan coated ELISA plate.

Discussion:
C3 deposition is a marker for complement activation. Here, for the first time, we explained the mechanism of C4-independent activation of the lectin pathway. This is a new addition to our understanding of the complement pathways and the new function of MASP-2 that is leading the direct activation of complement C3 prove its ability to activate complement even in absence of C2 or C4. Moreover, providing the alternative pathway of complement with an extra C3b needed for its functional activity.

Hussein Abbow
The interaction between complement and coagulation pathways
Supervisor: Prof. Wilhelm Schwaeble & Prof..Robert B. Sim.

The complement system is the main backbone of innate immunity and the coagulation system as a main backbone in hemostasis, both undergo enormous activation early after tissue injury. Although there are some work have been proposed to reveal the interconnections between these cascades, but the molecular interplay are still in the dark. The complement can be activated by three ways: the classical, the MBL and the alternative pathways. The complement system activity should be regulated, this regulation can be reached in host body by proteins which can be membrane or soluble. One of these regulators is factor H, which is the main regulator for the alternative pathway and recently the classical pathway. Fibrin is the main protein of blood clot. In few months some work have been done to detect the interaction between fibrin and the complement regulator factor H. results showed that factor H bind covalently to fibrin in ELISA plates. Factor H was purified from human serum in this period.

Emma Comber
Understanding the mechanisms of pore forming toxins
Supervisor(s): Prof. Peter Andrew, Andrew Hudson, Rana Lonnen

Introduction:
Streptococcus Pneumoniae is the bacteria responsible for a wide range of illnesses, including bacterial meningitis and pneumonia. It is still responsible for more than 2 million deaths worldwide each year. One of its major virulence factors is the large pore forming toxin, pneumolysin. The toxin binds to the cell membrane, oligomerizes on the surface to form a large pre-pore ring of around 50 monomers. It then undergoes conformational changes and inserts into the membrane, forming a large trans-membrane pore of around 50nm in diameter. Significant efforts have been made in order to understand the mechanism of pore formation however it is still not fully understood.

Methods:
The pore forming process has been investigated by measuring membrane potentials. This allows kinetic information about the process to be collected. Artificial cell membranes are labelled with a dye which is sensitive to the membrane potential. The toxin is added and the change in fluorescence is measured. The effect of temperature, membrane cholesterol concentration and toxin concentration has been investigated.

Results:
Binding of the toxin to the cell membrane has been observed independently of insertion and in real time. The rates of binding and insertion have been measured separately with the use of mutants which form the pre-pore ring but not a fully inserted pore. As well as providing information on the pore-forming process, the methods described above have proved useful for investigating the dynamics of the protein organisation on the cell membrane.

Raghad Hassan Hussein Sanyi
Characterisation of Mycobacterial Proteins Important for Survival in Macrophages Supervisor(s): Dr Galina Mukamolova

Introduction:
The success of *Mycobacterium tuberculosis* (*Mtb*) 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. Recent studies have shown that several mycobacterial proteins may play distinct roles during different stages of infection. My study is focused on investigation of the biological function of two mycobacterial proteins, Rv1219c and Rv3136, encoding PPE51 protein.

Methods:
Macrophage infection and growth during various stress conditions was used to study the role of BCG1279c in survival inside macrophages. The flexible cassette method (Parish and Stocker, 2000) was used to generate an *rv3136* deletion mutant in *Mtb* H37Rv.

Results and Discussion:
The results indicate that BCG1279c is important for *M. bovis* BCG survival at the initial stages of macrophage infection. Furthermore, a *bcg1279c* deletion mutant was

more sensitive to various stresses (nitric oxide, hydrogen peroxide and low pH). The *rv3136* deletion mutant did not show any growth defect in laboratory culture. Its replication in macrophages will be investigated in the near future.

Hasan Faisal Hussein Kahya
Studies on Pneumococcal Esterases
Supervisor(s): Dr.Hasan Yesilkaya and Professor Peter Andrew

The genome of pneumococcal strains contains 4 putative esterase genes, which encode 4 hypothetical esterase enzymes. These lipolytic enzymes have been reported to be important for bacterial physiology and virulence in other microorganisms. However, no detailed study has been done on pneumococcal esterases to understand their role in pneumococcal biology. Hence, to study their function, the putative esterase genes, SPD1239, SPD0534 (estA), SPD0932, and SPD1506 (axe) were mutated in two strain backgrounds, TIGR4 and D39. Non-specific esterase activity in the parental and the mutant strains, SPD1239M, SPD0534M, SPD0932M, and SPD1506M, was determined using chromogenic p-nitrophenyl esters. The highest level of esterase activity could be obtained with p-Nitrophenyl acetate (p-NPA), indicating that the pneumococcus has esterase specific for short acyl chains. The results also demonstrated that all mutants displayed significantly less esterase activity than the parental strains, however, the highest reduction in activity was observed in SPD0534M, indicating that EstA is the primary pneumococcal esterase. Genetic complementation of EstA and Axe isogenic mutants by intact copy of estA and axe restored esterase activity. estA and axe genes have been successfully expressed in E. coli BL21 using His-tag fusion, and the purified recombinant EstA and Axe were found to have optimal activity against p-NPA as a synthetic substrate compared to other p-nitrophenyl esters. In addition, EstA and Axe activity against Bovine Sub-maxillary Mucin (BSM), which is an organic highly acetylated substrate, showed that acetate release increased in a time and concentration dependent manner. We also found that pre-treatment of BSM by EstA or Axe increase sialic acid release by neuraminidase. To demonstrate esterases' role in potentiation of neuraminidase activity further, double esterase and neuraminidase (NanA) mutatants, □*estAnanA* and □*axenanA*were constructed. It was revealed that while □*estAnanA*showed a significant attenuation in growth compared to □*nanA*in medium containing BSM as the sole carbon source, no difference in growth could be seen between □*axenanA* and □*nanA* In addition the mutation of EstA alone or in combination with NanA reduced the pneumococcal virulence significantly after intranasal infection. Furthermore, nitrosative stress assay has been done to check whether theses esterases are involved in pneumococcal resistance against nitrosative stress. The growth of esterase isogenic mutants in CDM supplemented with S-nitrosoglutathione (GSNO), a nitrosative stress agent, revealed a clear attenuation of EstA and Axe mutants in the presence of GSNO in concentration dependent manner, whereas the other two

isogenic mutants, SPD932M and SPD1239M, grew as well as the wild type in the presence of GSNO.

David Ngmenterebo

The Virulent role of Type Six Secretion Systems in *Klebsiella pneumoniae*

Supervisor(s): Dr. Kumar Rajakumar & Dr. Yassine Amrani

Introduction:

Klebsiella pneumoniae is a non-motile Gram-negative enterobacterium and a significant agent in nosocomial infections. With Many evolving novel virulence mechanisms, the bacterium has a significant clinical impact (25%- 50% mortality rate) with adverse consequences in immuno-compromised individuals. Many bacteria including *Klebsiella pneumoniae* consistently employ secretion systems in the export of toxins/effectors. Type Six Secretion Systems, T6SSs (25% proteobacteria), is an inverted phage tail-like trans-envelope nano-syringe used to export toxin/effectors. While growing data suggest the virulence role of T6SS in bacteria, others are of the view that T6SS may be of much broader physiological role. Despite the clinical significance of *Klebseialla pneumoniae*, fewer virulence factors have been identified or characterized. We therefore seek to investigate the role of T6SSs in the virulence of *Klebsiella pneumoniae* as this could serve as novel drug targets.

Method:

Base on genomic *in silico* mining, identified T6SS clusters were used to create various inframe knockouts and to test our hypothesis using an array of *in vitro* and *in vivo* models. Competition assay and *Galleria* model among assays have been used to study these knockouts. We intend use other molecular techniques to study T6SS and innate immune response to these knockouts.

Results:

We created single and double mutants of T6SS clusters and specific T6SS-relate genes. Using T6SS-mediated antibacterial assay and *Galleria* model, we generated preliminary data for T6SS analysis.

Discussion:

Noticeably, the impact of T6SS can be observed in our assays though this is preliminary data and needs to be repeated for statistical significance but it seems promising.

Hastyar H. Najmuldeen
Studies on superoxides dismutases in *Klebsiella pneumoniae*
Supervisor(s): Dr.Hasan Yesilkaya

Introduction: *Klebsiella pneumoniae* is the causative agent of several nosocomial and community acquired infections. Some of its virulence determinants have been well studied but it is not known how it copes with reactive oxygen species. The analysis of *K. pneumoniae* genome revealed that this bacterium encodes three superoxide dismutase (SOD) Mn-, Fe- and CuZn co-factored SOD, which is responsible for removal of superoxide radical ($O_2^{-\bullet}$). We hypothesise that SOD activity is important for *in vitro* and *in vivo* biology of *K. pneumoniae.*

Methods: *K. pneumoniae* was routinely cultured in Luria-Bertoni broth at 37 ºC by constant shaking at 220 rpm. Qualitative and quantitative SOD activities under both aerobic and anaerobic conditions have been determined by native gel activity staining of whole cell lysates and using a commercial kit, respectively. For constructing markerless mutants, *in vitro* mutagenized gene fragments by overlap extension PCR (1) were transformed into strains containing arabinose inducible plasmid pKOBEG-apra (2) Antibiotic marker cassette in the mutant strain was removed by Flp-recombinase mediated excision of FRT (Flp recombinase target) using plasmid pFLP2.

Results and Discussion: Gel activity staining of whole cell lysate and subsequent treatment with sodium cyanide and hydrogen peroxide, which are used for differentiation of SOD metal co-factor, showed the presence of both cytoplasmic Mn- and Fe-SODs, but not periplasmic Cu/Zn-SOD activity. We also found that in aerobic growth total SOD activity increases relative to anaerobic growth. In order to study their function, markerless single, double and triple mutations to *sod* genes in one genetic background were introduced. Inactivation of either *sod* reduced total SOD activity, and attenuated *K. pneumoniae* to grow in aerobic conditions and in the presence of redox active compounds, such as paraquat.

Ibtihal Abdulhadi Majeed Al-Karaawi
The role of CL-11, a novel recognition component of the lectin activation pathway of complement in pneumococcal infection
Supervisor: Prof.Wilhelm Schwaeble

Streptococcus pneumoniae is a respiratory pathogen which may cause infectious disease. The Complement system plays a central role in host defense against invading microorganisms such as *S. pneumonia*e. Complement is activated via three pathways, the classical, the alternative and the lectin pathway. The lectin pathway is driven by soluble recognition molecules binding to specific pathogen associated

molecular patterns (PAMPS).One of these molecules is Collectin 11. CL- 11 was recently shown to have a role in the activation of the lectin pathway by binding to bacteria, fungi and influenza A virus. According to previous studies in our lab, CL-11 tend to bind to the *S. pneumoniae* and form complex with MASP -2 and it may have a critical role in driving the activation the lectin pathway on the surface of *S. pneumoniae*. Therefore , the role of CL-11 in activating the lectin pathway against *S. pneumonia* will be analyzed by assessing the susceptibility of CL-11 deficient C57BL/6 mice to experimental infections with *S. pneumoniae* D39. Another aspect of my study is to assess whether any of the SNPs of the human CL-11 gene correlates with a clinically established pre-disposition for frequent microbial infections.

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