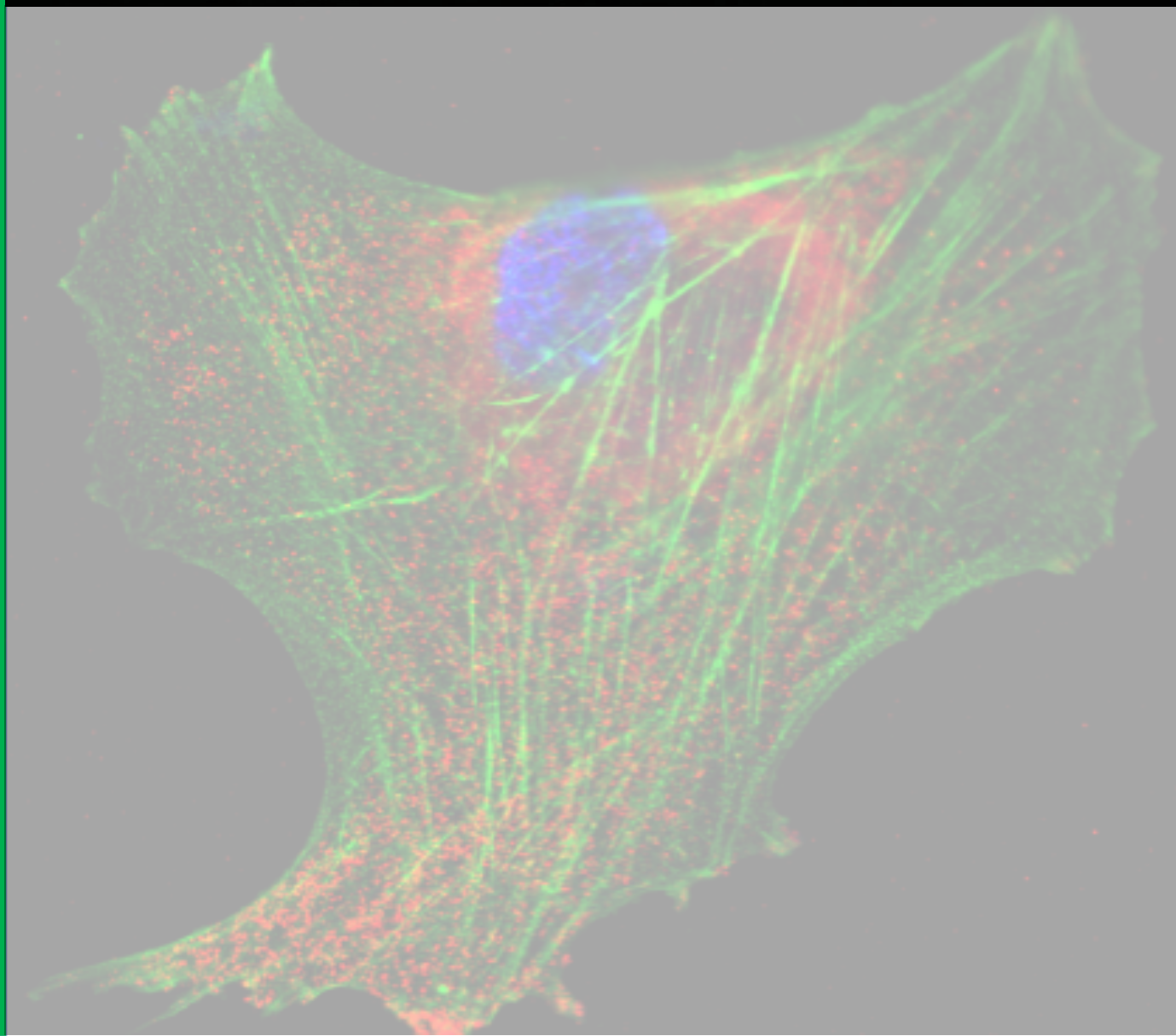
UK Cell Adhesion Society

31st UK Cell Adhesion Society Meeting

'A Day in the Life of Cellular Interactions'

University of Birmingham 1st-2nd July 2019

PROGRAMME



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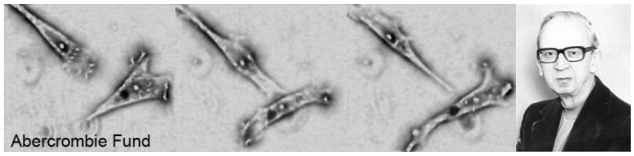

Thank you to the Abercrombie fund for supporting our keynote speaker Christoph Scheirmann

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Welcome to Birmingham

31st UKCAS Scientific Meeting

'A Day in the Life of Cellular Interactions'

University of Birmingham 1st-2nd July 2019

Dear Participants,

It is our great pleasure to welcome you to the University of Birmingham on behalf of the UK Cell Adhesion Society. The full and exciting scientific programme features a dedicated symposium on '*A Day in the Life of Cellular Interactions'*. Inflammation is a key feature of many diseases and so this meeting aims to showcase the latest discoveries in the basic biology and imaging of inflammatory processes and how they impact various body systems. Of equal importance are the 14 free oral communications and 19 posters selected from highly competitive abstract submissions.

We would like to thank our many sponsors for supporting this meeting. Without their generosity, the high standard of UKCAS meetings cannot be maintained. We encourage you all to visit the trade exhibitors who have contributed significantly to the success of this meeting. We hope you will also enjoy the meeting social events and use them as an opportunity to enhance scientific networking and develop new collaborations.

It only remains for us to thank all the registered delegates and invited speakers for attending this exciting meeting from local, national and overseas institutes. We wish you all a scientifically rewarding and enjoyable meeting.

Yours sincerely,

Myriam Chimen (UKCAS) and Helen McGettrick (UKCAS)

Organising committee

31st UKCAS Scientific Meeting

'A Day in the Life of Cellular Interactions'

University of Birmingham 1st-2nd July 2019

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General information

Registration: The Wolfson Centre, The Medical School, 8.30am onwards each day
Commercial Exhibition: Wolfson Centre common room. Trade exhibitors will be interspersed between the poster presentations. Sponsor quiz price will be awarded!

Oral Presentations: Oral presentations will take place in the CPD lecture theatre. Power Point presentation files should preferably be brought on a USB memory stick formatted for PC / Mac and loaded on the meeting projection computer at the start of the day of the presentation. We kindly request personal laptops are not used for presentations unless absolutely necessary (eg. if movies are shown) as this can cause delays in the schedule. Please contact the Event team if you intend to use your own laptop (med-cpdbookings@contacts.bham.ac.uk). Oral communications should be **15 minutes** in length allowing 5 minutes for questions.

Poster Presentations: Posters will be located in the Wolfson Centre common room. Posters should be **950x950mm**. Posters should be mounted by Monday morning and be displayed for the whole meeting duration. All poster presenters should be at their posters during lunch poster sessions on both days. At lunch time on Monday 1st of July and Tuesday 2nd of July, a panel of judges will select posters for poster prizes so please be at your posters at this time.

Refreshments and Lunch: Coffees/teas and buffet lunches for both days will be included in the registration and served in the Wolfson Centre common room.

Accommodation is available in local hotels including the Edgbaston Conference Hotel Centre and hostels within 30 minutes walk from the meeting venue. Alternatively, a number of luxury and budget hotels can be found along Broad Street, Five Ways. A local train from Five Ways travels to the University (2 mins) every 10 minutes. A list of hotels can be found here: https://www.birmingham.ac.uk/facilities/mds-cpd/conferences/ukcas-2019/hotels.aspx. Luggage can be left securely in a cloakroom over the meeting duration.

Travel information to the meeting venue, maps and instructions are included at the back of this booklet and on the 2019- UKCAS Conference website :
https://www.birmingham.ac.uk/facilities/mds-cpd/conferences/ukcas-2019/venue.aspx

Drinks Reception and Society Dinner will be at the **Banqueting Suite, Birmingham Council House, Victoria Square, B1 1BB** in the city centre from 19.00 onwards on Monday 1st July. Dinner tickets should be pre-booked at a cost of £50 per person, which includes a drinks reception, 3 course meal, wine and entertainment (http://www.thesla.org/wpfiles/wp-content/uploads/2013/10/Council-House-Map-and-Directions-2013-14.pdf)

Programme

MONDAY 1st JULY 2019

Wolfson Centre Common Room

	10:00 – 10:50	**Arrival & Registration** The Medical School – Poster Set-up

Leonard Deacon Lecture Theatre

	10:50 – 11:00	**Welcome Address** Helen McGettrick & Myriam Chimen – Local Organisers

Scientific Session 1: Chairs – Myriam Chimen and Ann Ager

S1	11:00 – 11:45	**KEYNOTE SPEAKER** **Arne Akbar** **University College London, UK** TARGETING INFLAMMAGEING TO ENHANCE IMMUNE ACTIVATION *IN VIVO*.
		Selected Oral Communications
OC1	11:45 – 12:05	**Speaker 1- Anna Barkaway (QMUL)** AGEING-ASSOCIATED SYSTEMIC INFLAMMATION SUPPORTS NEUTROPHIL REVERSE TRANSENDOTHELIAL MIGRATION *IN VIVO*
OC2	12:05 – 12:25	**Speaker 2- Laleh Pezhman (University of Birmingham)** ROLE OF T-CADHERIN IN THE ANTI-INFLAMMATORY EFFECTS OF ADIPONECTIN

Wolfson Centre Common Room

	12.25 – 14.00	Buffet Lunch, Poster Session and Trade Exhibitors

Scientific Session 2: Chairs – Victoria Ridger and Anna Barkaway

S2	14:00 – 14:45	**KEYNOTE SPEAKER** **Clare Howarth** **University of Sheffield, UK** A MOUSE MODEL OF NEUROVASCULAR COUPLING DURING OLD AGE.
		Selected Oral Communications
OC3	14:45 – 15:05	**Speaker 1- Aikaterini Kalargyrou (UCL)** IDENTIFYING NOVEL WAYS OF CELLULAR COMMUNICATION BETWEEN ROD PHOTORECEPTORS
OC4	15:05 – 15:25	**Speaker 2- Alana Cowell (University of Kent)** TALIN ROD DOMAIN-CONTAINING PROTEIN 1 (TLNRD1) IS A NOVEL ACIN-BUNDLING PROTEIN THAT LOCALISES TO THE TIPS OF FILOPODIA

Wolfson Centre Common Room

	15.25 – 16.00	**Tea/Coffee Break**

Programme

Scientific Session 3: Chairs – Diane Cooper and Asif Iqbal		
S3	16:00 – 16:45	**KEYNOTE SPEAKER** **Christoph Scheiermann** **University of Munich, Germany** CIRCADIAN RHYTHMS IN LEUKOCYTE MIGRATION AND FUNCTION
		Selected Oral Communications
OC5	16:45 – 17:05	**Speaker 1- Joshua Bourne (University of Birmingham)** PLATELETS INTEREACT WITH MACROPHAGES VIA THE CLEC-2-PODOPLANIN AXIS TO REGULATE IMMUNE CELL TRAFFICKING DURING INFECTION
OC6	17:00 – 17:25	**Speaker 2- Louise Jonhson (University of Oxford)** CD44 ORGANIZES THE HYALURONAN GLYCOCALYX ON DENDRITIC CELLS FOR EFFICIENT LYMPHATIC TRAFFICKING
Room TBC		
	17.30 – 18.30	**UKCAS Committee Meeting**
Banqueting Suite, Council House, City Centre Birmingham		
	19.00 – late	**Drinks Reception and Society Dinner (sitting 19.45 and dinner served at 20.00)**

TUESDAY 2ND OF JULY 2019

Scientific Session 4: Chairs – Mathieu-Benoit Voisin and Jon Hazeldine		
S4	9:00 – 9:45	**KEYNOTE SPEAKER** **Elizabeth Sapey** **University of Birmingham, UK** TAMING THE MIGHTY NEUTROPHIL - IMPROVING RESPONSES TO ACUTE INFECTION AND CHRONIC DISEASE
		Selected Oral Communications
OC7	9:45 – 10:05	**Speaker 1- Carolina Roque Silva (University of Sheffield)** THE EFFECT OF HUMAN NEUTROPHIL-DERIVED MICROVESICLES ON LUNG EPITHELIAL CELL FUNCTION
OC8	10:05 – 10:25	**Speaker 2- Franziska Krautter (Univeristy of Birmingham)** GALECTIN-9 CAUSES INCREASED ADHESION OF LEUKOCYTES FROM PERIPHERAL ARTERIAL DISEASE PATIENTS COMPARED TO HEALTHY INDIVIDUALS
OC9	10:25 – 10:45	**Speaker 3- Silvia Oggero (QMUL)** MONOCYTE EXTRACELLULAR VESICLES ACTIVATE THE HUMAN ATHEROSCLEROTIC PLAQUE
Wolfson Centre Common Room		
	10:45 – 11:00	**Tea/Coffee Break**

Programme

Scientific Session 4: Chairs – Ed Rainger and Julie Gibbs

S4	11:00 – 11.45	**Helen Weavers** **University of Bristol, UK** DISSECTING THE COMPLEX INTERPLAY BETWEEN TISSUE REPAIR AND INFLAMMATION USING *IN VIVO* MODELS
		Selected Oral Communications
OC10	11:45 – 12:05	**Speaker 1- Zania Stamataki (University of Birmingham)** HEPATOCYTES DELETE REGULATORY T CELLS BY ENCLYSIS: A CD4+ T CELL ENGULFMENT PROCESS
OC11	12:05 – 12.25	**Speaker 2- Ann Ager (University of Cardiff)** L-SELECTIN ENHANCED T CELLS PROLONG PROTECTIVE IMMUNITY TO INFLUENZA VIRUS INFECTION

Wolfson Centre Common Room

12.25 – 14.00 Buffet Lunch, Poster Session and Trade Exhibitors

Scientific Session 6: Chairs – Helen McGettrick and Patric Turowski

S6	14:00 – 14:45	**John O'Neill** **University of Cambridge, UK** CIRCADIAN ACTIN DYNAMYCS DRIVE RHYTHMIC FIBROBLAST MOBILISATION DURING WOUND HEALING
		Selected Oral Communications
OC12	14:45 – 15:05	**Speaker 1- Camilla Cerutti (King's College London)** CDC42 TARGETS REGULATE CANCER CELL INTERACTION WITH ENDOTHELIAL CELLS IN TUMOR PROGRESSION
OC13	15:05 – 15:25	**Speaker 2- Julie Gibbs (University of Manchester)** REGULATORY T CELLS CONFER A CIRCADIAN SIGNATURE TO INFALMMATORY ARTHRITIS
OC14	15:25 – 15:45	**Speaker 3- Fabian Spill (University of Birmingham)** MECHANICAL REGULATION OF ENDOTHELIAL CELL-CELL ADHESIONS AND FORMATION OF GAPS ASSISTING CANCER EXTRAVASATION

Leonard Deacon Lecture Theatre

15:45 – 16:00	**Presentation of Prizes** Announcement of 2020 UKCAS meetings
16:00	**Meeting Closes**

S1

Arne Akbar

Biography

Professor Akbar's work involves studies at the interface between academia, industry and clinical practice. He is internationally recognized for his studies on mechanisms that control the differentiation and senescence of human T lymphocytes and two of these studies were published in 2014 in Nature Immunology and the Journal of Clinical Investigation. In addition, he has made seminal observations about how different CD45R isoforms can be used to discriminate between primed and T cells and these markers are now used in routine diagnostic practice. His group was one of the first to identify human regulatory T cells. He was closely involved in the development of Basilizximab (Simulect), used for the prevention of acute solid organ graft rejection (Akbar is a joint patent holder) that has been used to treat ~300,000 patients. His group have also developed cutaneous recall antigen challenge models in humans for the study of immunity *in vivo* that have been adopted by researchers worldwide and by Glaxo Smith-Klein. His research group consists of basic scientists and clinicians facilitating the translational aspects of his work. The benefit of this combination is exemplified by the recent award of a highly competitive multidisciplinary MRC Experimental Medicine Grant (Akbar PI) to investigate whether blocking p38MAP kinase in older humans *in vivo* enhances their responses to recall antigen challenge in the skin. These studies are based on original mechanistic data that was generated in the Akbar Lab.

S2

Clare Howarth

Biography

Dr Clare Howarth is a Sir Henry Dale Research Fellow at the University of Sheffield. Her research focuses on understanding how the brain's blood flow is controlled in order to maintain brain function. Dr Howarth has previously discovered a novel mechanism of brain blood flow control acting at the capillary level (with Prof. Attwell, UCL), and has demonstrated a novel role for astrocytes in regulating hypercapnic vasodilation (with Prof. MacVicar, UBC, and Prof. Sibson, Oxford). In Sheffield, Clare's lab uses a multimodal approach to interrogate the relationship between neuronal activity and evoked blood flow changes, and how that relationship alters in conditions such as aging.

S3

Christoph Scheiermann

Biography

Christoph Scheiermann studied biochemistry at the FU Berlin. After having obtained his PhD at Imperial College London in 2008 in the group of Sussan Nourshargh he went to the Mount Sinai School of Medicine and the Albert Einstein College of Medicine in New York for postdoctoral work in the lab of Paul Frenette. In 2013 he started his own lab at the LMU Munich and in 2018 moved to the University of Geneva to take up an associate professorship. The research of the group focuses on the influence of time-of-day and the nervous system on the interaction of leukocytes with the vessel wall and the generation of acute and adaptive immune responses.

S3

Helen Weavers

Biography

After completing her developmental biology PhD studying tissue morphogenesis at the University of Cambridge (Prof Helen Skaer's lab), Helen moved into the field of tissue repair and inflammation during a postdoc with Profs Paul Martin and Will Wood. Helen has recently setup her own lab at the University of Bristol where she explores mechanisms driving tissue morphogenesis and repair, with a focus on the inflammatory response.

S5

Elizabeth Sapey

Biography

Dr Sapey is a Reader within the Institute of Inflammation and Ageing and an Honorary Respiratory Consultant Physician at the Queen Elizabeth Hospital, Birmingham. Her research group studies chronic inflammation associated with ageing and lung disease, in particular neutrophilic inflammation seen with COPD and Alpha 1 Anti trypsin Deficiency and acute lung diseases such as Pneumonia. She oversees the Chronic Disease Resource Centre COPD Cohort, which is a deeply phenotyped group of patients with COPD seen within the Centre for Translational Inflammation Research and is part of the Early COPD cohort, a national endeavour to understand COPD at its earliest manifestation.

Liz has established the UKs first research training programme in Acute Medicine with a number of translational and clinical research projects aimed at improving the care we provide to patients during unplanned admissions.

Liz's clinical work is based within the Queen Elizabeth Hospital, where she sees both respiratory and acute medical medical patients. Liz is the COPD Research Lead and Research Lead for Acute Medicine within this large NHS Trust.

Liz is the Managing Director of the NIHR/ Wellcome Clinical Research Facility (Adults), which supports a wide range of experimental research studies across Birmingham Health Partners (The University of Birmingham, University Hospital Birmingham NHS Foundation Trust and Birmingham Children's Hospital). She is the Chair of the British Thoracic Society Science Committee and a steering member of SUSTAIN, an AMS initiative to promote and retain woment in academic science.

Liz has achieved much of her successes while working part time (with young children).

S6

John O'Neill

Biography

John studied Biochemistry at New College, Oxford, and then did his PhD research on cAMP signalling and the mammalian circadian pacemaker at the MRC Laboratory of Molecular Biology in Cambridge with Michael Hastings. As a post-doc, he studied circadian rhythms in plants and algae with Andrew Millar (Edinburgh) and then human cells at the Institute of Metabolic Science in Cambridge. John was awarded a Wellcome Trust Career Development Fellowship in 2011, and in 2013 was recruited to become a group leader in the Cell Biology Division of the LMB.

The O'Neill group is interested in the fundamental mechanisms that sustain circadian rhythms in eukaryotic cells, how this endogenous clock evolved, and how daily timekeeping bestows an adaptive advantage upon specific mammalian cellular functions.

SESSION 1:

Keynote Speaker:

S1

ENHANCEMENT OF CUTANEOUS IMMUNITY DURING AGEING

Arne Akbar
University College London, UK

Immunity declines with age that leads to re-activation of varicella zoster virus (VZV). In humans, age associated immune changes are usually measured in blood leukocytes however this may not reflect alterations in tissue-specific immunity. We used a VZV antigen challenge system in the skin to investigate changes in tissue specific mechanisms involved in the decreased response to this virus during ageing. We assessed cutaneous immunity by the extent of erythema and induration after intradermal VZV antigen injection. We also performed immune histology and transcriptomic analyses on skin biopsies taken from the site of challenge in young (<40 yrs) and old (>65 yrs) subjects. Old humans exhibited decreased erythema and induration, $CD4^+$ and $CD8^+$ T cell infiltration and attenuated global gene activation at the site of cutaneous VZV antigen challenge compared to young subjects. This was associated with elevated sterile inflammation in the skin in the same subjects, related to p38 MAPK-related pro-inflammatory cytokine production ($p < 0.0007$). We inhibited systemic inflammation in old subjects by pre-treatment with an oral small molecule p38 MAP kinase inhibitor (Losmapimod), which reduced both serum C reactive protein (CRP) and peripheral blood monocyte secretion of IL-6 and TNF-□□□In contrast, cutaneous responses to VZV antigen challenge was significantly increased in the same individuals ($p < 0.0006$). Therefore, excessive inflammation in the skin early after antigen challenge retards antigen-specific immunity. However this can be reversed by inhibition of inflammatory cytokine production that may be utilized to promote vaccine efficacy and the treatment of infections and malignancy during ageing.

Key Messages:

1) Cutaneous immunity to VZV decreases during ageing
2) Associated with excessive early skin inflammatory response
3) The inflammation is linked to p38 MAP kinase activation
4) An oral p38 inhibitor (Losmapimod) inhibits systemic inflammation
5) Short-term p38 treatment enhances the VZV skin response in old subjects

Short talks:

OC1

AGEING-ASSOCIATED SYSTEMIC INFLAMMATION SUPPORTS NEUTROPHIL REVERSE TRANSENDOTHELIAL MIGRATION *IN VIVO*

Anna Barkaway, Loïc Rolas, Jenny Bodkin, Matthew Golding, Mathieu-Benoit-Voisin and Sussan Nourshargh

Queen Mary University of London

Ageing is the primary risk factor for all inflammatory disorders. Whilst many studies have investigated the impact of age on immune cell functions, less is known about age-associated changes in vascular responses. Here we investigated the impact of ageing on neutrophil-vessel wall interactions *in vivo*.

To address the above, neutrophil migration through IL-1β- and TNF-stimulated cremaster muscle post-capillary venules was analysed by brightfield and/or confocal intravital microscopy (IVM) in aged (>16 months) and young (2-4 months) mice. Whilst no gross significant change in total diapedesis was noted in aged vs young mice, aged animals exhibited significant levels of neutrophil adhesion and aberrant neutrophil transendothelial migration (TEM), most notably reverse TEM (rTEM; ~20% and ~1% in IL-1β –stimulated tissues of aged and young mice, respectively). Aged vasculature and interstitial tissue appeared to play a key role here, as aged chimeric animals transplanted with young *LysM-EGFP-ki* bone marrow also exhibited neutrophil rTEM (~15%).

In investigating the associated mechanisms, we noted that in response to locally-administered IL-1β, aged mice showed greatly enhanced tissue and plasma levels of CXCL1, as compared to young animals. Hypothesising a role for plasma CXCL1 in the induction of neutrophil rTEM in aged mice, two series of works were conducted. Initially, as a proof of concept experiment, since in young mice local IL-1β does not cause neutrophil rTEM, we considered that exogenous intravenous (i.v.) CXCL1 may promote this phenomenon. Indeed, whilst in young mice local IL-1β caused ~1% neutrophil rTEM, following i.v. CXCL1 this response was significantly enhanced to 36%. Furthermore, treatment of aged mice with i.v. anti-CXCL1 mAb significantly suppressed the frequency of neutrophil rTEM (~60% inhibition). On-going works are aimed at investigating the mechanisms through which CXCL1 promotes neutrophil rTEM in inflamed aged tissues. In this context, works to date have indicated increased expression of the atypical chemokine receptor ACKR1 at EC junctions in IL-1β-stimulated aged tissues. This expression profile was associated with altered patterning of endothelial cell (EC) CXCL1 in aged mice, with ECs exhibiting more CXCL1 in close proximity of cell-cell junctions in aged animals. Additionally, since we have previously associated neutrophil rTEM with remote organ damage, further works aimed to investigate whether this aberrant mode of neutrophil TEM in aged mice may be associated with distant organ (lung) damage. To address this, we investigated a model of cremasteric ischaemia-reperfusion (I/R) injury, a reaction that caused significantly more neutrophil rTEM in aged mice as compared to young. Within this model, whilst young mice exhibited a low level of lung permeability, aged mice showed extensive lung vascular leakage, a response that was suppressed by i.v. anti-CXCL1 mAb.

Collectively, we have identified a key role for systemic CXCL1 in mediating age-related neutrophil rTEM and lung permeability. These results offer novel insights to identification of therapeutics that may be of value in alleviating secondary organ complications in the elderly.
Supported by the Wellcome Trust, QMUL and the EU.

OC2

ROLE OF T-CADHERIN IN THE ANTI-INFLAMMATORY EFFECTS OF ADIPONECTIN

L. Pezhman[1], M. Hussain[1], J. Reyat[1], A. Odedra[1], H. McGettrick[2],G. Ed Rainger[1], M. Chimen[1]

[1] Institute of Cardiovascular Sciences, College of Medical and dental Sciences, University of Birmingham, UK
[2] Institute of Inflammation and Ageing, College of Medical and dental Sciences, University of Birmingham, UK

Adiponectin is an adipose tissue derived hormone with anti-inflammatory properties. Three adiponectin binding proteins have been cloned; Adiponectin receptors 1 and 2 (AdipoR1/2) and T cadherin (T-cad). AdipoR1/2 are expressed on B cells. Under adiponectin stimulation, B cells release a novel peptide, PEPITEM (PEPtide Inhibitor of Trans-Endothelial Migration) that imposes a tonic inhibition of T-cell trafficking during inflammation. Since T-cad does not have an intracellular domain it is not thought to exert a direct effect on adiponectin

cellular signalling or function, but rather may be an adiponectin-binding protein. Here, we aimed to assess whether T-cad plays a role in PEPITEM pathway and we hypothesise that T-cad presents adiponectin to the B-cells to trigger the PEPITEM pathway.

We recruited healthy participants and measured the effect of adiponectin on Peripheral Blood lymphocyte (PBL) transmigration across TNF-α/IFN-γ stimulated endothelial cells in presence of adiponectin. T-cad was knocked down using siRNA. We quantified surface and total T-cad expression, by western blot, flow cytometry and confocal microscopy on endothelial cells (EC).

Adiponectin reduced the transmigration of lymphocytes through the endothelial layer in a dose dependant manner. Knock down of T-cad interfered with the inhibitory effects of adiponectin on T-cell migration. Our western blot data showed that, T-cad was shed upon EC activation and adiponectin treatment increased T-cad expression in stimulated EC. Knockdown of T-cad decreased cellular levels of adiponectin.

Our data suggests that knock down of T-cadherin abrogates the inhibitory effects of adiponectin on T-cell migration. Our preliminary data suggest that T-cad is shed upon TNF-α/IFN-γ stimulation and adiponectin treatment seems to reverse this. This study suggests a crucial role of T-cad in mediating the anti-inflammatory effects of adiponectin via the PEPITEM pathway.

SESSION 2:
Keynote Speaker:

S2

A MOUSE MODEL OF NEUROVASCULAR COUPLING DURING OLD AGE

Clare Howarth
University of Sheffield, UK

In healthy brain, the energy demands of active neurons are met through increases in local blood flow. This is achieved through a dynamic process termed neurovascular coupling. In addition to providing the energy substrates (glucose, oxygen) required by neurons, these evoked changes in blood volume and oxygenation underlie functional imaging techniques, such as the BOLD fMRI signal which is commonly used as a surrogate measure of neural activity in humans.

Aging may lead to the impairment of neurovascular coupling. A precise understanding of the relationship between neural activity and local blood flow is essential in order to understand normal (and abnormal) brain function during aging. Such knowledge would also enable more accurate interpretation of BOLD fMRI data acquired in older human cohorts.
We have combined 2-dimensional optical imaging spectroscopy (2D-OIS) and electrophysiology to concurrently examine haemodynamic and neural responses, evoked by mechanical whisker stimulation or carbogen challenge, in the somatosensory cortex of adult (6 months) and old (>19 months) anaesthetised mice. In my talk, I will discuss the conditions under which we observe altered neurovascular coupling in old mice.

Short talks:

OC3

IDENTIFYING NOVEL WAYS OF CELLULAR COMMUNICATION BETWEEN ROD PHOTORECEPTORS.

Aikaterini Kalargyrou, Robin Ali, Rachael Pearson

University College London, Institute of Ophthalmology

Retina degeneration is a complex group of disorders that all culminate to the same final common path the loss of the light sensing cells of the eye the photoreceptors. Photoreceptor replacement strategies aim to reverse the loss of vision by transplanting healthy cells to replace those lost through degeneration. Over the past decade, we and others have shown that transplanting photoreceptor precursors into models of retinal dysfunction results in restoration of visual function. Until very recently, this outcome was thought to be attributed solely to donor photoreceptor cells integrating into the host retina. However, we recently demonstrated that, at least in host retinae where some photoreceptors remain, much of the observed rescue was instead largely due to exchange of RNA and/or protein between donor and remaining host photoreceptor cells, a mechanism we named material transfer (MT). Since this process appears to render host cells functional, the mechanisms by which this occurs are of significant interest. We hypothesised that MT may involve direct physical contacts or indirect shedding and uptake of information packaged in extracellular vesicles (EVs). We showed for the first time that primary rod photoreceptor precursors in culture release vesicles bearing all the phenotypical and molecular characteristics of EVs, accompanied with the signature of the cell of origin. By employing the Cre-loxP system we furthermore established that photoreceptor-derived EVs are able to alter gene expression in glia cells, both *in vitro* and *in vivo*, but not in other photoreceptors. However, live imaging, SEM and flow cytometry of photoreceptors in culture revealed transient tubulovesicular processes between photoreceptors. These processes are remarkably straight, thin and enriched with actin cytoskeleton. Moreover, they are capable of transferring endosomes, lysosomes, vesicles and fluorescent reporters that are tagged in the cytoplasm in a cell specific manner. Although these fine structures are typically destroyed during tissue processing, preventing comprehensive assessment *in vivo*, we provide pilot evidences of these structures to be the most like mechanism underlying cell-cell communication in the transplantation paradigm in retina *in vivo*.

OC4

TALIN ROD DOMAIN-CONTAINING PROTEIN 1 (TLNRD1) IS A NOVEL ACIN-BUNDLING PROTEIN THAT LOCALISES TO THE TIPS OF FILOPODIA

Alana Cowell[1], Guillaume Jacquemet[2], Abhimanyu Singh[1], Johanna Ivaska[2], Benjamin Goult[1]

[1]School of Biosciences, University of Kent, Canterbury, UK
[2]Turku Centre for Biotechnology, University of Turku, Finland

Talin rod domain-containing protein 1 (TLNRD1), formally known as mesoderm development candidate 1 (MESDC1), shares 22% homology with the R7R8 region of talin, a large mechanosensitive linker which connects integrins to the actin cytoskeleton and mediates focal adhesion formation. TLNRD1 is highly conserved throughout vertebrate evolution and is known to be overexpressed in a range of different cancers, however, little is understood about the proteins structure and function. Here we show that TLNRD1 is a novel actin-bundling

protein which forms an antiparallel dimer capable of interacting with Rap1-GTP-interacting adapter molecule (RIAM) and Kank proteins to regulate different aspects of the cytoskeleton. Actin co-sedimentation assays reveal that TLNRD1 is a strong actin binding protein, which dimerises via a conserved interface on a 4-helix module, to promote actin bundle formation. Using fluorescence polarization and NMR we have been able to identify protein interactions which TLNRD1 shares in common with talin that are known to be important for talin recruitment to the plasma membrane and microtubule recruitment to sites of focal adhesion formation. Overexpression of TLNRD1 in U2OS cells leads to increased filopodia formation, and its bundling capacity is required for this process, driving a more aggressive migratory phenotype. With these new discoveries we are getting closer to understanding the function of TLNRD1 in the cell and elucidating why overexpression of TLNRD1 leads to an unfavourable prognosis in cancer.

SESSION 3:
Keynote Speaker:

S3

CIRCADIAN RHYTHMS IN LEUKOCYTE MIGRATION AND FUNCTION

Christoph Scheiermann
University of Munich, Germany

The number of leukocytes circulating in blood is under circadian, i.e. ~24h, control. This talk will discuss our latest findings on the mechanisms governing leukocyte migration from the blood into various organs, focusing on the distinct leukocyte subtype- and organ vascular-specific molecules involved. A focus will be on the oscillatory expression patterns of adhesion molecules, chemokines and their receptors, expressed on endothelial cells and leukocytes, which are critical regulators of rhythmic leukocyte recruitment. Furthermore, the relevance of clock genes in endothelial cells and leukocytes for leukocyte function and migration will be discussed.

Short talks:

OC5

Platelets interact with macrophages via the CLEC-2-podoplanin axis to regulate immune cell trafficking during infection

Joshua Bourne, Ebrima Bojang, Elizabeth Haining, Ying Di, Steve Watson and Julie Rayes

Institute of Cardiovascular Science, University of Birmingham

Sepsis leads to life-threatening multi-organ dysfunction as a result of a dysregulated immune response to infection. Thrombocytopenia (low platelet count) is observed in septic patients and correlates with disease severity and mortality. Platelets have established roles in haemostasis and thrombosis and have immunomodulatory functions during infection. During bacterial peritonitis, we have shown that platelet CLEC-2 interacts with podoplanin upregulated on inflammatory macrophages in the peritoneum and regulates their recruitment/retention. The aim of the study is to investigate the stimuli for podoplanin expression on macrophages during infection and its functional significance. Our results show that the gram^{-ve} bacterial wall protein lipopolysaccharide (LPS) upregulates podoplanin on murine macrophage cell line RAW264.7 in a TLR-4-dependent manner. In addition, the

cytokine INFγ induces podoplanin expression on RAW264.7 cells. No significant upregulation is observed in the presence of peptides from gram^{+ve} bacteria such as PAM3CSK4 and PGN-SA. The upregulation of podoplanin on inflammatory RAW264.7 is further increased in the presence of wild type but not CLEC-2-deficient platelets. Platelet CLEC-2 binding to podoplanin-positive inflammatory macrophages initiated a rapid translocation of podoplanin from intracellular stores to the cell surface and increased macrophage spreading, elongation and pseudopodia formation. In a mouse model of LPS-induced peritonitis, injection of recombinant dimeric CLEC-2 (CLEC-2-Fc) inhibited macrophage accumulation in the peritoneum and increased clinical severity. In conclusion, infection and inflammation upregulate podoplanin on macrophages, promoting platelet CLEC-2 binding and altered macrophage migration and subsequent clinical severity.

OC6

CD44 ORGANIZES THE HYALURONAN GLYCOCALYX ON DENDRITIC CELLS FOR EFFICIENT LYMPHATIC TRAFFICKING

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Dendritic cells (DCs) play an important role in immunity by conveying antigens from peripheral tissues to draining lymph nodes, via afferent lymphatic vessels. Recently, we have demonstrated that the lymphatic endothelial vessel hyaluronan receptor LYVE1 acts as a docking receptor by engaging hyaluronan in the DC glycocalyx via transmigratory cups (Johnson et al, 2017, Nat. Immunol.). However, the manner in which the hyaluronan glycocalyx is anchored or organised on the leukocyte surface is unclear. Here we use super-resolution Airyscan confocal microscopy to image the DC hyaluronan glycocalyx and show that CD44 in bone marrow-derived and dermal DCs is essential for formation, retention and maintenance of a hyaluronan glycocalyx. Moreover, using CD44$^{-/-}$ DCs in a mouse model of oxazolone induced skin inflammation, we show that CD44 is required for efficient mobilization from the dermis and entry to dermal lymphatics, for subsequent migration of DCs to downstream lymph nodes.

SESSION 4:
Keynote Speaker:
S4

TAMING THE MIGHTY NEUTROPHIL - IMPROVING RESPONSES TO ACUTE INFECTION AND CHRONIC DISEASE

Elizabeth Sapey
University of Birmingham, UK

Short talks:

OC7

THE EFFECT OF HUMAN NEUTROPHIL-DERIVED MICROVESICLES ON LUNG EPITHELIAL CELL FUNCTION

Carolina Roque Silva, Merete Long, Victoria Ridger

Department of Immunity, Infection & Cardiovascular Disease (IICD), University of Sheffield

Background: Chronic obstructive pulmonary disease (COPD) is a leading cause of death, affecting 64 million people worldwide. It is based on a continued inflammatory response induced by inhaled harmful agents, with cigarette smoke being the major cause. Particularly, pro-inflammatory mechanisms driven by neutrophils are central to COPD pathogenesis. Amongst other processes, neutrophils respond to damaging stimuli by releasing neutrophil-derived microvesicles (NMVs) which can be internalised by and activate target cells. Thus, NMVs may promote airway inflammation and neutrophil recruitment, contributing to COPD development. Indeed, both neutrophils and NMVs have been found to be increased in the sputum of COPD patients, indicating that they might be involved in the disease.
Hypothesis: We hypothesised that NMVs have a role in COPD by activating lung epithelial cells. In addition, we postulated that different stimuli may produce NMVs with differential activity.
Methods: Neutrophils were isolated from venous blood samples from healthy participants using density-gradient centrifugation, and then stimulated with either 10% cigarette smoke extract (CSE; generated using Kentucky reference cigarettes 3R4F) or bacterial peptide (10 μM fMLP) to generate NMVs. NMVs were isolated by differential centrifugation, quantified by flow cytometry, and co-incubated with BEAS-2B cells (human bronchial epithelial cells) for varying time-periods (n=4). The resultant cell lysates and supernatants were analysed by qPCR and ELISA (IL-8 and CCL-2/MCP-1), respectively. Additionally, C57BL/6 mice were treated with vehicle (PBS) (n=4) or NMVs for 6h (either alone or after 16h exposure to low-dose LPS) (n=6), via intranasal instillation, and bronchial lavage fluid (BALF) and plasma were analysed by ELISA (CXCL-1/KC).
Results: Both NMV types (CSE and fMLP) induced secretion of epithelial cell IL-8 and CCL-2 (MCP-1) after co-incubation for 24h, but no differences in response magnitude were found. NMVs themselves had no detectable cytokine content. In our murine experiment, when NMVs were administered alone, no CXCL-1/KC (analogue for human IL-8) was detected in the BALF. LPS administration induced KC expression, and when NMVs were given in combination, levels of KC were increased in some mice, although these levels were variable and did not reach statistical significance. No KC was detected in the platelet-poor plasma.
Conclusion: NMVs activated lung epithelial cells *in vitro*, however, the two NMV types did not have a differential effect on chemokine secretion, suggesting that this pro-inflammatory response is initiated through a common mechanism. *In vivo,* NMVs alone did not elicit an inflammatory response in the lung, and these vesicles are likely rapidly cleared by alveolar macrophages. However, NMVs may be capable of augmenting an existing inflammatory response and may have a more important role in disease progression rather than pathogenesis. We will now look further into the mechanism of interaction between NMVs and lung epithelial cells to decipher how they induce cytokine secretion. In particular, we will investigate whether NMVs may be capable of delivering reactive oxygen species to target cells.

OC8

GALECTIN-9 CAUSES INCREASED ADHESION OF LEUKOCYTES FROM PERIPHERAL ARTERIAL DISEASE PATIENTS COMPARED TO HEALTHY INDIVIDUALS

F. Krautter[1], M. T. Hussain[1], D. R. Lezama[1], M. Chimen[1], D. Cooper[2], A. Iqbal[1]

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Atherosclerosis is an age-related, inflammatory disease driven by leukocyte recruitment. Galectins are a family of ß-galactose binding proteins which have a range of immunomodulatory functions. More recently Galectin-9 (Gal-9), a tandem repeat type galectin, has been proposed to play a role in leukocyte recruitment. Here, we aim to characterise the role of Gal-9 in atherogenesis.
Flow based assays were used to investigate the adhesion of neutrophils and peripheral blood mononuclear cells (PBMCs) of healthy individuals and patients with peripheral arterial disease (PAD) to immobilised Gal-9. Furthermore flow cytometry was used to analyse the expression of Gal-9 and its known receptors on leukocytes to determine differences in expression between healthy and aged individuals as well as patients with PAD.

We were able to show in vitro, that Gal-9 enhances the adhesion of leukocytes such as PBMCs and neutrophils under physiological flow. Not only cells of PAD patients showed an increase in adhesion, but also cells of aged individuals adhered to Gal-9 at a higher frequency than the cells of healthy young. However, no difference in the expression of known Gal-9 receptors were found which could have explained the higher frequency in adhesion. After activating neutrophils with Gal-9 however, total CD44 levels differed between healthy young and PAD patients. Additionally, higher Gal-9 serum levels were detected in aged individuals and PAD patients.

These results show that Gal-9 might drive leukocyte recruitment and therefore the plaque formation in atherogenesis. However, the underlying mechanisms leading to the increased adhesion of leukocytes of aged individuals and PAD patients have yet to be determined.

OC9

Monocyte extracellular vesicles activate the human atherosclerotic plaque.

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Abstract text (500 words maximum

Background Intercellular signalling by extracellular vesicles (EVs) has been defined as a route of cell-cell crosstalk that lets cells deliver biological messages to recipient cells. EVs

convey these messages through their different cargoes consisting of cytokines, proteins, nucleic acids and lipids. In atherosclerosis, EVs can contribute to disease development and progression by promoting endothelial dysfunction, vascular calcification, unstable plaque progression, rupture and thrombus formation.
Aim: To investigate the role of monocyte-derived EVs (mEVs) in promotion and progression of atherosclerosis.
Methods: Monocytes isolated with Rosette Sep™ technology (previously treated with 2 μM PGI2 to inhibit platelet aggregation), were stimulated for 1h with TNFα (50 ng/mL). Purity of monocytes was determined by flow cytometry while EV profile was studied with imaging-flow cytometry and Nanoparticle tracking analysis. Atherosclerotic plaques from 5 patients undergoing endarterectomy were dissected and cultured with either with $1x10^7$ mEVs or PBS. Cytokines released in the plaque culture media were measured by multiplex ELISA and protein content by liquid chromatography-mass spectrometry (LC-MS). Proteomics of the EVs prepared in different incubation settings was also conducted.
Results: Comparing Rosette Sep™ technology isolation with density gradient PBMC isolation by flow cytometry, revealed high degrees of platelet-monocyte aggregation ($n=4$, $p<0.01$) which was not modulated by PGI_2. mEV analyses identified EV from platelets (CD41+/CD14-) and monocytes (CD41-/CD14+) as well as a subset bearing both markers (CD41+/CD14+). Plaque culture media contained significant higher levels of TNFα, MIP1-α, IL-6, IL-13, INF-γ and GMCSF upon addition of TNF-α mEV. Proteomics analysis identified 26 proteins modulated by the mEV treatment, of which 19 were upregulated and 7 downregulated. Interestingly, most proteins were associated with metabolism and immune-cell recruitment and activation. Pathways analysis (PA) showed that NFkB, Vegf, TP53, PI3K and Pkc were positively regulated in the plaques treated with mEVs generated by TNF-α and prostacyclin had reduced effect on plaque reactivity. Proteomic analysis of mEVs revealed a distinctive composition when the cell preparation was activated with TNF-α alone or with prostacyclin.
Conclusion: In summary, mEV increased activation of patient atherosclerotic plaques through enhanced release of proinflammatory cytokines, overall upregulation of proteins and activation of several proinflammatory pathways. We also found that attenuating platelet activation has an effect on EV composition with downstream modulation of pro-inflammatory actions. These effects reflect the EV heterogeneity and highlight how this phenomenon might contribute to the increased risk for the development and progression of atherosclerosis.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675111.

SESSION 5:

Keynote Speaker:

S5

DISSECTING THE COMPLEX INTERPLAY BETWEEN TISSUE REPAIR AND INFLAMMATION USING *IN VIVO* MODELS

Helen Weavers
University of Bristol, UK

An effective inflammatory response is pivotal to orchestrate the repair of wounded tissues. We use precise genetic manipulation, live imaging and mathematical modelling within *Drosophila* to dissect the mechanisms that activate the inflammatory response to tissue damage and those that simultaneously protect the regenerating tissue from immunopathology.

Short talks:

OC10

HEPATOCYTES DELETE REGULATORY T CELLS BY ENCLYSIS: A CD4+ T CELL ENGULFMENT PROCESS

Scott P. Davies, Gary Reynolds, Alex L. Wilkinson, Xiaoyan Li, Rebecca Rose, Maanav Leekha, Yuxin S. Liu, Ratnam Gandhi, Emma Buckroyd, Joe Grove, Nicholas M. Barnes, Robin C. May, Stefan G. Hubscher, David H. Adams, Yuehua Huang, Omar Qureshi and Zania Stamataki

Institute of Immunology and Immunotherapy, Centre for Liver and Gastrointestinal Research, University of Birmingham, UK.

CD4+ T cells play critical roles in immunity, both as helper and regulatory cells (Tregs). Here we demonstrate a novel mechanism by which hepatocytes can modulate T cell populations, through the engulfment of live CD4+ lymphocytes. We termed this phenomenon enclysis, to reflect the specific enclosure of CD4+ T cells inside hepatocytes. Enclysis was selective for CD4+ but not CD8+ cells, independent of antigen-specific activation and occurred in human hepatocytes in vitro, ex vivo and in vivo. Intercellular Adhesion Molecule 1 (ICAM-1) facilitated T cell early adhesion and internalisation by hepatocytes, while hepatocytes formed membrane lamellipodia or blebs to mediate engulfment. T cell internalisation was unaffected by wortmannin and Rho kinase inhibition and led to the generation of T cell-containing vesicles connected to the extracellular space via the endocytic pathway. Beta-catenin but not alpha-catenin or E-cadherin -expressing endosomes associated with the T cell-containing vesicle. Hepatocytes engulfed Tregs more efficiently than non-Tregs, yet Treg-containing vesicles preferentially acidified overnight. Thus, enclysis represents a new biological process with potential impact for immunomodulation and opens a new field for research to fully understand CD4+ T cell dynamics in liver inflammation.

OC11

L-SELECTIN ENHANCED T CELLS PROLONG PROTECTIVE IMMUNITY TO INFLUENZA VIRUS INFECTION

Angharad Watson and Ann Ager

Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff CF14 4XN

L-selectin (CD62L) is expressed on naïve and central memory T cells and regulates recruitment into lymph nodes where T cells survey antigen presenting cells for activating, tolerogenic or homeostatic stimuli. Following engagement of the T cell receptor (TCR) by peptide-MHC complexes, L-selectin expression is downregulated by two independent mechanisms, ADAM17 dependent ectodomain shedding and transcriptional silencing. The impact of L-selectin downregulation on T cell activation and the potential role of L-selectin downregulation in the differentiation of effector and effector memory T cells are poorly understood. We hypothesised that L-selectin enhanced T cells which resist TCR-induced loss of L-selectin expression, would have continued access to lymph nodes via maintained expression of L-selectin, where interactions with antigen presenting cells impact on T cell activation, differentiation and generation of memory T cells.

Transgenic mice expressing a mutant form of L-selectin on T cells that is not downregulated (CD62Lmut) were used to determine the impact of L-selectin enhanced T cells

on the development of protective immunity to influenza virus. CD62Lmut and non-transgenic C57BL/6 mice expressing wild type (endogenous) L-selectin were infected intranasally with H17 strain influenza virus and subsequently challenged with a second virus, recombinant vaccinia virus expressing the H17 influenza nucleoprotein epitope NP68 (vacc-NP68), to measure CD8+ T cell dependent protective immunity (i.e., immunological memory) to influenza virus. In C57BL/6 mice, influenza-specific $CD8^+$ T cell memory wanes between 3 and 5 months following influenza infection since the ability to clear the second virus (vacc-NP68) was significantly reduced between 3 and 5 months following influenza infection. However, in CD62Lmut mice, protective immunity to influenza virus was maintained since vacc-NP68 was completely cleared at both 3 and 5 months following primary influenza infection. The numbers of influenza-specific $CD8^+$ T cells in CD62Lmut mice before and after the secondary virus challenge with vacc-NP68 were similar to those in C57BL/6 mice indicating that the persistence of $CD8^+$T cell memory was not simply related to increased numbers or altered homing of L-selectin enhanced, influenza-specific T cells. To determine whether the effect of L-selectin enhancement is intrinsic to CD62Lmut T cells, $CD8^+$ T cells co-expressing the influenza-specific F5 TCR were activated *in vitro* with influenza peptide, transferred to naïve C57BL/6 mice and challenged 5 months later with vacc-NP68. C57BL/6 mice that received F5/CD62Lmut T cells completely cleared virus whereas mice receiving F5/B6 T cells expressing wild type (endogenous L-selectin) did not.

These findings suggest that downregulation of L-selectin on effector and effector memory T cells limits the persistence of protective CD8+ T cell dependent immunity to influenza virus. Further studies will be required to dissect the underlying mechanism(s) but enhancing L-selectin expression on T cells may have therapeutic potential in vaccination strategies for viruses damaging to human and animal health.

SESSION 5:

Keynote Speaker:

S5

CIRCADIAN ACTIN DYNAMYCS DRIVE RHYTHMIC FIBROBLAST MOBILISATION DURING WOUND HEALING

John O'Neill
University of Cambridge, UK

Fibroblasts are primary cellular protagonists of wound healing. They also exhibit circadian timekeeping, which imparts an approximately 24-hour rhythm to their biological function. We interrogated the functional consequences of the cell-autonomous clockwork in fibroblasts using a proteome-wide screen for rhythmically expressed proteins. We observed temporal coordination of actin regulators that drives cell-intrinsic rhythms in actin dynamics. In consequence, the cellular clock modulates the efficiency of actin-dependent processes such as cell migration and adhesion, which ultimately affect the efficacy of wound healing. Accordingly, skin wounds incurred during a mouse's active phase exhibited increased fibroblast invasion in vivo and ex vivo, as well as in cultured fibroblasts and keratinocytes. Our experimental results correlate with the observation that the time of injury significantly affects healing after burns in humans, with daytime wounds healing ~60% faster than nighttime wounds. We suggest that circadian regulation of the cytoskeleton influences wound-healing efficacy from the cellular to the organismal scale.

Short talks:

OC12

CDC42 TARGETS REGULATE CANCER CELL INTERACTION WITH ENDOTHELIAL CELLS IN TUMOR PROGRESSION

Camilla Cerutti[1,2], Serena Lucotti[3#], Sofia Tirados[1], Ruth J Muschel[3], Anne J Ridley[1,2]

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Interaction of cancer cells with endothelial cells, which line blood vessels, is a critical event in metastasis formation. Previously, we showed that the small Rho GTPase Cdc42, a key regulator of actin dynamics, acts via □1-integrin to mediate cancer cell adhesion to endothelial cells[1]. We have found that □1-integrin contributes to the adhesion of prostate and breast cancer cells to endothelial cells under shear stress conditions *in vitro*, and to the lung endothelium *in vivo.* We screened a panel of downstream targets of Cdc42 to identify those that affect □1-integrin expression. We identified a role for IQGAP1 and NWASP, known to regulate actin organization, cell migration and cell adhesion. Similar to Cdc42, depletion of IQGAP1 and NWASP in cancer cells decreased interaction with endothelial cells *in vitro* and the lung vasculature *in vivo,* and reduced metastatic lung nodule formation *in vivo.* These data suggest IQGAP1 and NWASP as potential therapeutic targets to inhibit breast and prostate cancer progression and metastasis.

[1]Reymond N et al. (2012) Cdc42 promotes transendothelial migration of cancer cells through β1 integrin. J Cell Biol 199, 653-668.

OC13

REGULATORY T CELLS CONFER A CIRCADIAN SIGNATURE TO INFALMMATORY ARTHRITIS

Hand LE, Dickson SH, Ray DW, Hepworth MR and Gibbs JE

University of Manchester, Manchester UK

Chronic inflammatory disorders, such as rheumatoid arthritis (RA), show daily variation in disease activity. The circadian clock is an intrinsic oscillator that imparts 24h rhythms onto physiological processes, including immunity. We previously identified that this clock drives rhythmic repression of inflammatory arthritis during the night (active period) in mice. The mechanisms by which joint inflammation is dampened at night remain undefined, and the therapeutic potential of this circuit unexploited.

Here we show that anti-inflammatory regulatory T cells (Tregs) within the joints show marked diurnal variation, with both numbers and markers of activation peaking during the nadir of joint inflammation (mid-night). Depletion of Tregs resulted in enhanced expression of pro-inflammatory cytokines within the joints at night. Further studies showed that the anti-inflammatory action of Tregs on innate immune cells contributes significantly to the repression of inflammation at night. Finally, we identify that Tregs are not intrinsically circadian rhythmic, suggesting their rhythmic function may be a consequence of external signals.

In conclusion we report a novel circadian rhythmic network in which non-rhythmic cells, Tregs, are driven to rhythmic activity by systemic signals to confer a circadian signature to chronic arthritis.

OC14

MECHANICAL REGULATION OF ENDOTHELIAL CELL-CELL ADHESIONS AND FORMATION OF GAPS ASSISTING CANCER EXTRAVASATION

Jorge Escribano, Michelle B. Chen, Emad Moeendarbary, Xuan Cao, Vivek Shenoy, Jose Manuel Garcia-Aznar, Roger D. Kamm, Fabian Spill

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Endothelial cells constantly push and pull on neighbouring cells, leading to forces on VE-cadherin mediated cell-cell adhesions. These adhesions are highly dynamic, and their rupture may lead to the autonomous formation of gaps in the endothelium. We present a novel mathematical model that predicts the frequency, lifetime and size of these gaps, and validate the predictions with experiments of HUVEC monolayers. We find that gaps occur more often at the vertices of three or more cells, as opposed to the borders between two cells. Interestingly, cancer cells follow this trend and primarily extravasate at the vertices. Notably, they do so even when they first adhere on the two cell border, where they subsequently migrate towards the endothelial vertices. This indicates that the cancer cells exploit the autonomously forming gaps, and do not necessarily rely on signalling to the endothelium to initiate gap formation.

POSTER PRESENTATIONS

P1

CHANGES IN CARGO OF CIRCULATING MICROVESICLES IN STEMI PATIENTS

Darcy Sidebotham, Merete Long, Dr Ever Grech, Dr Victoria C Ridger

Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Medical School, Beech Hill Rd, Sheffield S10 2RX

Background: Research has shown that microvesicles (MVs), extracellular vesicles which bleb from the plasma membrane, are involved in various underlying disease mechanisms. The role of MVs in cardiovascular disease (CVD) has been increasingly studied, however their effect during ST elevation myocardial infarction (STEMI) is currently unknown. Thin cap fibro atheromas (TCFA) are considered to be the most vulnerable plaque phenotypes, causing ~60% of all sudden coronary deaths when ruptured (Virmani, 2000). Degradation of the thin fibrous cap overlying the plaque by certain metalloproteases (MMPs) can catalyse the rupture, exposing the thrombogenic interior of the plaque, causing STEMI. Neutrophils are the most abundant innate immune cell in the human body. MVs derived from neutrophils contain enzymatically active MMP-9, a protease which has been associated with plaque rupture (Kobayashi et al, 2016). We hypothesised that STEMI patients have higher numbers of circulating neutrophil microvesicles (NMVs) and express a higher concentration of MMP-9 compared with healthy controls. Through adhesion to and interaction with endothelial cells, we believe that these NMVs excerpt protease activity and contribute to plaque rupture.
Methods: Antibody concentrations and laser voltages for MV flow cytometric analysis were initially titrated and optimised using human platelet poor plasma (PPP) from healthy controls by calculating the staining index (SI). PPP from venous and arterial blood was obtained from

STEMI patients (n=7) upon admission following the infarction and after percutaneous coronary intervention and compared with venous samples from healthy controls (n=7). Plasma MVs were labelled with fluorescently-conjugated primary antibodies against CD41a (platelet), CD66b (neutrophil), CD14 (monocyte) and CD144 (endothelial cell) respectively, and quantified by flow cytometry. MMP-9 activity of the plasma MVs was determined using Gelatin Zymography and total MMP-9 was investigated using ELISA.

Results: STEMI patients tended to have higher number of total NMVs, although this difference was not statistically significant. There were significantly higher levels of platelet-derived MVs in STEMI patient arterial samples post-infarction compared with healthy controls (one way ANOVA, p=0.039). There were no statistically significant differences in monocyte and endothelial MV numbers in either arterial vs. venous samples or between patients and controls. Preliminary results show higher concentrations of active MMP-9 in the plasma MVs of STEMI patient than those from healthy controls (n=2)

Conclusion: In our current cohort, although we did not detect significantly increased levels of NMVs in STEMI patients, levels of circulating platelet MVs may be consequential after STEMI or as a result of percutaneous coronary intervention. Our preliminary data suggests that the content, rather than the number of MVs may be important. A higher concentration of plasma MV MMP-9 could contribute to the transition from a vulnerable plaque phenotype to a ruptured plaque. We are currently investigating whether MV-MV interactions, particularly platelet-neutrophil doublets, are different in STEMI patients due to the inflammatory nature of myocardial infarctions. We will further investigate MV interactions with endothelial cells and myocytes.

P2

DO NEUTROPHIL MICROVESICLES AFFECT PLAQUE EROSION?

Reece Dow, Merete Long, Prof Paul Evans and Dr Victoria Ridger

Infection, Immunity and Cardiovascular Disease, University of Sheffield Medical School, Beech Hill Rd, S10 2RX.

Cardiovascular disease (CVD) is the single biggest cause of mortality. An underlying cause of CVD is atherosclerosis, where plaques develop in the arterial walls in areas of the vasculature exposed to disturbed blood flow. Two categories of plaques are known to form: highly inflammatory, lipid-rich rupture-prone plaques and less inflammatory, fibrous erosion-prone plaques. Disruption of either can result in thrombosis, arterial blockage and myocardial infarction. Whilst plaque rupture has been extensively researched and can be relatively effectively managed, plaque erosion has received less attention and its mechanisms are poorly undestood. Erosion is thought to occur through loss of endothelial cells (ECs) overlying the plaque as a result of increased apoptosis and extracellular matrix (ECM) degradation. Loss of ECs and exposure of the ECM may induce thrombosis by triggering coagulation and the accumulation of platelets. Neutrophils are associated with plaque development and disruption but are rarely detected in plaques themselves. Activated neutrophils release 0.1-1 □m neutrophil microvesicles (NMVs) from their membanes. These NMVs contain a library of proteases, reactive oxygen species and cyokines derived from the parent cell, which could affect EC integrity and detachment. NMVs have been shown to preferentially adhere to ECs in atheroprone regions and induce EC activation under flow conditions.

We hypothesised that NMVs affect plaque erosion by inducing endothelial cell apoptosis and facilitating the degradation of the underlying extracellular matrix through the action of neutrophil-derived proteases.

Primary human coronary artery ECs (HCAECs) were cultured in chambers under atheroprone flow conditions using an Ibidi pump system. The concentration and activity of

NMV-derived proteases was assessed by ELISA and gelatin zymography. A fluorescent gelatin conjugate was used to assess NMV ability to degrade analogues of the ECM using a microplate assay and fluorescence microscopy. Alongside this we used fluorescence microscopy to determine caspase 3 activity in HCAECs incubated with NMVs under different flow conditions.

NMVs were found to contain active MMP-2 and MMP-9. In addition, NMVs were able to effectively degrade gelatin. However, NMV incubation with HCAEC resulted in no significant difference in rates of apoptosis and preliminary results indicate that endothelial cell proliferation is not changed by NMVs.

These results indicate that NMVs do not induce endothelial cell apoptosis but do contain proteases capable of degrading components of the ECM, potentially contributing to endothelial cell detachment and plaque erosion.

P3

GLUCOCORTICOID REGULATION OF THE PEPITEM PATHWAY

Ailbhe Ni Chosgora, Asif Iqbal, Helen McGettrick, Ed Rainger, Rowan Hardy, Myriam Chimen

University of Birmingham

Dysregulated recruitment of leukocytes across endothelial cells into target tissues is a hallmark of the pathology of most chronic inflammatory diseases (CIDs). We have recently identified a novel homeostatic pathway that regulates T-cell migration during inflammation. The pathway is impaired in patients with CIDs such as type-1-diabetes and rheumatoid arthritis (RA), and is also attenuated in the elderly. The dysregulation of this pathway across all conditions is caused by a lack of the adiponectin receptors (AdipoRs) on B-cells, which are not able to secrete PEPITEM (PEPtide Inhibitor of Trans-Endothelial Migration) in response to adiponectin. It is therefore important to understand how to this endogenous pathway is down-regulated and how it might be restored to develop therapies that will re-balance T-cell recruitment during inflammation. We hypothesise that changes in AdipoRs expression on the surface of B-cells is caused by a common process across these distinct CIDs. Here, we aimed to determine whether potent anti-inflammatory glucocorticoids such as cortisol and their associated modulating enzymes, 11β-hydroxysteroid dehydrogenase 1 and 2 (11β-HSD1 and 2), regulate the PEPITEM pathway.

Isolated B-cells were cultured in presence of cortisol and other leukocytes for 48 hours and the expression of AdipoR1/2 was measured using flow cytometry. Expression of 11β-HSD1, 11β-HSD2, AdipoR1/2, and glucocorticoid associated genes was measured using qPCR.

We observed an up-regulation of AdipoR1/2 on B-cells in presence of cortisol and monocytes but not on B-cells alone. In addition, we observed a reduction in the expression of AdipoR1/2 in the 11β-HSD1 knock-out mouse. The expression of 11β-HSD1 is very low in B-cells but is up-regulated in presence of stimulatory signal such as Interleukin-4. Our preliminary data indicate that in patients with RA, the expression of 11β-HSD1 tend to be lower than in gender and age-matched healthy controls.

Our data indicate a potential role of cortisol in the regulation of the PEPITEM pathway via a cross-talk with monocytes and a potential role for 11β-HSD1.

P4

Claudio Mauro

Institute of Inflammation and Ageing (IIA)
College of Medical and Dental Sciences
University of Birmingham

The research in my group focusses on the interconnections between metabolic and inflammatory pathways and how systemic and cellular metabolic alterations in diseases with an inflammatory component lead to aberrant immune cell responses, which favour both the establishment and the propagation of inflammation. In particular, we investigate the mechanisms of metabolic control of T cell-mediated immune responses, including migration, differentiation and cytokine production in physiology and under metabolic stress.

One key area of our research is how small metabolites, like lactate, which accumulates locally in the inflamed tissue or systemically during acute and chronic inflammation, can impact the fate of the immune-inflammatory response via induction of intracellular metabolic rewiring with immediate effect on broad range human diseases. I will present our latest data, which are at a final stage of revision.

Another important aspect of the research is how fatty acids can impact the outcomes of an immune response, with repercussions on obesity-related diseases. I will present our latest data on the impact of omega-3 fatty acids on T cell responses in the adipose tissue, again data at a final stage of revision prior to publication.

Our published studies so far indicate that interfering with metabolic pathways (i.e., lipid, glucose and oxidative metabolism) alters immune cell effector functions and can be exploited for therapy (PLoS Biol 2015, Cell Metab 2017, Nat Comm 2017).

P5

THE EFFECT OF CIGARETTE SMOKE ON HUMAN NEUTROPHIL-DERIVED MICROVESICLE ACTIVITY AND CONTENT

Magdalena Grudzien, Merete Long, Dr Victoria Ridger

Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Medical School, Beech Hill Road, Sheffield, S10 2RX

Background: Chronic obstructive pulmonary disease (COPD) is a progressive lung disease caused by alveolar damage and airway constriction leading to breathlessness and in later stages, death. Cigarette smoke is one of the major COPD risk factors and has been associated with increased levels of neutrophils-pro-inflammatory phagocytic cells-in patients' blood and sputum. Neutrophils release proteases such as matrix metalloproteinase-9 (MMP-9), that are responsible for extracellular matrix degradation, therefore alveolar damage and emphysema. It has been shown that neutrophils stimulated with harmful stimuli, e.g. the bacterial peptide (fMLP) are able to generate small de-nucleated vesicles (i.e. microvesicles) containing parental miRNA and proteases. Neutrophil-derived microvesicles (NMVs) have previously been detected in COPD patient sputum and are able to be internalised by other cells, delivering their content. However, it is currently unknown whether cigarette smoke has any effect on NMV generation or activity.

Aims: This study investigated whether stimulation with cigarette smoke extract (CSE) can induce NMV generation and, if so, whether their content, activity and uptake by target cells differ from those of the better-characterised NMVs produced by fMLP stimulation.

Methods: CSE was generated by bubbling smoke from a research-grade cigarette through PBS with $^{Ca2+}$ using a Masterflex pump. Neutrophils were isolated from peripheral blood of healthy human participants using Histopaque-1077 and density-gradient separation. NMVs were generated by stimulating cells with PBS (control), 1, 5, or 10% CSE, 10μM fMLP or 10%CSE+10μM fMLP. Numbers of generated microvesicles were quantified using flow cytometry and SpheroTech AccuCount counting beads. Total levels of MMP-9 in neutrophils exposed to different stimuli and their resulting microvesicles were measured with enzyme-linked immunosorbent assay (ELISA).

Results: Exposure to different CSE concentrations and fMLP induced generation of NMVs. There was no significant difference in NMV numbers between different stimuli, however 1, 5 and 10% CSE had a concentration-dependent effect on NMV production. fMLP, CSE and CSE+fMLP generated NMVs containing more MMP-9 than derived from untreated (PBS) neutrophils. Higher levels of MMP-9 were detected in microvesicles that were generated with fMLP rather than CSE. No differences were found in the total MMP-9 levels of the parent neutrophils after stimulation.

Conclusions: CSE and fMLP had differential effects on NMV content, indicating that NMVs from these two stimuli may play different roles in inflammation and tissue damage. Since intracellular MMP-9 levels were unaffected by these stimuli, this supports the action of NMVs as a mode of delivery for specific neutrophil content. Investigations into levels of the pro- and active forms of MMP-9 in these NMVs as well as MMP-9 activity are currently ongoing. Further experiments will test if NMVs generated by CSE can be internalised by lung epithelial cells (BEAS-2B) *in vitro* and compare their uptake and effect on cellular MMP-9 expression between different stimuli (fMLP vs. CSE).

P6

The Collagen Toolkits provide ligands to manipulate cell behaviour

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The Collagen Toolkits are libraries of 56 and 57 triple-helical synthetic peptides spanning the length of the collagen II and collagen III helices. These have been used in solid-phase binding assays to locate sites where collagen receptors and extracellular matrix components bind to collagens. Truncation and substitution allowed exact binding sites to be identified, and corresponding minimal peptides to be synthesised for use in structural and functional studies. 170 sites where over 30 proteins bind to collagen II have been mapped, providing firm conclusions about the amino acid distribution within such binding sites. This strategy has been applied to the major collagen receptors: the four collagen-binding integrins, the three known collagen-binding immune receptors, platelet glycoprotein VI, LAIR1 and OSCAR, both discoidin domain receptors and GPR56. Protein binding to collagen II is not

random, but displays a periodicity of about 28nm, with several prominent nodes where multiple proteins bind. Notably, the vicinity of the collagenase-cleavage site in Toolkit peptide II-44 is highly promiscuous, binding over 20 different proteins. This may reflect either the diverse chemistry of that locus or its diverse function, together with the interplay between regulatory binding partners. Peptides derived from Toolkit studies have been used to determine atomic level resolution of interactions between collagen and several of its binding partners and are finding practical application in tissue engineering.

P7

IMMUNE CELL RESPONSE TO INFCETION IN SEPSIS

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Sepsis arises when the body's response to an infection damages its own tissues and organs; defined as life threatening organ dysfunction caused by a dysregulated host response to infection. The body's entire system is engulfed by a deleterious inflammatory response as opposed to local inflammation resulting from a local infection. This can lead to septic shock and death if not recognised early and treated promptly. Eicosanoids, including prostaglandins and leukotrienes, are a family of lipids that play key roles in inflammation including helping leukocytes fight infection. Cells of the innate immune system including tissue macrophages, neutrophils and sentinel dendritic cells are major contributors of local eicosanoids. It is known that exposure to varying bacterial components results in a different profile of lipids and cytokines, and by characterising this lipid signature it may be possible to define a biomarker fingerprint predictive for sepsis. This study will address the hypothesis that the profile of the eicosanoid and cytokine response varies depending on the nature of the bacterial pathogen.

The experimental aim is to identify signatures of eicosanoids and cytokines that may represent useful prognostic indicators of bacterial infection.

Primary cell responses to bacterial components were analysed using neutrophils isolated from healthy human donors. Isolated neutrophils were exposed to different bacterial toll-like receptors (TLR) agonists and the cells and supernatants were tested for lipids and cytokines.

Generation of the eicosanoid, 5-hydroxyicosatetraenoic acid (5-HETE), and proinflammatory cytokines, IL-8 and TNF-alpha, by isolated human neutrophils were detected following exposure to a Gram-positive (TLR2) and Gram-negative (TLR4) bacterial agonist. Data indicates inherent variation within the population. Now the ability to detect eicosanoid and cytokine profiles in isolated human neutrophils has been established, these approaches will be employed to characterise further the innate immune responses to bacterial infection using whole bacteria with a view to identifying pathogen specific profiles.

P9

Interplay of cell-cell and cell-matrix adhesion, stiffness and cell crowding regulates YAP/TAZ in benign and cancer cells

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The YAP/TAZ signalling pathway emerged as a key pathway sensing multiple mechanochemical stimuli– including cell-matrix adhesion and stiffness, cell-cell adhesion and crowding. While these stimuli are often simultaneously altered during tumour progression, little

is understood about the mechanisms of signal integration of these stimuli. We present a mathematical model that can predict the patterns of integration of such stimuli and infer how YAP/TAZ activity is differentially integrated in benign and cancer cells. Given the importance of YAP/TAZ signalling during tumour progression, this work is yielding novel insights into the complexity of the physical tumour microenvironment and its role in driving tumour progression

P10

REGULATION OF LEUKOCYTE TRAFFICKING DURING ACUTE INFLAMMATION: IMPLICATIONS OF ADVANCING AGE ON THE PEPITEM PATHWAY

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Ageing is associated with exacerbated systemic inflammation (inflammageing) and the progressive decline of immune system function (immnosenescence). Leukocyte migration is necessary for effective immunity; however, dysregulated trafficking of leukocytes into tissue contributes to inflammageing and the development of age-related inflammatory diseases. During acute inflammation, B-cells are able to regulate T-cell trafficking into tissue. At the heart of this B cell-mediated pathway, circulating adiponectin binds adiponectin receptors 1 and 2 (AdipoR1/2) on peripheral blood B-cells, stimulating the release of PEPITEM (PEPtide Inhibitor of TransEndothelial Migration) which indirectly inhibits the transendothelial migration of T-cells into tissue. However, the PEPITEM pathway is reportedly suppressed in aged individuals, which would contribute to dysregulated T-cell trafficking in ageing and disease. Here, we investigated further the dysregulation of the PEPITEM pathway in aged wild-type mice.

We measured the number of leukocyte subsets in the blood, spleen and bone marrow and the expression of AdipoR1/2 on B-cells from young and aged animals using flow cytometry.

We found an age-related reduction in the frequency of circulating murine B-cells expressing AdipoR1, however, the frequency of B-cells expressing AdipoR2 was unchanged. Furthermore, we found that splenic lymphocyte and dendritic cell numbers decrease with age, whilst peritoneal-resident B-cell numbers increase with age. Ageing was also associated with decreased B-cell and dendritic cell numbers in the bone marrow.

Our results indicate an age-related blunting of the PEPITEM pathway and demonstrate how ageing affects resident leukocyte populations in a tissue-specific manner. We anticipate that our findings will offer a novel avenue of investigation in the pursuit of re-establishing regulated leukocyte trafficking in ageing. Restoration of the PEPITEM pathway through targeting AdipoR1 expression on aged B-cells will contribute to the re-establishment of regulated T-cell trafficking.

P11

TCR-INDUCED SEQUENTIAL PROTEOLYSIS OF L-SELECTIN BY ADAM17 AND PRESENILIN 1

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The lymph node homing receptor L-selectin is proteolytically shed in response to TCR engagement. Loss of cell surface L-selectin is thought to redirect activated T-cells away from lymph nodes to sites of infection. However, we have shown that activated T-cells re-express L-selectin before exiting lymph nodes and use L-selectin to locate to virus-infected tissues (Mohammed et al, 2016, doi:10.1016/j.celrep.2015.12.0900). We considered other roles for L-selectin proteolysis and found that ADAM17-dependent proteolysis of L-selectin promotes clonal expansion of virus-specific
T- cells (Mohammed et al, 2019, doi:10.1038/s41598-019-41811-z). This led us to hypothesise that the ADAM17 generated membrane-retained fragment of L-selectin has intracellular signalling potential.

The aim of this study was to determine the fate of the membrane-retained fragment of L-selectin. Using human T leukaemic cells expressing V5-His cyto-tail tagged L-selectin, we found that, following cleavage by ADAM17, the membrane-retained fragment of L-selectin was subject to presenilin 1 (PS1) dependent cleavage. To identify L-selectin binding partners, Co^{2+} magnetic beads were bound to the V5/His tag at the carboxy terminus of L-selectin in pull-down assays. We found that full length L-selectin forms a complex with ADAM17 in unactivated T-cells which, on TCR activation, leads to rapid cleavage of L-selectin and dissociation of the L-selectin-ADAM17 complex. The membrane-retained fragment of L-selectin then binds nicastrin, the substrate recognition component of γ-secretase, as well as PS1 in the active site, resulting in rapid cleavage.

Future studies will isolate the PS1 cleavage product of L-selectin, determine its signalling capacity and its' role in the rapid expansion of T-cells following virus infection.

P12

NEW THERAPEUTIC AVENUES IN BONE REPAIR: HARNESSING A NOVEL ENDOGENOUS MOLECULE TO BOOST BONE AND PREVENT BONE LOSS IN INFLAMMATORY DISEASE

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Introduction: During bone homeostasis, osteoblasts interact with and produce a mineralisation matrix in order to form new bone. This is coupled with osteoclasts binding and resorbing bone, which is essential for maintenance. Imbalance of these functions leads to severe bone damage. We investigated the role of a novel peptide, Pro-B1X, in homeostatic and diseased bone.

Methods: *In vivo:* Normal 6-week (M, C57Bl/6J, n>3) mice were given daily intraperitoneal injections of Pro-B1X or control (PBS) for two weeks. Cortical and trabecular parameters were analysed in long bones by micro-CT, while 3-point bend testing and TRAP staining of osteoclasts was assessed.
In vitro: Primary murine calvarial and MC3T3-E1 cell line osteoblasts were cultured in differentiation media with Pro-B1X and analysed for mineralisation by Alizarin red.
Results: In normal mice, Pro-B1X significantly increased bone volume density, trabecular number and trabecular thickness, coupled with increased bone stiffness and failure load compared to control. No differences were observed in cortical bone parameters over this time-period. *In vitro*, Pro-B1X promoted mineralisation of osteoblasts after 12 days in culture which persisted through to day 21.
Importantly, Pro-B1X caused a reduction in mean bone erosion scores and a decrease in osteoclast number in mice with inflammation induced bone loss, compared to control-treated animals.
Conclusion: Pro-B1X was able to increase trabecular bone parameters in vivo, leading to stronger, stiffer bone, possibly through increasing osteoblast mineralisation. Additionally, Pro-B1X reduced bone damage during inflammation mediated bone loss, providing potential for its use as a novel treatment.

P13

AUTOINHIBITION OF KANK PROTEINS: REGULATING THE LINK BETWEEN INTEGRIN-MEDIATED ADHESIONS AND MICROTUBULES

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Many cellular activities rely on interactions between cells and the extracellular matrix (ECM) and these attachments are mediated by the integrin family of transmembrane receptors. Talin, a cytoplasmic adapter protein, activates integrin by binding to the cytoplasmic tails and ultimately couples integrin to the actin cytoskeleton. Once the adhesion has formed, talin can then act as a mechanosensitive signalling hub and recruit additional proteins in a force-dependent manner.

KANK proteins, via a direct interaction with talin and with the kinesin KIF21A, mediate the connection of these integrin-based adhesions with dynamic microtubules. A complex of proteins termed the cortical microtubule stabilising complex (CMSC), containing CLASPs, liprins and LL5β, is recruited upon talin:KANK interaction. This complex subsequently recruits KIF21A which stabilises microtubules in the vicinity of adhesions. This talin:KANK connection results in mechanosensitive crosstalk between the actin and microtubule cytoskeletons, contributes to microtubule polarity, and provides a mechanism which allows for turnover of adhesions during cell migration.

Many of the core proteins in adhesions including integrin, talin, vinculin and FAK are regulated by autoinhibition and, here, we investigate the role played by autoinhibition of KANK proteins in regulating interactions with talin and the CMSC. Using structural and biochemical techniques, we show that KANK proteins are autoinhibited and have identified the autoinhibitory domains, including a key region comprised of nine residues that is essential for this regulatory interaction. Our current focus is to explore the cellular consequences of constitutively active KANKs and how this affects adhesion dynamics.

P14

BIOCHEMICAL INSIGHT INTO PROTEIN:PROTEIN INTERACTIONS AT INTEGRIN-MEDIATED CELL ADHESIONS.

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Integrin-mediated cell adhesions are highly regulated structures forming between the cell cytoskeleton and the extracellular matrix. These adhesions are regulated by a complex network of proteins and lipids, allowing for an ever-expanding diversity in types of adhesions. This network forms on a simple core consisting of integrin linked to actin via the large adapter protein talin. Talin is comprised of a FERM domain comprised of four subdomains in an atypical linear form linked to a long mechanosensitive rod domain. This property of talin allows it to act as a mechanosensitive signalling hub, dictating the composition of the adhesions. We have developed a suite of biochemical assays to unravel the increasingly complex nature of integrin-mediated adhesions. This is allowing us to study the interactions and properties of proteins which were previously difficult. Using this suite, we have identified a mechanism by which pathogenic bacteria hijack the adhesome to assist cell invasion. Additionally, we have identified a novel interaction between talin and kindlin, which may prove vital for integrin activation. Furthermore, we have a novel crystal structure of the talin-2 head, revealing an alternative conformation which alludes to a new form of regulation of integrin activation. Overall, employing this suite is enabling us to unravel the complex nature of adhesions, advancing our understanding of how they are controlled.

P15

NEUTROPHIL-DERIVED MICROVESICLES CONTAIN POTENTIALLY DAMAGING PROTEASES AND INDUCE LUNG EPITHELIAL CELL DYSFUNCTION

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Rationale: Neutrophilic infiltration of the airways is central to the pathogenesis of chronic obstructive pulmonary disease (COPD). COPD patient sputum contains high concentrations of neutrophil-derived microvesicles (NMVs), which express a variety of neutrophil proteases. However, very little is known about the role of NMVs in COPD.

Hypothesis: NMVs generated locally and/or systemically induce lung epithelial cell dysfunction by delivering harmful, active proteases to the lung tissue.

Aim: To investigate the role of NMVs in inflammation and COPD progression.

Methodology: Healthy participant NMVs produced from peripheral blood neutrophils were characterised by electron microscopy and nanoparticle-tracking analysis. Matrix metalloproteinase-9 (MMP-9) content was investigated by flow cytometry, western blotting, and substrate degradation assays. NMV internalisation by BEAS-2B lung epithelial cells was quantified using confocal microscopy and flow cytometry, and MMP-9 and/or pro-inflammatory

cytokine expression was measured by qPCR and ELISA. Cell apoptosis was assessed using fluorescence microscopy and CellEvent caspase3/7 detection reagent, and cell loss/detachment was determined by flow cytometry.

Plasma MVs from two COPD patient cohorts were analysed (exacerbating COPD patients (n=6) and COPD patients with co-morbid pulmonary arterial hypertension (n=13)), and compared with age-matched controls (n=5 and 12, respectively) using multi-colour flow cytometry to determine cellular origin and surface MMP-9 expression. MMP-9 content of whole plasma MVs was determined by ELISA.

Results: Stimulated neutrophils released NMVs that contained active MMP-9. This protease was detected on the surface of NMVs, along with the adhesion molecule CD44. NMVs time-dependently degraded collagen IV, an effect inhibited by tissue inhibitor of metalloproteinase 1 (TIMP-1). Increased MMP-9 levels were detected both in BEAS-2B cell supernatant and intracellularly after 24 and 48h treatment with NMV. Over 90% of BEAS-2B cells internalised NMVs after 2h, and 24h co-incubation induced IL-8 and MCP-1 secretion and epithelial cell apoptosis.
NMVs expressing surface MMP-9 were detected in plasma samples from healthy volunteers and patients with COPD, but no significant differences were found between groups.

Conclusion: NMVs contain active proteases capable of extracellular matrix degradation and also express an adhesion molecule linked with anchoring active MMP-9 on the cell surface. These vesicles interact with and activate the lung epithelium. However, since there was no difference in plasma NMV MMP-9 expression in COPD patient samples, local rather than systemic NMV production may be key. Future work will determine the mechanism of NMV-induced epithelial activation and apoptosis, and further characterise COPD-patient NMV content. Additionally, we are currently using an *in vivo* model to decipher the role of NMVs in cellular activation and neutrophil recruitment during LPS-induced lung inflammation.

P16

PODOPLANIN REGULATES THE MIGRATION OF MESENCHYMAL STROMAL CELLS AND THEIR INTERACTION WITH PLATLETS

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Mesenchymal stromal cells (MSC) up-regulate podoplanin at sites of infection, chronic inflammation, and cancer. We investigated the functional consequences of podoplanin expression on the migratory potential of MSC and their interactions with circulating platelets. Expression of podoplanin significantly enhanced the migration of MSC compared to MSC lacking podoplanin. Rac-1 inhibition altered the membrane localisation of podoplanin and in turn significantly reduced MSC migration. Blocking Rac-1 activity had no effect on the migration of MSC lacking podoplanin, indicating it was responsible for regulation of migration through podoplanin. When podoplanin-expressing MSC were seeded on the basal surface of a porous filter, they were able to capture platelets perfused over the uncoated apical surface and induce platelet aggregation. Similar microthrombi were observed when endothelial cells were co-cultured on the apical surface. Confocal imaging shows podoplanin-expressing MSC extending processes into the EC layer in vitro and in vivo (umbilical cord), which could interact with circulating platelets. In both models, platelet aggregation induced by podoplanin-expressing MSC was inhibited by recombinant soluble CLEC-2. Thus, podoplanin may

enhance the migratory capacity of tissue-resident MSC and enable novel interactions with cells expressing CLEC-2.

P17

IMPAIRED NEUTROPHIL MIGRATION IN ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Patients with Chronic Obstructive Pulmonary Disease (COPD) suffer from repeated lower respiratory tract infections known as acute exacerbations of COPD (AECOPD), associated with decreased health status and accelerated decline in lung function. Neutrophils are thought to be key to the pathogenesis of COPD, and in stable COPD (sCOPD), neutrophils migrate through the lung with greater speed but less accuracy, and therefore take longer to reach sites of infection and have increased potential for tissue damage. This can be corrected by inhibition of Phosphoinositide 3-kinase (PI3K). During AECOPD, bacterial clearance is impaired despite an influx of neutrophils into the airways. However, neutrophil migration in AECOPD has not been characterised.
Objectives: We aimed to assess neutrophil migration and the effect of PI3K inhibitors during AECOPD, as well as investigate potential mechanisms for impairments in migration.
Methods: Patients on day 0 of AECOPD were recruited from the acute medical unit of a large tertiary hospital. sCOPD patients were recruited from general respiratory clinics at the same hospital. An Insall chamber and time-lapse microscopy was used to assess the speed and velocity (a measure of accuracy) of peripheral neutrophils migrating towards Interleukin-8 (IL8) or formyl-methionyl-leucyl-phenylalanine (fMLP). The effect of isoform specific class 1 PI3K inhibitors (PI3K□□and PI3K□□□were also studied. Neutrophil viability was assessed by staining with Annexin V and 7-Aminoactinomycin-D (7AAD). Expression of the key receptor for IL8, C-X-C motif chemokine receptor 2 (CXCR2), and 2 markers of activation (CD11b and CD66b) was assessed by flow cytometry.
Results: 33 AECOPD and 33 sCOPD patients, matched for age and FEV1% predicted, were recruited. Compared to sCOPD, neutrophils from patients on day 0 of AECOPD migrated towards IL8 and fMLP with both a lower speed and a lower velocity. Neutrophil velocity towards IL8 inversely correlated with serum C-reactive protein concentration, a marker of systemic inflammation. In a subset of 10 patients followed up after clinical recovery from AECOPD (day 56), speed of migration had not improved. However, velocity towards IL8 was further decreased after recovery. Incubation with PI3K□ or -□ inhibitors was unable to rescue neutrophil migration during AECOPD. Neutrophils from patients with AECOPD had significantly lower CXCR2 expression than sCOPD. There were no significant differences in cell viability between AECOPD and sCOPD. Expression of CD11b and CD66b was increased during AECOPD compared to sCOPD.

Conclusion: Slow and inaccurate neutrophil migration may partly explain why bacterial clearance is impaired during AECOPD, despite increased numbers of neutrophils in the lung. Reduced speed of migration does not improve after recovery, and velocity of migration towards IL8 is further reduced, which may predispose patients to further infection. Unlike stable disease, inhibition of PI3Kγ or –δ was unable to normalise migration during AECOPD. However, expression of CXCR2 was reduced in AECOPD compared to stable disease, offering a putative mechanism for the observed migration defects. Slow migration and increased neutrophil activation during AECOPD may lead to an increased potential for by-stander damage, contributing to accelerated lung function decline.

P18

Therapeutic potential of syndecan-2$^+$ MSC in treating inflammatory arthritis.

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Background: Rheumatoid arthritis (RA) is an autoimmune disease characterised by aberrant leukocyte infiltration and synovial hyperplasia. Despite advancements in treatment options, a significant proportion of patients do not respond to treatment and there is no known cure for RA. Mesenchymal stem cells (MSC) are multipotent cells with immunomodulatory properties, and reparative functions making them an attractive potential therapeutic tool for RA. Previous studies have largely examined bulk heterogeneous populations of MSC which has contributed to inconsistencies between preclinical studies and are highly unlikely to be approved for clinical use in humans by regulatory bodies.

Objectives: We investigated the efficacy of therapeutic systemic administration of a homogeneous syndecan-2$^+$ MSC population to ameliorate the effects of inflammatory arthritis in mice

Methods: Collagen induced arthritis was trigger in DBA/1 mice by immunisation with bovine type II collagen. A single dose of syndecan-2$^+$ MSC at 100 or 200 x10^3 cells/injection by intraperitoneal injections therapeutically at the first signs of inflammation. Disease onset and severity were evaluated daily. Bone morphology and leukocyte infiltration were assessed by microCT, immunohistochemistry, flow cytometry and qPCR.

Results: Therapeutic administration of syndecan-2$^+$ MSC significantly reduced footpad and ankle swelling, but had no effect on the global clinical score of arthritis when compared to control mice. Furthermore, we observed no difference in bone architecture, cartilage erosion or immune cell infiltration in MSC treated mice when compared to PBS controls.

Conclusions: Thus syndecan-2$^+$ MSC are able to modify the level of swelling induced during inflammatory arthritis through mechanisms as yet unknown.

P19

INHIBITION OF ENDOTHELIAL CELL-OSTEOBLAST INTERACTION INCREASES TRABECULAR BONE FORMATION

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