Medical Research Council

UNIVERSITY OF OXFORD

Oxford-MRC Doctoral Training Partnership

Oxford-MRC DTP Symposium

18 June 2020

Programme

10:00 **Welcome – Professor Ester Hammond,** Director of Oxford-MRC DTP

10:15 Student Talks – Session 1 (Chair – Keanu Paphiti)
Gurpreet Bharj – Department of Physiology Anatomy and Genetics
"Insights into Otitis Media: Dissecting the interaction of C-Reactive Protein with Non-Typeable Haemophilus influenzae"
Sonali Munshaw – Department of Physiology Anatomy and Genetics
"Thymosin b4 - A novel therapeutic that protects against aortic aneurysm"
Athena Cavounidis – Nuffield Department of Medicine
"A pathogenic inflammatory loop in Hermansky-Pudlak Syndrome"

11:15 **Coffee Break**

11:30 Student Talks – Session 2 (Chair – Helena Meyer-Berg)
Lilli Hahn – Sir William Dunn School of Pathology
"Investigating the mechanisms of nuclear envelope re-assembly during mitosis"
Rose Hodgson – MRC Harwell Institute
"A rare genetic disorder models human lupus-like disease"
Henry Bailey – Nuffield Department of Medicine
"Cryo-electron microscopy reveals new functional states of the Succinyl-CoA synthetase complex"

12:30 **Lunch Break**

13:30 Keynote Lecture

Professor Chas Bountra – Nuffield Department of Clinical Medicine and Structural Genomics Consortium Oxford

"The world needs many more great leaders, innovators and entrepreneurs"

14:15 3 Minute Thesis Competition (Chair – Jan Boehning)

Katie Mellor – Centre for Statistics in Medicine; Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences

"Investigating the use of pre-specified criteria to inform progression from a randomised pilot study to a definitive RCT: A first year DPhil outline"

Amy Southern – Department of Biochemistry and MRC Harwell Institute

"Deciphering Deafness in Down Syndrome: Finding the Otitis Media Gene"

Sophie Hughes – Oncology

"Investigating immune infiltrate in the pancreatic tumour microenvironment using a 17-colour flow cytometry panel"

Daan Paget – Pharmacology

"Plasma extracellular vesicle proteomes are altered by the type of isolation method"

Shahd Fouad – Oncolocgy

"Investigating the role of cyclin F in oncogene-induced DNA replication stress"

15:00 **Dr. Mike Moss**

"How to think like an inventor to make better decisions about career and work"

15:45 **Conclusion and Prizes**

Student Talk Abstracts

Session 1

Insights into Otitis Media: Dissecting the interaction of C-Reactive Protein with Non-Typeable Haemophilus influenzae

Gurpreet Bharj, Dr Derek Hood, Professor Marisa Martin-Fernandez

Department of Physiology Anatomy and Genetics

Otitis Media (OM) is inflammation of the middle ear (ME). Infection by the bacterium Non-typeable Haemophilus influenzae (NTHi) is one of the most common causes of OM. Phosphocholine (PCho) on the NTHi lipopolysaccharide (LPS) influences host interaction and disease. C-reactive protein (CRP), an acute phase response molecule, binds PCho and can initiate bacterial killing. PCho expressed on NTHi can therefore influence the immune response elicited against these bacteria. However, some strains of NTHi survive despite the presence of CRP. The aim of this project is to study the interaction of CRP with NTHi to understand its role in bacterial survival and disease. CRP was detected 7-days post-inoculation at similar levels in ME fluid (MEF) samples from Junbo mice (a characterised NTHi infection model) infected with PCho-expressing and PCho non-expressing NTHi variants. Higher but comparable levels of CRP were detected in the serum from both Junbo and wild-type mice infected with each NTHi strain. Control Junbo mice inoculated with PBS showed a baseline level of CRP in MEF that was significantly higher than that observed in mice inoculated with NTHi strains, suggesting that CRP may be binding to infecting bacteria in vivo. IL-1β expression levels were elevated in MEF of NTHi inoculated mice. The binding assays suggest that mouse rCRP may interact with NTHi independent of PCho expression and its location for NTHi 375 isogenic strains. The number of viable, intracellular NTHi recovered from macrophages 24h after uptake was lower for rCRP treated than non-treated NTHi, supporting a role for CRP in opsonophagocytosis. The interaction of CRP (human and mouse) with NTHi is currently under investigation to advance our understanding of its role in the complex biological processes that influence bacterial killing and the onset, progression and resolution of OM caused by NTHi.

Thymosin b4 - A novel therapeutic that protects against aortic aneurysm

Sonali Munshaw, Susann Bruche, Jyoti Patel, Andia Redpath, Karina N. Dubé, Regent Lee, Ashok Handa, Keith M. Channon, Nicola Smart

Department of Physiology Anatomy and Genetics

Introduction: Aortic aneurysm (AA) is a degenerative vascular disease and a leading cause of mortality. Poor understanding of underlying mechanisms limits pharmacological treatment. Dysregulation of smooth muscle cell (VSMC) phenotype critically impairs vascular stability. Low density lipoprotein receptor related protein 1 (LRP1), an endocytic regulator of PDGFRβ signalling in VSMCs, is associated by GWAS with AA risk. Thymosin β4 (Tβ4) is an actin binding peptide with roles in embryonic VSMC differentiation. We identified a putative interaction between Tβ4 and LRP1 in VSMCs. Rationale: As a regulator of VSMC differentiation, we hypothesised that Tβ4 may interact with LRP1, to maintain healthy vasculature postnatally and protect against disease. Methodology and

Results: Global and VSMC-specific Tβ4KO mice, like LRP1 KO, demonstrated predisposition to AA (1mg/kg/day Angiotensin II model), with aortic dilatation and rupture in <5 days. Accelerated disease progression was not caused by exacerbated inflammation, rather by enhanced VSMC phenotypic switching, elastin degradation and dysregulated LRP1/PDGFRβ signalling. The therapeutic potential of exogenous Tβ4 to prevent AA was evaluated. Significant reduction in aortic dilatation and rupture associated with preserved VSMC and elastin phenotype and normalised PDGFRβ signalling. Ongoing studies will define the molecular mechanisms by which Tβ4 controls PDGFRβ signalling to protect against AA.

A pathogenic inflammatory loop in Hermansky-Pudlak Syndrome

Athena Cavounidis, Sumeet Pandey, Melania Capitani, Fiona Powrie, Bernadette Gochuico, William Gahl, Louis Cohen and Holm H. Uhlig

Nuffield Department of Medicine

Inflammatory bowel diseases (IBD) are complex and chronic diseases characterized by intestinal inflammation due to a dysregulated interplay between the host immune system and the microbiota. Mendelian diseases that present with intestinal inflammation can provide important insights into the mechanisms of disease. Hermansky-Pudlak Syndrome (HPS) types 1 and 4 are classified as lysosomal storage disorders and may present with intestinal inflammation. We found that myeloid cells had high expression levels of the HPS1 and HPS4 genes compared to other immune cell types. We show that monocyte-derived macrophages from HPS1 patients express an inflammatory gene signature, consistent with polygenic IBD signatures. Additionally, HPS1 macrophages have a defective anti-microbial defect in a gentamicin protection assay and confocal microscopy experiments. Our data suggest that an endosomal trafficking defect caused by HSP1 deficiency affects anti-microbial activity. This mechanism is relevant for the phagocyte foam cell formation seen in HPS1 patients and explains the tissue inflammation in the absence of a clinically relevant immunodeficiency.

Session 2

Investigating the mechanisms of nuclear envelope re-assembly during mitosis

Lilli Hahn, Sabrina Liberatori, Logesvaran Krshnan, Pedro Carvalho

Sir William Dunn School of Pathology

The nuclear envelope (NE) separates the nucleoplasm from the cytoplasm and is involved in numerous biological processes such as gene regulation and repair. It is made up of two lipid bilayers, which are continuous with the endoplasmic reticulum (ER). Interestingly, the inner nuclear membrane (INM) harbours a distinct set of proteins, and disruption in INM homeostasis has been linked to muscular dystrophies and premature ageing syndromes. During mitosis, the elaborate structure of the NE is dismantled and INM proteins disperse throughout the peripheral ER. Upon re-establishment of the NE in late mitosis, INM proteins have to migrate back to the NE. However, the molecular mechanisms involved in this process are poorly understood. Using the APEX2 proximity biotinylation assay coupled to quantitative proteomics, this project aims to investigate the underlying mechanisms of INM protein re-location to the newly formed NE after mitosis. Targeting APEX2-fusion proteins to various locations within the INM and the peripheral ER at distinct time points during NE re-assembly, I will create a temporally and spatially resolved understanding of NE reassembly.

A rare genetic disorder models human lupus-like disease

Rose Hodgson, Tanya Cheetham, Eleanor Cawthorne, Lucie Abeler-Dorner, Adrian Hayday, Katherine Bull, Richard Cornall

MRC Harwell Institute

Rare genetic variants provide a tractable way to discover novel genes and regulatory pathways involved in maintaining immunological tolerance. Individuals with loss-of-function mutations in the prolidase (PEPD) gene present with a range of clinical manifestations including lower limb skin ulcerations, recurrent respiratory infections and, in some patients, systemic lupus erythematous (SLE) characterised by anti-nuclear antibodies (ANAs). Prolidase drives the hydrolysis of dipeptides containing proline or hydroxylproline and is required in the breakdown of proline-rich substrates including collagen, however the mechanism of how prolidase regulates immune tolerance is unknown. A high-throughput screen for autoimmune phenotypes at the Wellcome Trust Sanger Institute showed that Pepd-/- mice have higher incidence of class-switched ANAs than wildtype counterparts, indicating they may provide a good model to study the role of prolidase in autoimmunity. Flow cytometric immunophenotyping has demonstrated that Pepd-/- CD4+ and CD8+ T cells present an activated effector phenotype, with enlarged proportions of CD44hiCD62L- T cells, and CD4 Treg lymphocytes. Multi-dimensional mass cytometry has confirmed the affected T cell sub-populations, and indicated that effector T cells have an altered surface phenotype. Furthermore, the combination of single cell proteomic and transcriptomic sequencing has enabled identification of pathways affected by prolidase loss in T cell subsets.

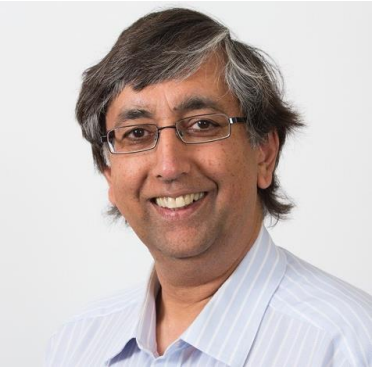
Cryo-electron microscopy reveals new functional states of the Succinyl-CoA synthetase complex

Henry J. Bailey, Elzbieta Rembeza, Leela Shrestha, Nicola Burgess-Brown, Wyatt W. Yue

Nuffield Department of Clinical Medicine

Mitochondrial Succinyl-CoA synthetase (SCS) catalyses the reversible conversion of succinyl-CoA to succinate in the citric acid cycle. SCS is composed of a catalytic α-subunit (SUCLG1) that interacts with one of two possible beta subunits (SUCLA2 or SUCLG2). The different subunit pairs allow substrate-level phosphorylation producing either ATP via the SUCLA2-SCS complex or GTP by the SUCLG2-SCS complex. SUCLG2-SCS has been well characterised from a structure function perspective revealing heterodimeric arrangement of two subunits. However, disease mutations resulting in rare mtDNA depletion syndromes (MDS) are found only within in SUCLG1 and SUCLA2 subunits. Here, the crystal structure of the human SUCLA2-SCS complex at 2.6 Å resolution has been determined revealing a similar heterodimeric arrangement to the homologous SUCLG2-SCS complex. Surprisingly, in-solution studies of the complex revealed a previously uncharacterised substrate specific inter conversion between the hetero-dimer and a higher order oligomeric state. Cryo-electron microscopy data at 6.5 Å resolution unveils novel interaction interfaces within the complex, divergent from the SUCLG2-SCS, that allow octamerization of the hetero-dimer. This work provides a starting point to better understand catalytic mechanism and the role disease mutations leading to MDS.

Keynote Lecture



The world needs many more great leaders, innovators and entrepreneurs

Professor Chas Bountra

Nuffield Department of Clinical Medicine and Structural Genomics Consortium Oxford

Professor Chas Bountra has worked in both industry and academia. Chas previously held the roles of Vice President and Head of Biology for the pharmaceutical company GlaxoSmithKline, where he was responsible for developing drugs for a range of inflammatory and gastrointestinal diseases. Chas is now chief scientist for the Stuctural Genomics Consortium in Oxford and Professor of Translation Medicine for Nuffield Department of Clinical Medicine. His current research interests are the identification of novel human drug targets for diseases including cancer and Alzheimer's using genetic and epigenetic approaches, and the utilisation of target crystal structures to direct drug design. Chas is also an advisor for many biotech and pharma drug discovery programmes and aims to improve the efficiency of drug development by building and strengthening links between acadaemia, industry, investors, regulatory bodies, hospitals and patients.

Invited Speaker

How to think like an inventor to make better decisions about career and work

Dr. Mike Moss

Inventor and Careers Advisor

Dr. Mike Moss has had a widely varied career, from research to music production. After completion of a PhD in chemistry, Mike undertook post doctoral research at the California Institute of Technology. Mike then spent 22 years working for Procter and Gamble in Research and Product Development where he developed 54 patentented inventions, including 3 in 1 laundry detergent pods. Mike is now an Oxford University careers advisor and provides coaching and lectures on confidence, creativity and innovation.

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- Bridget, TMS patient

"*I feel like a new person and my family says that they are glad to finally have 'me' back.*"
- Kathy, TMS patient

"[TMS Therapy] gives me the opportunity to actually live my life for the first time."
- Rob, TMS patient

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MRC Harwell Institute is at the international forefront of the use of mammalian models to study genetics and human disease.

MRC Harwell Institute is an international research centre at the forefront of the use of mammalian models to study genetics and human diseases. Located on the Harwell Campus, just south of Oxford, it is nestled amongst a vibrant community of leading science organisations. We aim to advance medicine through cutting-edge research into the genetic basis of disease. Our research programmes span an entire lifetime, from embryonic development to diseases of ageing.

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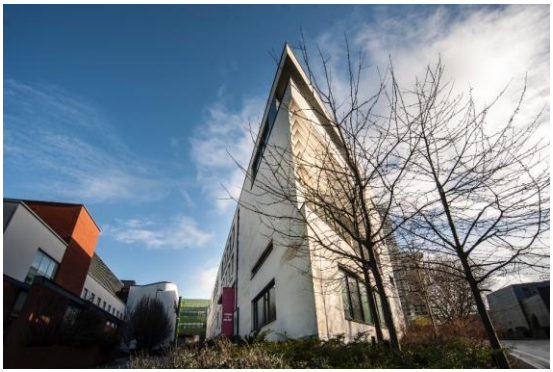
MRC Molecular Haematology Unit

UNIVERSITY OF OXFORD

MRC Molecular Haematology Unit (MRC MHU) aims to understand how mature blood cells are normally made from stem cells and how this is perturbed in common blood disorders. Ultimately, their purpose is to improve the prognosis of patients with inherited and acquired blood disease.

The Unit includes 14 research teams with over 100 scientists who share a common interest in understanding the process by which multipotential haemopoietic stem cells become committed and differentiate into the highly specialised cells found in the peripheral blood (red cells, granulocytes, lymphocytes and platelets). They also study how these processes are perturbed in acquired and inherited blood diseases such as thalassaemia, myelodysplasia and leukaemia.

The Medical Research Council Population Health Research Unit (MRC PHRU) generates and disseminates reliable evidence from randomised trials and genetic or classical epidemiological studies that lead to practical methods of avoiding premature death and disability, or to an understanding of disease mechanisms.



The Unit has a particular focus on cardiovascular and metabolic diseases, including diabetes mellitus and chronic kidney disease. It has facilitated some of the world's largest and most informative observational studies and randomised trials.

MRC PHRU is part of the Clinical Trial Service Unit & Epidemiological Studies Unit in the Nuffield Department of Population Health. For further information, please visit https://www.mrc-phru.ox.ac.uk/

Vertex is a global biotechnology company that invests in scientific innovation to create medicines for people with serious diseases. The company has three approved medicines in the UK that treat the underlying cause of cystic fibrosis (CF) – a rare, life-threatening genetic disease — and has several ongoing clinical and research programs in CF. Beyond CF, Vertex has a robust pipeline of investigational medicines in other serious diseases where it has deep insight into causal human biology, such as sickle cell disease, beta thalassemia, pain, alpha-1 antitrypsin deficiency, Duchenne muscular dystrophy and APOL1-mediated kidney disease.

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