Joint Meeting of British Microcirculation Society & UK Cell Adhesion Society

Monday 3rd - Tuesday 4th April 2017
The Medical School, University of Birmingham, UK

Featuring a symposium on
'The Many Facets of Inflammation'

Guest Speakers
Yvonne Alexander, Jan Nilsson, Mathieu-Benoit Voisin
Joseph Stiktzi, Paul Kubes, Britta Engelhardt
Dear Participants,

It is our great pleasure to welcome you to the University of Birmingham on behalf of the British Microcirculation Society and the UK Cell Adhesion Society. This is the first joint meeting of these Societies and so we hope to offer the respective members of both something new and different. We have approximately 150 eminent scientists, postdoctoral fellows, postgraduate and graduate students, clinicians, and commercial exhibitors attending this event. The full and exciting scientific programme features a dedicated symposium on 'The Many Facets of Inflammation'. Inflammation is a key feature of many diseases and so this meeting aims to highlight the latest discoveries in the basic biology and imaging of inflammatory processes and how they impact various body systems. Of equal importance are the 21 free oral communications and 58 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 BMS & 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,

Neena Kalia (BMS Hon.Secretary) and Helen McGettrick (UKCAS)
JOINT MEETING

67th Annual Conference of the British Microcirculation Society
& 29th Meeting of the UK Cell Adhesion Society

LOCAL ORGANISERS

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Email: agera@cardiff.ac.uk
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.

Oral Presentations: Oral presentations will take place in the Leonard Deacon 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 necessary (e.g. 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-cpddbookings@contacts.bham.ac.uk). Oral communications should be 10 minutes in length allowing 5 minutes for questions.

Poster Presentations: Posters will be in the Wolfson Centre common room. Posters should be A0 Portrait (841x1189mm). 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 3rd April, a panel of judges will select posters for several 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 and hostels within 30 minutes’ walk from the meeting venue. Alternatively, several 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 listing of hotels can be found on the 2017 BMS-UKCAS meeting website. 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 2017 BMS-UKCAS Conference website:

www.birmingham.ac.uk/bms-ukcas2017

British Microcirculation Society Annual General Meeting will be held in the main meeting Leonard Deacon lecture theatre at 17.30-18.30 on Monday 3rd April 2017. The UKCAS Committee Meeting will be held at the same time but in the West Wing Meeting room.

Drinks Reception and Society Dinner will be at the Banqueting Suite, Birmingham Council House in the city centre from 19.30 onwards on Monday 3rd April. Dinner tickets should be pre-booked at a cost of £40 per person which includes a drinks reception, 3 course meal, wine, and entertainment.
Meeting Sponsors and Trade Exhibitors

The meeting has been generously supported by:

Invited Speakers have also been sponsored by:

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- publishing leading peer-reviewed journals
- facilitating scientific meetings and communities
- providing travel grants for young researchers
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Meeting Sponsors and Trade Exhibitors

There will be a number of commercial exhibitors in the poster hall. Please take time to visit their stands and talk to their representatives.

Further information on these sponsors and links to their company webpages can be found at the meeting webpage at:

www.birmingham.ac.uk/bms-ukcas2017
### 2017 BMS-UKCAS Scientific Meeting Programme

**MONDAY 3rd APRIL 2017**

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<thead>
<tr>
<th>Location</th>
<th>Time</th>
<th>Session/Summary</th>
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<tr>
<td>Wolfson Centre Common Room</td>
<td>09:00 – 10:50</td>
<td>Arrival &amp; Registration: The Medical School – Tea/Coffee &amp; Poster Set-up</td>
</tr>
<tr>
<td>Leonard Deacon Lecture Theatre</td>
<td>10:50 – 11:00</td>
<td>Welcome Address: Neena Kalia &amp; Helen McGettrick – Local Organisers</td>
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#### Scientific Session 1: Chairs – Nicola Brown and Mathieu-Benoit Voisin

**S1** 11:00 – 11:45 **KEYNOTE SPEAKER**
M. Yvonne Alexander  
Manchester Metropolitan University, UK  
ENDOTHELIAL-DERIVED MICROVESICLES AS IMMUNE EFFECTORS IN DISEASE

**Selected Oral Communications**

**OC1** 11:45 – 12:00  
Marie O’Connor – University College London  
TARGETING LRG1 POTENTIATES VASCULAR NORMALISATION AND REDUCES GROWTH IN SUBCUTANEOUS TUMOURS

**OC2** 12:00 – 12:15  
Aleksandar Ivetic – Kings College London  
L-SELECTIN SHEDDING IS THE "GO" SIGNAL FOR NEUTROPHIL CHEMOTAXIS AND EFFECTOR FUNCTION: A POSSIBLE THERAPEUTIC TARGET FOR STERILE INJURY?

**OC3** 12:15 – 12:30  
Chris Hansell – University of Glasgow  
THE ATYPICAL CHEMOKINE RECEPTOR, ACKR2, SCAVENGES CHEMOKINES IN THE LUNG AND CONSTRAINS NK CELL MIGRATORY ACTIVITY WHICH PROMOTES METASTASIS

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<thead>
<tr>
<th>Wolfson Centre Common Room</th>
<th>12:30 – 14:00</th>
<th>Buffet Lunch, Poster Session and Trade Exhibitors</th>
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#### Scientific Session 2: Chairs – Helen Arthur and Melissa Gammons

**S2** 14:00 – 14:45 **KEYNOTE SPEAKER**
Jan Nilsson  
University of Lund, Sweden  
IMMUNITY AND REPAIR AS NOVEL TARGETS FOR INTERVENTION IN ATHEROSCLEROSIS
### Selected Oral Communications

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
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<tbody>
<tr>
<td>**OC4</td>
<td>14:45 – 15:00**</td>
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<tr>
<td></td>
<td>INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2 (IGFBP-2) AND ITS ROLE IN ANGIOGENESIS</td>
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<tr>
<td>**OC5</td>
<td>15:00 – 15:15**</td>
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<tr>
<td></td>
<td>HUMAN LEUKOCYTE PECAM-1 AND L-SELECTIN CO-CLUSTER IN CIS TO REGULATE TRANSENDOTHELIAL MIGRATION UNDER FLOW CONDITIONS</td>
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<tr>
<td>**OC6</td>
<td>15:15 – 15:30**</td>
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<tr>
<td></td>
<td>INTRAVITAL IMAGING OF LEUKOCYTE, PLATELET AND STEM CELL TRAFFICKING IN VIVO IN THE CARDIAC MICROCIRCULATION FOLLOWING MYOCARDIAL ISCHAEMIA-REPERFUSION INJURY</td>
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**Wolfson Centre Common Room**

15.30 – 16.00  | Tea/Coffee Break

### Scientific Session 3: Chairs – Gerard Nash and Carolyn Staton

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<tr>
<td>**S3</td>
<td>16:00 – 16:45**</td>
</tr>
<tr>
<td></td>
<td>Mathieu-Benoit Voisin</td>
</tr>
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<td></td>
<td>William Harvey Institute, London, UK</td>
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<td>NEUTROPHIL MIGRATION INTO LYMPHATIC VESSELS DURING ANTIGEN CHALLENGE IN VIVO</td>
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### Selected Oral Communications

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<tr>
<td>**OC7</td>
<td>16:45 – 17:00**</td>
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<td>VALIDATION OF THE GLYCOCHECK DEVICE FOR INDIRECT MEASUREMENT OF GLYCOCALYX INTEGRITY IN VIVO</td>
</tr>
<tr>
<td>**OC8</td>
<td>17:00 – 17:15**</td>
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<tr>
<td></td>
<td>SEQUENTIAL CHEMOTACTIC CUES GUIDE NEUTROPHILS THROUGH DISTINCT CELLULAR BARRIERS OF BLOOD VESSEL WALLS DURING INFLAMMATION</td>
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<tr>
<td>**OC9</td>
<td>17:15 – 17:30**</td>
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<td>IMPAIRED SKELETAL MUSCLE PERFORMANCE CORRESPONDS TO ACUTE REDUCTIONS IN CAPILLARY PERFUSION</td>
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17.30 – 18.30  | BMS Annual General Meeting - UKCAS CoM  |
19.00 – late  | Drinks Reception and Society Dinner (served 20.00)  |
|              | Banqueting Suite, Council House, City Centre |
**TUESDAY 4<sup>th</sup> APRIL 2017**

### Scientific Session 4 : Chairs – Ann Ager and Kenton Arkill

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<th>Time</th>
<th>Speaker</th>
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<tr>
<td>S4</td>
<td>09:00 – 09:45</td>
<td><strong>KEYNOTE SPEAKER</strong></td>
<td>Joseph Skitzi</td>
<td>Roswell Park Cancer Institute, USA</td>
</tr>
<tr>
<td>OC10</td>
<td>09:45 – 10:00</td>
<td>Alexander Brill</td>
<td>University of Birmingham</td>
<td>MAST CELLS EXACERBATE DEEP VEIN THROMBOSIS IN MICE: A NOVEL LINK BETWEEN THROMBOSIS AND INFLAMMATION</td>
</tr>
<tr>
<td>OC11</td>
<td>10:00 – 10:15</td>
<td>Ross King</td>
<td>William Harvey Institute, London</td>
<td>INVESTIGATING THE ROLE OF NEUTROPHIL-DERIVED TNF IN PATHOPHYSIOLOGICAL MICROVASCULAR HYPERPERMEABILITY</td>
</tr>
<tr>
<td>OC12</td>
<td>10:15 – 10:30</td>
<td>Sarah Fawaz</td>
<td>University of Bristol</td>
<td>GLOBULAR ADIPONECTIN SIGNALS TO GLOMERULAR ENDOTHELIAL CELLS TO ACTIVATE THE AMP-ACTivated PROTEIN KINASE PATHWAY</td>
</tr>
<tr>
<td>OC13</td>
<td>10:30 – 10:45</td>
<td>Joanna Jung</td>
<td>University of Alberta, Canada</td>
<td>DISRUPTED ENDOPLASMIC RETICULUM PROTEOSTASIS PREVENTS PROGRESSION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALITIS (EAE) IN A MURINE MODEL OF MULTIPLE SCLEROSIS</td>
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**Wolfson Centre Common Room**

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<tr>
<td>10:45 – 11:15</td>
<td>Tea/Coffee Break</td>
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### Scientific Session 5 : Chairs – Sussan Nourshagh and Kim Gooding

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<tbody>
<tr>
<td>S5</td>
<td>11:15 – 12:00</td>
<td><strong>KEYNOTE SPEAKER</strong></td>
<td>Paul Kubes</td>
<td>University of Calgary, Canada</td>
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<tr>
<td>OC14</td>
<td>12:00 – 12:15</td>
<td>Angharad Watson</td>
<td>University of Cardiff</td>
<td>MAINTAINING L-SELECTIN ON ADOPTIVELY TRANSFERRED T CELLS AS A CANCER IMMUNOTHERAPY STRATEGY</td>
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<td>Session</td>
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<td>Speaker</td>
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<tr>
<td>C15</td>
<td>12:15</td>
<td>Sarah Marsh – University of Newcastle</td>
<td>CIRCULATING NON-CLASSICAL MONOCYTES ARE PREFERENTIALLY DEPLETED FROM THE CIRCULATION IMMEDIATELY AFTER REPERFUSION IN STEMI PATIENTS AND ARE ASSOCIATED WITH PATIENT OUTCOMES</td>
<td></td>
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<tr>
<td>OC16</td>
<td>12:30</td>
<td>Alexandra Daniel – University of Leeds</td>
<td>SKIN MICROVASCULAR FLOW ASSESSED BY DYNAMIC OCT: FIRST NON-INVASIVE QUANTITATIVE OUTCOME MEASURE OF MICROVASCULAR DAMAGE IN SYSTEMIC SCLEROSIS</td>
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<tr>
<td>OC17</td>
<td>12:45</td>
<td>Lauren Quinn – University of Birmingham</td>
<td>INVESTIGATING THE EFFECT OF AGE ON T CELL TRANS-ENDOTHELIAL MIGRATION</td>
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**Wolfson Centre Common Room**

13:00– 14:30 **Buffet Lunch, Poster Session and Trade Exhibitors**

### Scientific Session 6: Chairs – Angela Shore and Helen McGettrick

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<th>Speaker</th>
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<tr>
<td>S6</td>
<td>14:30</td>
<td>KEYNOTE SPEAKER Britta Engelhardt – sponsor: Abercrombie Fund University of Bern, Switzerland</td>
<td>IMMUNE CELL TRAFFICKING ACROSS THE BLOOD BRAIN BARRIER</td>
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<tr>
<td>OC18</td>
<td>15:15</td>
<td>Zofia Tuharska – University of Dundee</td>
<td>β-AMYLOID PROMOTES DIABETES-LIKE VASCULAR DYSFUNCTION IN MICE</td>
</tr>
<tr>
<td>OC19</td>
<td>15:30</td>
<td>Khalid Naseem – University of Hull</td>
<td>PLATELET-DERIVED THROMBOSPONDIN-1 MODULATES cAMP SIGNALING TO PROMOTE PLATELET ACTIVATION</td>
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<tr>
<td>OC20</td>
<td>15:45</td>
<td>Roy Bicknell – University of Birmingham</td>
<td>MULTIMERIN-2 IS A LIGAND FOR THREE C-TYPE LECTINS AND SPANS THE ENDOTHELIAL PERICYTE INTERFACE</td>
</tr>
<tr>
<td>OC21</td>
<td>16:00</td>
<td>Robert Byers – University of Sheffield</td>
<td>NON-INVASIVE VISUALISATION OF THE MICROVASCULATURE WITHIN SUBCUTANEOUSLY IMPLANTED TUMOURS USING OCT</td>
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<tr>
<td></td>
<td>16:15</td>
<td>Presentation of Prizes</td>
<td>Announcement of 2018 BMS and UKCAS meetings</td>
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<tr>
<td></td>
<td>16:30</td>
<td>Meeting Closes</td>
<td><strong>Meeting Closes</strong></td>
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**SYMPOSIUM COMMUNICATION ABSTRACTS (S1-S6)**

**S1**

**ENDOTHELIAL-DERIVED MICROVESICLES AS IMMUNE EFFECTORS IN DISEASE**

*M. Yvonne Alexander*

Translational Cardiovascular Science Research Group, Biomedical Research Centre, Manchester Metropolitan University, Manchester, UK

Endothelial microvesicles (EMVs), (previously known as microparticles), are phospholipid-rich vesicles released from cells when activated or injured, and although increased circulating levels of EMVs have been identified in several inflammatory disorders, we have shown they can be reduced following anti-inflammatory treatment both *in vitro* and *in vivo*. EMVs are released into the extracellular space and are recognised as cell-to-cell communicators, carrying soluble cytosolic material and nuclear components which in turn act both locally and remotely. A fundamental understanding of the interaction between EMVs, endothelial dysfunction and inflammation is critical for our comprehension of the mechanisms leading to the derangement of endothelial homeostasis and vascular dysfunction. Our work focuses on identifying the phenotype of EMVs in plasma of RA and Lupus patients, correlating their relation to endothelial dysfunction and identifying biological pathways activated by EMVs stimulated under inflammation conditions. I will present data to demonstrate that EMPs potentially play a role in the feedback loop of damage and repair during homeostasis, and can also be protective under oxidative stress conditions, confirming that EMV function is reflected by their micro-environment.
Development of atherosclerotic plaque vulnerability involves an accumulation of cytotoxic lipids, an inflammation-driven degradation of the plaque extracellular matrix and a loss of cells with repair function. There is a clinical need for good circulating biomarkers that reflect these processes. While different types of inflammatory biomarkers have been extensively studied biomarkers reflecting cell death as well as extracellular matrix degradation and repair are less well characterized. We have recently shown that activation of apoptotic cell death is associated with a release of soluble death receptors such as Fas and TRAIL receptor 2 that can be measured in plasma and that subjects with high plasma levels of death receptors have an increased risk of cardiovascular disease. Moreover, an increased risk is also observed in subjects with high levels of the matrix metalloproteinases MMP-7 and -12 as well as in subjects low levels of growth factors for vascular smooth muscle cells and vascular progenitor cells.\(^1,2\) These observations provide clinical support for an important role of vascular injury and repair in the development of cardiovascular disease. Indeed, there is evidence that impaired repair vascular is a key factor in cardiovascular complications to diabetes.\(^3\) An additional factor that has been implicated in plaque development is autoimmune responses against lipoproteins that have accumulated and oxidized in the artery wall. There is strong evidence that Th1-type immunity against these lipoproteins is atherogenic while regulatory T cells have a protective role. Several investigators have developed vaccines based on apolipoprotein B (apoB)-100 derived peptides to promote tolerance.\(^4\) The most studied peptide, p210, has been shown to reduce atherosclerosis by up to 50% when administered subcutaneously or intra-nasally in different animal models of atherosclerosis.

References:


TNFα DRIVES NEUTROPHIL MIGRATION AND CRAWLING INTO AFFERENT LYMPHATIC VESSELS DURING ANTIGEN CHALLENGE IN VIVO

Mathieu-Benoit Voisin

William Harvey Research Institute, London, UK

Neutrophils are now viewed as key effectors of both innate and adaptive immunities in many physiological and pathological conditions. Whilst the trafficking of neutrophils through blood vessels has been extensively studied, the mechanism that control their entry into the lymphatic system is poorly understood. For this purpose, we have developed a murine model of cremasteric inflammation to visualise by intravital confocal microscopy the interactions of neutrophils with tissue-associated afferent lymphatic vessels in vivo. In the present study, we report that neutrophils migrate rapidly into the lymphatic vessels upon both antigen challenge and TNFα-induced inflammation of murine cremaster muscles in a CCR7-dependent manner. Interestingly, neutrophil intravasation into lymphatics (but not extravasation through blood vessels) upon antigen challenge is inhibited (~70%) in mice genetically deficient in both receptors for TNFα; a response associated with a reduced expression of CCR7 on the surface of tissue-infiltrated neutrophils. Furthermore, in WT mice pre-treated with an anti-TNFα blocking antibody, ICAM-1 expression by lymphatic vessels was reduced; leading to an alteration of the directional crawling of neutrophils onto the lumen of lymphatic endothelial cells. Collectively, our results demonstrate the critical role of TNFα in promoting neutrophil recruitment into the lymphatic vasculature in vivo during the inflammatory response of antigen challenge.
INTRA-OPERATIVE INTRAVITAL MICROSCOPY TO STUDY HUMAN TUMOUR MICROVESSELS

Joseph Skitzi

Roswell Park Cancer Institute, New York, USA

Tumour vessels have been studied extensively as they are critical sites for drug delivery, anti-angiogenic therapies and immunotherapy. As a preclinical tool, intravital microscopy (IVM) allows for in vivo real-time direct observation of vessels at the cellular level. However, to date there are no reports of intravital high-resolution imaging of human tumours in the clinical setting. Here we report the feasibility of IVM examinations of human malignant disease with an emphasis on tumour vasculature as the major site of tumour-host interactions. Consistent with preclinical observations, we show that patient tumour vessels are disorganized, tortuous and ~50% do not support blood flow. Human tumour vessels diameters are larger than predicted from immunohistochemistry or preclinical IVM, and thereby have lower wall shear stress, which influences delivery of drugs and cellular immunotherapies. Thus, real-time clinical imaging of living human tumours is feasible and allows for detection of characteristic within the tumour microenvironment.
VISUALIZING IMMUNE CELLS DURING INFLAMMATION, INFECTION AND TISSUE INJURY

Paul Kubes

Snyder Institute for Chronic Diseases, Faculty of Medicine, University of Calgary, Canada

The recruitment of immune cells to sites of infection and sterile injury have many commonalities but differ in certain ways. To understand these differences we have developed a very simple model of sterile injury and have begun to visualize the progression of cell recruitment and the mechanisms by which these events occur. We found that during a focal sterile thermal injury in the liver, platelets were immediately recruited to the site and paved the vessel lumen. Neutrophils followed a gradient of chemokines along the sinusoids to the injury site. In addition rapid recruitment of macrophage from the peritoneum was also noted. After 24 hrs, many of the neutrophils disappeared and a switch from inflammation to repair occurred, in part mediated by iNKT cells that sampled the environment and released IL4 but not IFNgamma. This led to hepatocyte growth, revascularization as well as inflammatory monocytes (CCR2 high Ly6C high) surrounding the injury site but becoming locally educated to become Ly6C low and CX3CR1 high repair monocytes. Comparing this model to models of either chronic inflammation or infection reveal key differences that could explain why repair may or may not occur under some circumstances.
Recent rediscoveries of the lymphatic vessels within the dura mater surrounding the brain by modern live cell imaging technologies have provoked dismissal of the existence of immune privilege of the central nervous system (CNS). I will explain that understanding immune privilege of the CNS requires intimate knowledge of its unique anatomy including the endothelial, epithelial and glial brain barriers. The brain barriers establish compartments within the CNS that differ strikingly with regard to their accessibility to immune cell subsets. I will focus on describing the unique cellular and molecular mechanisms mediating immune cell migration into the CNS during immunosurveillance and neuroinflammation in the context of animal models for multiple sclerosis and ischemic stroke.
TARGETING LRG1 POTENTIATES VASCULAR NORMALISATION AND REDUCES GROWTH IN SUBCUTANEOUS TUMOURS

Marie O’Connor

Institute of Ophthalmology, University College London, London EC1V 9EL, UK

Co-Authors: D.M. Kallenberg, A. Watson, J. Ohme, L. Dowsett, J. George, N. Jeffs, A. Ager, S.E. Moss, J. Greenwood

Tumour vessels are often chaotic, dysfunctional and poorly-perfused. Normalising tumour vasculature to enhance vessel patency, reduce hypoxia and vascular leakage, and improve delivery of cytotoxic drugs has thus become an additional therapeutic objective. In this study we investigate the role of leucine-rich alpha-2-glycoprotein 1 (LRG1) in vessel formation, structure and function in experimentally induced tumours. Tumours were established in wild-type and Lrg1-/- mice through subcutaneous injection of mouse melanoma (B16/F0) cells. The growth rate of B16/F0 tumours in Lrg1-/- mice was significantly reduced compared to WT mice. Upon histological examination we observed a significant decrease in vessel number, especially small-diameter vessels, in tumours from Lrg1-/- mice or from mice treated systemically with the LRG1 blocking antibody 15C4. Furthermore, in Lrg1-/- mice and in mice treated with 15C4, we observed a larger proportion of endothelium in contact with pericytes and an increase in vessel perfusion by FITC-lectin. To establish whether this vascular normalisation improves cytotoxic drug delivery we treated WT tumour-bearing mice with 15C4 antibody in combination with a suboptimal dose of cisplatin. Tumours treated with 15C4 in combination with cisplatin exhibited a significant reduction in tumour growth compared with cisplatin alone. Our data indicate that LRG1 is involved in the promotion of dysfunctional tumour angiogenesis and that LRG1 knockdown or inhibition results in a more normalised vasculature. We propose, therefore, that LRG1 is a potential therapeutic target in cancer, and that its inhibition may aid the delivery and efficacy of tumour therapeutics.
L-SELECTIN SHEDDING IS THE "GO" SIGNAL FOR NEUTROPHIL CHEMOTAXIS AND EFFECTOR FUNCTION: A POSSIBLE THERAPEUTIC TARGET FOR STERILE INJURY?

Aleksandar Ivetic

BHF Centre of Research Excellence, Cardiovascular Division, King's College London, London, SE5 9NU, UK, ¹University of Calgary, Calgary, Alberta, T2N 4N1

Co-Authors: J. Davies, I. Rahman, J.J. Pombo, B. Petri, F. Zemp, G. Charras, ¹P. Kubes

L-selectin is a cell adhesion molecule expressed on all circulating neutrophils to regulate tethering and rolling. We have recently reported in monocytes that ectodomain shedding of L-selectin occurs specifically during transendothelial migration and not before. We found this event to be critical in regulating front-back polarity once monocytes enter the subendothelial space. Our more recent (unpublished) studies reveal that neutrophils respond in a similar way. To better understand what drives L-selectin shedding in neutrophils in vivo, we systematically challenged the cremasteric muscle first with chemokine (2-4h) and then with laser injury local to the site of neutrophil emigration. To our surprise, neutrophils emigrating exclusively in response to chemokine were L-selectin positive and adopted clear front-back polarity. However, laser injury, or superfusion of the cremasteric muscle with FMLF, led to rapid L-selectin shedding. Modelling these inflammatory responses in vitro revealed that human neutrophil chemotaxis through 3D collagen scaffolds towards fMLF, but not the intermediary chemoattractant, IL-8, was significantly attenuated when L-selectin shedding was genetically or pharmacologically blocked. Additionally, release of superoxide in response to fMLF stimulation or phagocytosis of opsonised zymosan particles was significantly attenuated when L-selectin shedding was blocked. Taken together, we hypothesise that L-selectin shedding is the “go” signal for neutrophil chemotaxis towards end-stage chemoattractants (e.g. fMLF) and effector function (e.g. phagocytosis and superoxide production). Future work will explore whether blocking L-selectin shedding is a suitable therapeutic target for minimising unwanted neutrophil effector function in the setting of sterile injury.
THE ATYPICAL CHEMOKINE RECEPTOR, ACKR2, SCAVENGES CHEMOKINES IN THE LUNG AND CONSTRAINS NK CELL MIGRATORY ACTIVITY WHICH PROMOTES METASTASIS

Chris Hansell

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Dissemination of cells from a primary tumour site to secondary destinations is essential for the establishment of metastasis, a process which is responsible for the majority of cancer-related deaths. Inflammatory chemokines have been shown to be essential players both in orchestrating the immune response to cancer cells and paradoxically driving the processes of metastasis. The atypical chemokine receptor ACKR2 binds and destroys many of these inflammatory chemokines. Here we demonstrate that mice deficient in the atypical chemokine receptor Ackr2 display impaired development of lung metastasis in vivo in both cell line and spontaneous models. Further analysis reveals that the protection afforded to the ACKR2-/- depends upon collaboration between lung stroma and NK cells. We demonstrate for the first time that ACKR2 is abundantly expressed by lung blood endothelial cells and actively scavenges luminal inflammatory chemokine. At the same time ACKR2 deletion also leads to an unexpected increase in the expression of the chemokine receptor CCR2 specifically by KLRG1+ NK cells from the Ackr2-/- mice. Thus metastatic lung tumours expressing the relevant ligand CCL2 are efficiently targeted in ACKR2-/- due to the absence of chemokine scavenging locally by endothelial cells and the enhanced sensitivity of KLRG1+ NK cells to CCR2 chemotactic gradients. Our data have important implications for our understanding of the roles for chemokines in the metastatic process and highlight Ackr2 and CCR2 as potentially manipulable therapeutic targets in metastasis.
INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2 (IGFBP-2) AND ITS ROLE IN ANGIOGENESIS

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Ischaemic heart disease and peripheral arterial disease present a significant burden on global mortality. Novel therapeutic strategies for these diseases are urgently needed. Insulin-like growth factor binding proteins (IGFBP1-7) demonstrate great potential in therapeutic angiogenesis. IGFBPs primarily modulate IGF bioactivity and bioavailability but several IGFBPs also exert IGF-independent effects. IGFBP-2 possesses IGF-binding domains, Arg-Gly-Asp integrin recognition motif, heparin-binding domains and a nuclear localisation sequence. Nuclear entry of IGFBP-2 may lead to possible activation of vascular endothelial growth factor. However, the specific mechanism which IGFBP-2 uses to exert its angiogenic properties remains unclear. Effects of IGFBP-2 on angiogenic signalling pathways were investigated in Human Umbilical Vein Endothelial Cells (HUVECs) and other cell types, using immunoblotting. In vitro and in vivo angiogenic models were employed to determine the effect of IGFBP-2 on cell adhesion, migration, tube formation and blood flow restoration. In HUVEC, phosphorylation of Akt stimulated by IGFBP-2 was maximal at 15 mins (200ng/ml). HUVEC adhesion to fibronectin displayed a 1.7 fold increase in the presence of 200ng/ml IGFBP-2. Chronic stimulation of 500ng/ml IGFBP-2 exhibited significant increase in HUVEC wound closure in the absence and presence of Mitomycin C, as well as tube formation. Recovery of hind limb perfusion in mice subjected to hind limb ischaemia was greater in transgenic mice overexpressing IGFBP-2 compared to wild-type littermate controls. IGFBP-2 significantly enhances angiogenic activity and may provide a potential additional acute treatment for ischaemic conditions. Future works involve investigation of each domain and their roles in IGFBP-2 mediated angiogenesis.
HUMAN LEUKOCYTE PECAM-1 AND L-SELECTIN CO-CLUSTER IN CIS TO REGULATE TRANSENDOTHELIAL MIGRATION UNDER FLOW CONDITIONS

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Directional migration of circulating leukocytes towards damaged tissue is fundamental to the inflammatory response. The “multi-step adhesion cascade” lists the chronological contribution of cell adhesion molecules involved in the successful exit of leukocytes from the circulation into damaged tissue. Importantly, very little is known about how multiple cell adhesion molecules cluster in cis and contribute to unique signalling pathways that drive successive steps of the adhesion cascade. We recently showed that ectodomain “shedding” of L-selectin occurs exclusively as monocytes undergo transendothelial migration (TEM). The biological significance of L-selectin shedding ensures monocytes entering the subendothelial space adopt front-back polarity that is essential for interstitial chemotaxis towards a site of injury. Using a FRET-based functional screen in monocytes and neutrophils, we identified PECAM-1 as a cell adhesion molecule that can co-cluster in cis with L-selectin specifically during TEM. We found that the clustering relationship between leukocyte PECAM-1 and L-selectin in cis was unidirectional: clustering of PECAM-1 triggered co-clustering of L-selectin, but clustering L-selectin did not promote co-clustering with PECAM-1. The functional significance of PECAM-1/L-selectin clustering induced faster TEM rates, which was negated if L-selectin shedding was blocked. Furthermore, PECAM-1/L-selectin clustering was cytokine specific and occurred on TNF-stimulated, but not IL-1β-stimulated, endothelial monolayers. Finally, small nucleotide polymorphisms (SNPs) in monocyte PECAM-1 associated with increased risk of coronary heart disease show increased adhesion and TEM across TNF-activated endothelial monolayers. We will discuss the impact of these PECAM-1 SNPs on co-clustering with L-selectin, TEM rates and their potential contribution to coronary heart disease.
Background: The clinical success of stem cell (SC) therapy for myocardial infarction is compromised by their poor cardiac homing following systemic delivery. Since SC therapy may depend on beneficial paracrine effects, it is a further hindrance that little is known about the inflammatory response dynamics within myocardial microcirculation in vivo. Methods: 3D-printed stabilisers were bonded to the beating heart of anaesthetised (ketamine/xylazine) mice. This enabled confocal intravital imaging of ventricular microcirculation in their centre. PE-anti-Gr-1 and APC-anti-CD41 antibodies were injected to label neutrophils and platelets respectively with FITC-BSA enabling blood flow visualisation. In some mice, haematopoietic SCs (HSCs; HPC-7s) were introduced intra-arterially. Ischaemia-reperfusion (IR) injury was induced by 45min (reversible) ligation of the LAD artery. Results: Neutrophil adhesion and platelet accumulation were both significantly (p<0.001) and rapidly increased in IR injured microvessels with platelet accumulation increasing with time. No difference in the number or velocity of free-flowing neutrophils was observed. A significant (p<0.05) decrease in functional capillary density was also observed in injured hearts. Although HSC adhesion was not significantly enhanced following injury, a time-dependent increase in adhesion was observed in both sham and injured hearts. No significant change in the number or velocity of free-flowing HSCs was observed following injury. Interestingly, despite reduced capillary perfusion, approximately 10-20 free-flowing HSCs were observed trafficking though the heart at each time point throughout reperfusion. Discussion: Intravital microscopy has allowed successful visualisation of the microvascular inflammatory response and HSC homing events in the beating mouse heart post-reperfusion. Subsequent experiments will allow us to assess whether HSC administration can confer vasculoprotection.

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VALIDATION OF THE GLYCOCHECK DEVICE FOR INDIRECT MEASUREMENT OF GLYCOCALYX INTEGRITY IN VIVO

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Perturbation to the endothelial glycocalyx can contribute to vascular dysfunction in health and disease. Sidestream dark field videomicroscopy connected with GlycoCheck software allows indirect measurements of glycocalyx integrity in vivo. However, validation data is lacking. The aims of this study were to compare in vivo assessment of glycocalyx integrity using the GlycoCheck system against an established confocal fluorescence microscopy technique in a rodent model. Measurements of the glycocalyx using confocal microscopy and the GlycoCheck system were taken at baseline, and after enzymatic treatments of glycocalyx using neuraminidase (1.6 IU/kg body wt.). Mesenteric microvessels of six male Sprague Dawley rats were superfused with trimethylamine-diphenylhexatriene (TMA-DPH, 1µM, for labelling vessel wall) and perfused with fluoresceinisothiocyanate conjugated wheat germ agglutinin (FITC-WGA lectin, 6.25 mg/kg body wt., for labelling glycocalyx) for confocal imaging. The differences of the peak TMA-DPH and the peak FITC-WGA fluorescence intensities represent the glycocalyx. These vessels were also assessed by the GlycoCheck system, in which perturbed glycocalyx is reflected by an increase in perfused boundary region (PBR). The majority of measurements were obtained from vessels with RBC column width between 10 and 19 µm. An inverse correlation was observed between the measurements of glycocalyx on GlycoCheck and confocal imaging (p=0.03, Pearson r=−0.64) (10–19 µm). Reduced percentage red blood cell filling of microvessels was observed post-neuraminidase (p<0.05) which is inversely correlated with PBR (p<0.0001, Pearson r=−0.97) (10–19 µm). Results from this study confirmed that PBR measured by GlycoCheck may serve as a validated non-invasive measure of glycocalyx integrity in vivo.
SEQUENTIAL CHEMOTACTIC CUES GUIDE NEUTROPHILS THROUGH DISTINCT CELLULAR BARRIERS OF BLOOD VESSEL WALLS DURING INFLAMMATION

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The passage of circulating neutrophils through venular walls is a key component of effective immune responses. Although the importance of chemokines in this process is well established, little is known about the contribution of individual chemokines in guiding neutrophils through distinct cellular components of the venular wall. To address this, we investigated the potential roles of the chemokines CXCL1 and CXCL2, in guiding neutrophils through endothelial cells (ECs) and the pericyte layer in vivo. For this purpose we employed a confocal intravital microscopy (IVM) platform developed in our laboratory for the concomitant tracking of neutrophil interactions with ECs and pericytes in cremasteric venules of a-SMA-RFPcherry;LysM-eGFP-ki mice (express RFP+ pericytes/EGFP+ neutrophils). In this model local injection of blocking anti-CXCL1 and anti-CXCL2 mAbs similarly reduced neutrophil entry into the extravascular space of TNF-stimulated tissues (72.6% and 61.7% inhibition, respectively). IVM analyses showed that CXCL1 was required for efficient neutrophil adhesion and crawling on ECs and sub-EC crawling on pericytes, whereas CXCL2 was dispensable for these responses. In contrast, CXCL2 was found to be specifically important in guiding neutrophils through EC junctions in a luminal-to-abluminal direction. Further, immunofluorescence staining of TNF-activated cremaster muscles demonstrated that venular ECs and pericytes are an abundant source of CXCL1, but not CXCL2. In contrast, CXCL2 was primarily associated with neutrophils. Collectively, our data suggests that the chemokines CXCL1 and CXCL2 are derived from distinct cellular sources and exhibit non-redundant functions to cooperatively mediate efficient neutrophil extravasation during an acute inflammatory reaction in vivo.
IMPAIRED SKELETAL MUSCLE PERFORMANCE CORRESPONDS TO ACUTE REDUCTIONS IN CAPILLARY PERFUSION

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Microvascular rarefaction (loss of functional capillaries) may contribute to skeletal muscle dysfunction, but the relative importance for performance decline is unknown because concurrent declines in, e.g. fibre size, oxidative capacity, occur in pathologies such as chronic heart failure. To investigate the effect of microvascular rarefaction per se on muscle performance in otherwise healthy tissue, capillary perfusion was reduced in rat extensor digitorum longus (EDL) by stochastic arteriolar blockade using microsphere injections. Microspheres (15µm diameter) were washed and resuspended in 4% bovine serum albumen to avoid localised vasodilatory effect of the carrier solution. Bilateral EDL twitch force and fatigue-resistance were determined by indirect stimulation of isometric contractions (10Hz, 180s). Carotid pressure and femoral artery flow were monitored simultaneously. Fatigue index (FI; relative force decline) in control EDL was 60.6±1.6%, and decreased in proportion to microspheres injected: a linear dose-response relationship was found in the ipsilateral EDL (r²=0.454; P=0.016) while contralateral EDL had enhanced FI (r²=0.362; P=0.039). A reduction in exercise hyperaemia was associated with increasing microsphere dose (1 min after stimulation, P=0.004). Cryosections of EDL correlated impaired muscle performance with a reduction in perfused capillaries; injection of 1.8 million microspheres effected a 29.4% decline in FI and a reduction in perfusion index of 14.3%. These data highlight the sensitivity of muscle endurance to acute changes in microvascular perfusion, while the methodology offers a convenient model to assess short and long-term effects of constrained microcirculation upon active muscle.
Deep vein thrombosis (DVT) and its devastating complication, pulmonary embolism, are a frequent cause of morbidity and mortality around the world. Although precise mechanisms of DVT initiation remain unclear, it has been demonstrated that DVT develops similarly to sterile inflammation. In this work, we hypothesized that mast cells (MCs), which contain both proinflammatory (i.e., potentially prothrombotic) and anticoagulant (i.e., potentially antithrombotic) mediators, are involved in DVT development. Using a murine DVT model of inferior vena cava (IVC) stenosis, we demonstrate that two independent strains of MC-deficient mice were fully protected against thrombosis. Adoptive transfer of in vitro differentiated MCs restored DVT in these mice. Staining for MC granules revealed MCs in the venous wall and their number decreased with thrombosis. Pharmacological ablation of MC granules or prevention of their release prevented DVT. Deficiency in MCs lessened endothelial activation, liberation of Weibel-Palade body content and recruitment of cells to the vessel wall. Local application of a MC degranulator (compound 48-80) or histamine on the IVC promoted DVT in wild-type mice and histamine induced DVT in MC-deficient animals. In conclusion, MC are implicated in DVT initiation and development. Mice deficient for MCs have unchanged normal hemostasis and therefore MCs can be considered a potential target for DVT prevention in humans.
INVESTIGATING THE ROLE OF NEUTROPHIL-DERIVED TNF IN PATHOPHYSIOLOGICAL MICROVASCULAR HYPERPERMEABILITY

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Tumour necrosis factor (TNF) is a pro-inflammatory cytokine that is released by numerous cell types, in particular cells of the immune system, following infection and injury. Previous studies from our laboratory have identified neutrophil-derived TNF as a key regulator of neutrophil-dependent vascular permeability in response to neutrophil chemoattractants. To build on these novel findings, the present work aimed to investigate the role of neutrophil-derived TNF in pathophysiological models of aberrant microvascular permeability, through the use of a novel mouse model exhibiting selective deletion of TNF in neutrophils (TNFf/f MRP-8 Cre-/-). Initial characterisation of the TNFf/f MRP-8 Cre+ mice indicated ~60% deletion of TNF in mouse neutrophils but not in other leukocyte subtypes. In vivo, using a dorsal skin permeability assay, TNFf/f MRP-8 Cre+ mice showed reduced vascular permeability in response to intradermal leukotriene B4, but not histamine, confirming a key role for neutrophil-derived TNF in induction of vascular permeability by neutrophil chemoattractants. Furthermore, mice lacking neutrophil TNF were protected from vascular hyperpermeability post induction of ischaemia/reperfusion injury in the mouse cremaster muscle. Collectively, these results support our hypothesis that neutrophil-derived TNF is an important pro-permeability factor that may contribute to pathophysiology characterised by excessive oedema.

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GLOBULAR ADIPONECTIN SIGNALS TO GLOMERULAR ENDOTHELIAL CELLS TO ACTIVATE THE AMP-ACTIVATED PROTEIN KINASE PATHWAY

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Introduction: Adiponectin (Ad), an adipocyte-derived hormone, implements its effects through two transmembrane receptors (AdipoR1 and AdipoR2). It is well established that a marked decrease in Ad levels is characteristic of type 2 diabetes. This decrease has been implicated in the development of albuminuria, indicative of the dysfunction of the renal glomerular filtration barrier and therefore of the cells which make up the barrier: glomerular endothelial cells (GEnC) and podocytes. It has also been shown that Ad increases glucose metabolism through the 5' AMP-activated protein kinase (AMPK) signalling pathway and enhances fatty acid oxidation through phosphorylation of acetyl-CoA carboxylase (ACC). Aim: Our aim is to determine whether Ad acts on GEnC and whether this is disturbed in diabetes. To this end, we performed in vitro studies in well-characterised conditionally immortalised human GEnC line (CiGEnC).

Results: Our data show that AdipoR1 and AdipoR2 were expressed in CiGEnC. We also show that globular adiponectin (gAd) induces phosphorylation and activation of the alpha subunit of AMPK (AMPKa). Consecutively, gAd also stimulates phosphorylation of ACC. Furthermore, full-length adiponectin was shown to increase the trans-endothelial electrical resistance across a CiGEnC monolayer using an electronic cell impedance sensing (ECIS) system, showing that adiponectin enhances endothelial barrier properties. We found that expression levels of AdipoR1 and R2 were decreased after a 24h exposure a high level of glucose (25mM). Discussion: Taken together these initial findings suggest that adiponectin may be important in maintaining the CiGEnC phenotype and that disturbed adiponectin signalling may contribute to glomerular disease in diabetes.
Calnexin (CANX) is an endoplasmic reticulum (ER) membrane chaperone involved in the quality control and trafficking of glycoproteins. We showed that CANX plays a functional role in myelination and that changes in expression of ER chaperones influence cell surface protein stabilization and function in the nervous system [1, 2]. Multiple sclerosis is a chronic, progressive disease characterized by the destruction of central nervous system (CNS) myelin and often the underlying axons. Leukocyte infiltration into the CNS contributes to the disease pathology. Leukocyte crossing requires cascade of physiological changes in brain blood barrier (BBB) endothelial cells and cells of immune system. Here we found that mice lacking the CANX are capable of a number of activated T-cell responses but were highly resistant to the induction of EAE. In contrast brain tissue of MS patients showed increased abundance of CANX. Conditional knockout of CANX from CD4 and CD8 T-cells did not protect mice from developing EAE suggesting that deletion of CANX from T-cells alone is insufficient to account for the protective nature of CNX deletion in murine models of EAE. CRISPR/Cas9-dependent knockout of CANX from bEnd.3 endothelial cells prevented T-cells from binding to endothelial cell. We concluded that there is a requirement of CANX for the regulatory attachment of T-cells to brain endothelial cells that may impact on their ability to cross the BBB.

MAINTAINING L-SELECTIN ON ADOPTIVELY TRANSFERRED T CELLS AS A CANCER IMMUNOTHERAPY STRATEGY

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L-selectin is an adhesion molecule expressed on naïve T cells, and proteolytically shed upon T cell activation. Despite activated cytotoxic T cells being routinely characterised as L-selectin negative, it has been demonstrated that T cells lacking L-selectin are not able to control solid tumour growth in an adoptive transfer model as well as wildtype T cells. We have previously shown that L-selectin is re-expressed on activated T cells after lymph node egress, and that activated T cells expressing non-shedding L-selectin are better able to home to sites of viral infection and clear virus. We therefore propose that T cells with non-shedding L-selectin will be better able to home to tumours, and, furthermore, that maintaining expression of this key adhesion molecule will improve the ability of transferred T cells to extravasate into the tumour stroma. Improving the ability of T cells to infiltrate solid tumours will help to expand the clinical applications of cell-based therapies such as genetically modified Chimeric Antigen Receptor (CAR) T cells, which are currently most often used to treat more accessible leukaemias, lymphomas and myelomas.
CIRCULATING NON-CLASSICAL MONOCYTES ARE PREFERENTIALLY DEPLETED FROM THE CIRCULATION IMMEDIATELY AFTER REPERFUSION IN STEMI PATIENTS AND ARE ASSOCIATED WITH PATIENT OUTCOMES

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Background: In patients with acute myocardial infarction (MI), monocytes are rapidly recruited to the injured tissue, where they contribute to the inflammatory milieu. Different monocyte subsets exhibit distinct roles. Classical monocytes (CD16- CCR2+ CX3CR1-) migrate to sites of injury in response to CCL2 and differentiate into inflammatory macrophage, whilst non-classical monocytes (CD16++ CCR2- CX3CR1+) patrol the endothelium where they potentially interact with fractalkine. The aim of this project was to characterise these circulating monocyte subpopulations dynamics in ST elevation MI patients immediately following reperfusion, and evaluate their prognostic value. Methods: Flow Cytometry was used to quantify monocyte subpopulations in the blood of 50 STEMI patients at the time of primary percutaneous coronary intervention for acute MI and at different times thereafter. Infarct size and microvascular obstruction (MVO) were assessed using cardiac MRI. Results: STEMI patients showed a significant drop in circulating CD16++ monocyte counts at 90 minutes post-reperfusion, whereas the CD16- monocyte counts remained unchanged at this early stage. This rapid decrease in CD16++ monocytes was greater in individuals with a larger infarct size and MVO. Conclusion: This data suggests that CD16++ monocytes during the acute phase post-MI may hold predictive value for myocardial injury and long-term patient outcome. Further work using monocyte cell migration/adhesion studies and a mouse model of MI are being used to investigate the mechanisms that underpin these findings.
SKIN MICROVASCULAR FLOW ASSESSED BY DYNAMIC OPTICAL COHERENCE TOMOGRAPHY: FIRST NON-INVASIVE QUANTITATIVE OUTCOME MEASURE OF MICROVASCULAR DAMAGE IN SYSTEMIC SCLEROSIS

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The aim of our work was to determine the face and content validity of Dynamic Optical Coherence Tomography (D-OCT) as an outcome measure of the skin microvascular disease in Systemic Sclerosis (SSc), assuming the presence of current digital ulcers (DUs) as gold standard for ischemic peripheral vasculopathy. A total of 54 patients were enrolled in this cross-sectional study, including 18 SSc patients with current DUs; 18 SSc patients without current DUs and 18 patients with secondary Raynaud’s phenomenon. For each patient, we performed a D-OCT scan on both index and middle fingers, employing Vivosight Scanner. The speckle variance signal of D-OCT images within the first mm of skin depth was extracted, quantified as area under the curve and defined as Micro Vascular Flow (MVF). The distribution of MVF was not significantly different among the four fingers within each group (DU: p=0.459, no DU: p=0.953 and SRP: p=0.616). On the contrary, the distribution and median MVF for all fingers was significantly different among the 3 groups: DU=0.134(IQR 0.121-0.134), no DU=0.153(IQR 0.132-0.153) and SRP=0.167(IQR 0.148-0.167) (p<0.0001). Sub analysis of the DU group showed that 10/20 DUs were on the left index finger. Within this subgroup the MVF of patients on Sildenafil (n=6) was significantly higher than the rest of the group (0.148±0.021 vs. 0.113±0.019, p=0.03). MVF assessed by D-OCT is a quantitative, non-invasive surrogate outcome measure of severe peripheral ischemic vasculopathy in SSc.
INVESTIGATING THE EFFECT OF AGE ON T CELL TRANS-ENDOTHELIAL MIGRATION

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The ageing of the immune system predisposes adults to chronic inflammatory diseases (CIDs) where inappropriate accumulation of T-cells contributes to pathogenesis. We have characterised a unique regulatory peptide, PEPITEM (PEPptide Inhibitor of Trans-Endothelial Migration) that imposes a tonic inhibition of T-cell trafficking during inflammation. The PEPITEM pathway is impaired in CIDs. Here, we aimed to assess the effect ageing on the PEPITEM pathway by investigating levels of adiponectin receptors (AdipoRs) on B-cells that release PEPITEM under adiponectin stimulation as well as the capacity of adiponectin and PEPITEM to inhibit T-cell transmigration. We quantified surface AdipoRs expression using flow cytometry in a healthy cohort of 20-40 and above 60 years-old. We performed in vitro static migration assays to measure T-cell transmigration in the presence or absence of PEPITEM or adiponectin. We showed that B cells numbers significantly declined with advancing age. We also demonstrated that AdipoRs expression was significantly lower in older men. While lymphocyte total adhesion was unaffected, lymphocyte transmigration was significantly higher in older subjects. Adiponectin significantly inhibited lymphocyte transmigration across all age groups in women but not in older men. PEPITEM retained capacity to inhibit lymphocyte transmigration regardless of age or gender. The data presented here suggests that T-cell trafficking in inflammation changes with advancing age depending on gender. In addition, control of T-cell migration mediated is retained in women but compromised in men, and this can be rescued by treatment with PEPITEM, suggesting an age and gender-related deficit in controlling T-cell trafficking during inflammation.
β-AMYLOID PROMOTES DIABETES-LIKE VASCULAR DYSFUNCTION IN MICE

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Aims: β-amyloid production, via BACE1 activity, results in the formation of amyloid plaques, a hallmark pathology in Alzheimer’s disease. However it is becoming apparent that β-amyloid can also play a role in type 2 diabetes and cardiovascular disease progression. Our aim was to determine whether manipulating the levels of β-amyloid in mice alters vascular function. Methods: Measurements of blood pressure and endothelial function, determined by the vascular response to Acetylcholine using laser Doppler imaging in two separate studies; (i) wild-type (C57Bl/6) mice infused with murine Aβ1-42 or scrambled peptide (ScP) (3.36 µg/kg) for 6 weeks or (ii) diet-induced obese (DIO) C57Bl/6 mice infused with a BACE1 inhibitor (M-3; 10 mg/kg) or vehicle (DMSO/PBS) for 4 weeks. Results: BACEi given to DIO mice improved endothelial function and reduced hypertension. In contrast, β-amyloid per se promoted impaired vascular responses and high blood pressure on regular chow. Increased Endothelin-1 (ET-1) and reduced NO levels promote vascular dysfunction and diabetic retinopathy and nephropathy. In line with our hypothesis, β-amyloid infusion increased, while M-3 treatment of DIO mice reduced, the ET-1/NO ratio. Conclusions: We previously reported that mice lacking BACE1 are protected from diet-induced endothelial dysfunction. We now show that pharmacological inhibition of BACE1 also reverses diet-induced endothelial dysfunction, via reducing serum β-amyloid levels. This suggests that β-amyloid processing has a role in normal vascular function with aberrant processing leading to endothelial dysfunction and hypertension. Accordingly serum β-amyloid could be a novel bio-marker for diabetes-induced vascular disease, with inhibiting BACE1 an innovative treatment.
PLATELET-DERIVED THROMBOSPONDIN-1 MODULATES cAMP SIGNALING TO PROMOTE PLATELET ACTIVATION

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Background: Previously we showed that recombinant thrombospondin-1 (TSP-1) blocks the inhibitory effects of prostacyclin (PGI2) to promote platelet activation. Here we examined the role of endogenous TSP-1 on platelet regulation by PGI2. Methods: Biochemical and functional analyses, including in vivo thrombosis and adoptive transfer protocols, of genetically modified mice were used. Results: Agonist-induced platelet aggregation was consistent WT, CD36-/- and TSP-1-/- platelets. In contrast, PGI2-mediated inhibition of aggregation was greater in TSP-1-/- and CD36-/- platelets, suggesting that TSP-1-/- and CD36-/- platelets were hypersensitive to cAMP signalling. Importantly, intravenous injection of the PGI2 mimetic iloprost prolonged bleeding time in TSP-1-/- but not in WT mice. A plasma crossover approach was used to determine the importance of different pools of TSP-1. Platelet hypersensitivity to cAMP signalling in TSP-1-/- platelets was maintained when they were resuspended in plasma from WT mice. However, incubation of TSP-1-/- platelets, with releasate from WT platelets or purified TSP-1 decreased their sensitivity to PGI2 to that observed with WT platelets. PGI2-induced cAMP levels in WT platelets were significantly lower than those in TSP-1-/- platelets, with those observations PDE3A activity was lower in TSP-1-/- than in WT platelets, enhancing cAMP accumulation. To confirm the role of platelet TSP-1 in vivo we demonstrated that TSP-1-/- mice exhibited defective thrombus formation and longer bleeding times, which was corrected by infusion of WT platelets. Conclusion: Platelet-derived TSP-1 regulates PDE3A activity downstream of CD36 to modulate cAMP signalling to promote platelet activation by reducing sensitivity to PGI2.

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MULTIMERIN-2 IS A LIGAND FOR THREE C-TYPE LECTINS AND SPANS THE ENDOTHELIAL PERICYTE INTERFACE

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Introduction: Multimerin-2 (MMRN2) is an endothelial specific secreted matrix protein involved in angiogenesis. We recently showed that MMRN2 is a ligand for the endothelial specific c-type lectin CLEC14A. CLEC14A is one of the four type14 lectin family that also includes CD93 and thrombomodulin on endothelial cells and CD248/endosialin/TEM1 on pericytes. Despite being first identified 25 years ago, no ligand for CD248 is known. The purpose of this study was to investigate potential interactions between MMRN2 and the other type14 family lectins.

Methods: Using recombinant proteins and antibodies in a combination of far western analysis, FACS and confocal microscopy we have dissected the interaction of MMRN2 with each member of the type14 family c-type lectins. Recombinant proteins have been studied in angiogenesis and tumour assays.

Results: Far western analysis has shown that MMRN2 binds directly to CLEC14A, CD93 and CD248 but not thrombomodulin. By a series of truncations we have shown that CLEC14A and CD93 bind to the same region of MMRN2 that is different to the region that binds CD248. Co-localisation of MMRN2, CLEC14A and CD248 in the vessels was confirmed by immunostaining and confocal microscopy. We have identified the sites of MMRN2 and CLEC14A that bind each other. The binding fragment of MMRN2 is shown to be anti-angiogenic and to inhibit tumour growth.

Conclusion: This work shows that MMRN2 acts to bring the endothelial cell and pericyte together. Breaking this interaction disrupts angiogenesis and inhibits tumour growth.
NON-INVASIVE VISUALISATION OF THE MICROVASCULATURE WITHIN SUBCUTANEOUSLY IMPLANTED TUMOURS USING OPTICAL COHERENCE TOMOGRAPHY (OCT)

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Rationale and Objective: Recently developed processing methodologies have enabled the extraction of depth resolved angiographic data from four-dimensional OCT scans. Furthermore, OCT's unique combination of high-resolution (<10µm) and moderate tissue depth-penetration (~1mm) may allow for visualisation of the superficial microvasculature within subcutaneous (sc) tumours, omitting the need for an optically transparent "window" through skin. This study aimed to assess and validate the suitability of angiographic OCT for the longitudinal study of tumour microcirculation within such models.

Methods and Results: Angiographic OCT volumes of two fibrosarcoma tumour types were acquired directly through the dorsal-skin of immune-deprived mice. Each tumour type expressed a single VEGF isoform (188 or 120) such that morphological differences could be visualised. Layers of the skin were correlated with histology to validate the imaging and vessel detection depth. To compare tumours, quantitative vessel parameters such as vessel diameter, length and position were extracted from each dataset, with significant differences in mean vessel diameter being found between tumours expressing only the VEGF120 isoform (63.8±31µm), VEGF188 (53.0±27µm) and healthy dorsal skin (27.8±12.1µm).

Conclusion: The ability of angiographic OCT to discern differences in vessel diameter between healthy tissue and tumours expressing differing VEGF isoforms suggest that the modality is capable of visualising the superficial vasculature of subcutaneous tumours unlike intravital microscopy. This provides an opportunity to study the tumour microcirculation in an orthotopic, three-dimensional environment.

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PLATELET CLEC-2 PROTECTS AGAINST LUNG INJURY VIA EFFECTS OF ITS LIGAND PODOPLANIN ON INFLAMMATORY ALVEOLAR MACROPHAGES

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No therapeutic interventions have been proven to prevent the acute respiratory syndrome (ARDS). Novel mechanistic insights into the pathophysiology of ARDS are therefore required. Platelets have been implicated in regulating many of the pathogenic processes which occur during ARDS, however the mechanisms remain elusive. The platelet receptor C-type lectin-like 2 (CLEC-2) has been shown to regulate vascular integrity and neutrophil recruitment at sites of acute inflammation. Therefore, the purpose of this study was to establish the role of CLEC-2 and its ligand podoplanin in a mouse model of ARDS. Platelet-specific CLEC-2-deficient, as well as alveolar epithelial type I cell (AECI)-specific- or hematopoietic-specific podoplanin deficient mice were established using cre-loxP strategies. Combining these with intratracheal (IT) instillations of lipopolysaccharide (LPS), we demonstrate that the decline in arterial oxygen saturation in response to IT-LPS in platelet-specific CLEC-2-deficient mice is significantly augmented. An increase in bronchoalveolar lavage (BAL) neutrophils and protein permeability index (PPI) is also observed 48h post IT-LPS, with significant increases in pro-inflammatory chemokines detected in BAL of CLEC-2 deficient animals. Deletion of podoplanin from hematopoietic cells but not AECIs also reduces lung function and increases PPI as well as pro-inflammatory chemokine expression following IT-LPS. Furthermore we demonstrate that following IT-LPS, platelets are present in BAL in aggregates with neutrophils with allows for CLEC-2 interaction with podoplanin expressed on BAL inflammatory alveolar macrophages. The CLEC-2-podoplanin signaling axis therefore regulates the severity of lung injury and is a possible novel target for therapeutic intervention.
Far Infra-Red (FIR) is an invisible part of the infrared spectrum, thought to have beneficial effects on the cardiovascular system. FIR irradiation has been shown to improve microvascular blood flow in animals and to improve arteriovenous fistula patency in dialysis patients, independently of heat. However, the underlying mechanisms remain unclear. Endothelial function is the central regulator of the cardiovascular system, and dysfunction may lead to the development of myocardial infarction, peripheral vascular disease and diabetic complications. A single-blinded randomised study was conducted on 40 normal healthy volunteers with 29 receiving FIR and 11 in the control group receiving no irradiation. Volunteers received three 40-minute sessions of FIR over 4-6 days. The primary outcome measure was iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) with laser speckle contrast imaging (LSCI). Secondary measures were post-occlusive reactive hyperaemia (PORH) with LSCI and two venous blood tests of oxidative stress; derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP). ACh iontophoresis, an endothelium-dependent measure, caused a statistically significant increase in skin microvascular perfusion immediately after one and three FIR sessions, compared to no change in controls, which suggests an acute improvement in microvascular endothelial function. PORH, d-ROMs and BAP tests were unchanged following FIR. Our results suggest that FIR caused an acute improvement in endothelial function, which did not seem to be due to reduced oxidative stress. Further investigation is required to clarify the biological mechanisms of FIR and establish whether it could be a useful treatment for cardiovascular disease.
Cancer cell:endothelial cell interaction is a critical event for metastasis formation, which is the primary cause of cancer patient death. Here, we used an in vitro assay under shear forces mimicking blood flow in vivo, to study cancer cell adhesion to endothelial cells. Using this method, we show that β1 integrin mediate breast (MDAMD231), prostate (PC3) and T cell leukemia (Jurkat) cell firm adhesion to human brain endothelial cells, hCMEC/D3. It has been shown that β1 integrin is regulated by the Rho GTP-ase Cdc42. We found that two direct Cdc42 targets, IQGAP1 and N-WASP regulate β1 integrin total and membrane-associated expression both in breast and prostate cancer cells. These results suggest a new insight in the regulation of β1 integrin by IQGAP1 and N-WASP in breast and prostate cancer cells, thus these two targets may be a new potential therapeutic tool to prevent cancer cell adhesion to endothelium.
Vorinostat (SAHA) is a histone deacetylase (HDAC) inhibitor that is licensed for treatment of recurrent cutaneous T cell lymphoma, and is currently in trial for metastatic breast cancer. Vorinostat has been shown to directly inhibit cancer via multiple mechanisms, including cancer cell apoptosis, and previous data have suggested that they may have an indirect anti-angiogenic effect via inhibition of HIF-1α expression and resultant downregulation of VEGF. This study aimed to test the hypothesis that Vorinostat acts directly to inhibit angiogenesis by altering endothelial cell phenotype. Treatment of HUVECs with increasing concentrations of Vorinostat ranging from 0-10µM resulted in a dose-dependent, significant reduction of proliferation, demonstrated in both MTS and clonogenic assays (p<0.0038, EC50=1µM). Vorinostat also significantly (p<0.0001) inhibited HUVEC migration by 30% and significantly (p<0.002) decreased the number of tubules formed on Matrigel (control=51+/-1.6; 10?M=12+/-1.2) and the number of branch points (control=30+/-1.5; 10?M=6+/-0.8). In contrast Vorinostat significantly increased tubule length (p=0.042) by 25% in the same assay. Cytoskeletal staining suggested that Vorinostat treated HUVECs exhibit a rearrangement of actin filaments that presented as stress fibres. These data suggest that SAHA caused a direct inhibition on the proliferation, tubule forming and migration steps of angiogenesis in HUVECs, although further work is required to validate these data.
PC5

GALECTIN-1 KO MICE EXHIBIT REDUCED DISEASE IN A NEUTROPHIL-DRIVEN MODEL OF ARTHRITIS

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Galectin-1 (Gal-1), an endogenous ß-galactoside binding lectin, has been attributed with numerous immunomodulatory properties. Specifically shown to influence apoptosis, cytokine secretion, adhesion and leukocyte trafficking, Gal-1 also has protective effects in several models of auto-immune disease. In models such as collagen-induced arthritis and experimental autoimmune encephalomyelitis the effects of Gal-1 are largely due to its influences on pathogenic T cell subsets. Although shown to limit neutrophil trafficking and induce apoptosis of synovial fluid neutrophils, the impact of Gal-1 on neutrophil-driven pathologies remains less studied. We have therefore chosen to investigate the role of endogenous Gal-1 in the KBxN serum transfer model of rheumatoid arthritis, the development of which is neutrophil-dependent. Arthritis was induced in WT (C57/Bl6) and Gal-1 KO mice by intraperitoneal injection of arthritogenic serum. The disease profile was assessed by weight loss, paw oedema and clinical scoring of inflammation. Gal-1 KO mice developed arthritis at a slower rate, taking longer to reach the peak of their disease, as evidenced by clinical score, weight loss and oedema. WT mice lost significantly more weight and had significantly increased oedema compared to their knockout counterparts. These findings indicate a divergence in the roles of Gal-1 in neutrophil-driven pathologies compared to T cell-driven models, such as collagen-induced arthritis, where Gal-1 KO mice exhibit increased disease severity. Collectively this suggests the role of Gal-1 in inflammatory pathologies might be dependent on the specific mediator(s) responsible for disease pathogenesis and the dominance of different cell types over the course of the inflammatory response.
BONE MARROW ENDOTHELIAL CELLS AND BREAST CANCER CELLS ADHESIVE INTERACTIONS MODELLED USING PREDICTIVE MECHANO-BIOLOGICAL MODELS

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In this study, we developed a mechano-biological model combined with experimental studies to characterize and predict adhesive interactions between human bone marrow endothelial cells (HBME) and human breast cancer cells (MB435). Atomic force microscopy (AFM) was used to measure adhesion forces occurring between a single MB435 cell (attached to the AFM tip) and a monolayer of HBMEC-60 cells. Adhesion was characterized for differing times of contact. A computational 2D discrete stochastic-elastic model was developed of the adhesive interactions to simulate AFM experiments. Using the model, detailed quantification of binding dynamics, location, lifetime, and strength of cell-cell adhesions were determined. Cell-cell interactions in our model are described by considering intracellular actin in each cell and adhesion proteins in the cell membranes as: freely diffusing, actin-bound to the first cell, actin-bound to the second cell and fully-bound to cytoskeleton of both cells. Adhesions between the cytoskeletons of cells are connected to form a dynamic network of springs, which obey stochastic binding/unbinding events. Free adhesion molecules diffuse randomly and the dynamically changing network structure is coupled to the local mechanical environment through force-dependent unbinding propensities. Experimentally, we found that peak adhesion forces increased with increasing contact time between MB435 and HBMEC-60 cells and that total adhesion force increased dramatically initially and then plateaued. Additionally, we found that our stochastic computational model recapitulated these experimental findings, suggesting that important mechanisms have been captured in the model thus, validating our combination of an experiment and modelling approach.
ADHESIVE INTERACTIONS BETWEEN BREAST CARCINOMA CELLS TO BONE MARROW ENDOTHELIAL CELLS STUDIED BY ATOMIC FORCE MICROSCOPY

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In this study cell-cell force spectroscopy performed with atomic force microscopy (AFM) was used to characterize adhesion between human breast cancer cells (MDA-MB-231) and human bone marrow endothelial cells (HBME-60). A single MDA-MB-231 cell was attached to the AFM cantilever tip and brought in contact with HBME for different contact times then retracted. Force required to separate MDA-MB-231 and HBME increased progressively as cell-cell contact time increased from 0.5 to 300 sec. Specific adhesion receptors involved were investigated by pre-incubation of HBME with function blocking antibodies against integrin b1, integrin a3, galectin-3 or Thomsen-Friedenreich-antigen (TF-Ag). Adhesion force between MDA-MB-231 and HBME-60 was measured at 0.5, 30 and 60-sec of contact before and after antibody treatment. Results showed increased involvement of b1 integrin and galectin-3 with increasing contact time but not TF-AG or integrin a3. As a positive control, adhesion significantly increased after application of integrin β1 function-activating antibody. In summary: 1. Increasing contact time leads to enhanced adhesion between MDA-MB-231 and HBME; 2. The increased adhesion over time appears mediated, in part, by integrin b1 and galectin-3. Adhesion characterization using cell-cell force spectroscopy provides a useful method to investigate the temporal aspects of adhesion between HBME and MDA-MB-231.
ROBUST MEASUREMENTS OF SUBCLINICAL INFLAMMATION IN SKIN AFFECTED BY ATOPIC DERMATITIS USING ANGIOGRAPHIC OPTICAL COHERENCE TOMOGRAPHY

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Background and Aim: With inflammatory skin conditions such as atopic dermatitis (AD), epidermal thickening is likely to influence both the depth and shape of the underlying microcirculation in the skin. Optical coherence tomography (OCT) provides a non-invasive view into the tissue, however structural measures of epidermal hyperplasia (thickening) are made challenging due to the lack of a delineated dermal-epidermal junction in AD patients. Alternatively, angiographic extensions to OCT may facilitate direct measurement of vascular depth, potentially presenting a more robust method of estimating the degree of subclinical inflammation. Methods and Results: To investigate microcirculatory changes within AD patients, volumes of angiographic OCT data were collected from 5 healthy volunteers and compared to that of 5 AD patients (Mean eczema area and severity index of 8.5±3.3). Test sites included the cubital and popliteal fossa, which are commonly affected by AD. Quantitative parameters such as capillary loop depth and superficial vascular plexus depth were derived from each dataset and compared between groups. Capillary loops were significantly (p=0.003) deeper in AD patients (80.5±19.9µm) compared to healthy controls (59.9±18.2µm). Similarly, the superficial vascular plexus is deeper in AD (199.6±60.5µm) than healthy patients (108.2±19.27µm), likely because of subclinical inflammation. Conclusions: Quantifying subtle changes within vascular morphology and depth may give clinicians an indication of subclinical disease progression and aid in evaluating the efficacy of treatments.

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Mesenchymal stem cells (MSC) may be used therapeutically via injection into the blood, where their adhesive properties and interactions with other blood cells may influence their fate. We reported previously that MSC isolated from human bone marrow (BMMSC) or umbilical cord (UCMSC) were both able to adhere from flow to matrix proteins but had distinct interactions with platelets in blood, with only UCMSC causing platelet aggregation and a marked decrease in platelet count if infused into mice. Upon further investigation, we found that UCMSC, but not BMMSC, expressed podoplanin (PDPN) which is a ligand for the receptor CLEC-2 on platelets. Levels of expression of PDPN varied between UCMSC donors; most yielded podoplanin-positive cells, but cells from some donors lacked expression. Only the PDPN+UCMSC were able to aggregate with platelets and caused a drop in platelet count when mixed with blood in vitro. When recombinant CLEC-2 was added to blood, there was significant inhibition of aggregation induced by the PDPN+ UCMSC. Human UCMSC caused reduction of platelet count when mixed with mouse blood, but the response was lost with blood taken from mice lacking expression of CLEC-2. PDPN- USMC caused a lesser effect on platelet count when infused into mice, but responses when PDPN+ cells were infused into the mice lacking CLEC-2 were variable. Thus, the origins of MSC and their expression of PDPN may have impact on their behaviour in the blood. During therapy, interactions with platelets could be thrombotic, but might also promote targeting to damaged tissue.
Metastasis to bone is a significant problem for breast cancer patients because such metastases are difficult to detect until clinical symptoms arise. Metastasis to bone rapidly results in debilitating pain and bone destruction, for which there are very few effective treatments. Disseminated tumour cells (DTCs) home to three overlapping niches within the metaphysial region of bone called the vascular, stem cell and endosteal niches. The first of these is characterized by the recently identified H-type capillary, around which the DTCs reside. These bone metastatic niches comprise many cell types including endothelial cells, stem cells, osteoblasts, osteoclasts, and such inflammatory cells as macrophages, all embedded in extracellular matrix. Homeostasis in the bone microenvironment is co-ordinated by complex networks of soluble factors and mechanical forces that arise from cell-cell and cell-matrix interactions. A growing canon of evidence demonstrates that these homeostatic processes can regulate the dormancy and outgrowth of DTCs. Consequently, there is an urgent need for additional mechanistic studies that further elucidate mechanisms regulating DTC dormancy and reactivation, to improve therapeutic strategies for metastatic breast cancer patients. Here, we use confocal microscopy, flow cytometry, qRT-PCR and proteomic methods, to examine the effects of conventional anti-cancer therapies on the cellular composition of the bone microenvironment; the vascular, endosteal and stem cell niches and the homeostatic processes linked with cancer cell dormancy and outgrowth. Insights gained from these studies will help to prevent either colonization of the bone microenvironment by DTCs or prevent reactivation of dormant cells.

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Psoriasis is a common chronic skin inflammation affecting 2-3% of the UK population. Using the Aldara™-induced model of psoriasis, we aimed to elucidate the role of small diameter sensory nerves in driving the erythema and skin inflammation, focusing on the roles of TRPV1 and a-CGRP. In vivo procedures were carried out in accordance to the UK Home Office Animals (Scientific Procedures) Act 1986. Male WT C57BL/6J, TRPV1 KO, or a-CGRP KO (n=5-7 per group, 6-8 weeks) mice were anaesthetised under 2% isoflurane during procedures. Following dorsal skin hair removal, 75mg of Aldara™ (5% imiquimod) cream (Meda Pharma, UK) or Vaseline (control) was applied over a 2x2cm² area daily for 4 days. On the last day, daily Aldara treatment significantly increased cutaneous blood flow, as measured by the Full Field Laser Perfusion Imaging technique, consistent with increased skin inflammation and other skin pathological changes, including increased skin thickness and epidermal thickness. Sensory-denervated mice (0.3mg/kg resiniferatoxin (RTX) daily s.c for 4 days) showed significantly attenuated erythema and blood flow, associated with reduced skin inflammation. A significant reduction in TRPV1 and a-CGRP mRNA expression in the dorsal root ganglion (DRG) was observed following denervation. Hence, to confirm their potential roles in vivo, global TRPV1 KO and a-CGRP KO were used. Interestingly, all aspects of in vivo and ex vivo parameters of Aldara™-induced skin changes, including increased blood flow, were not attenuated in either KO. We conclude that the beneficial effects of sensory denervation are independent of TRPV1 and a-CGRP expression.
THE ROLE OF FRACTALKINE AND CX3CR1-EXPRESSING LYMPHOCYTES DURING MYOCARDIAL ISCHAEMIA/REPERFUSION INJURY

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Background: Primary percutaneous coronary intervention (PPCI) is the standard care for treatment of acute myocardial infarction (MI), reducing both mortality and morbidity. However, ischaemia/reperfusion (I/R) injury remains an important complication, contributing up to 50% of the final infarct size. Lymphocytes expressing the fractalkine receptor (CX3CR1) have been proposed to be key mediators of I/R pathogenesis. This project aims to investigate the role of fractalkine in lymphocyte-mediated myocardial I/R injury and evaluate whether this damage can be reduced by inhibiting fractalkine/receptor (CX3CL1/CX3CR1) interaction.

Methods: We used a mouse model of myocardial I/R injury to study lymphocyte infiltration following MI. Flow cytometry, immunofluorescence staining and qPCR were used to evaluate the recruitment of CX3CR1-expressing lymphocytes.

Results: Similar to MI patients who underwent PPCI, our mouse model showed marked lymphopenia following myocardial I/R. This drop in circulating lymphocytes may be the result of the immune cells infiltrating the myocardium. This is supported by our data showing endothelial fractalkine expression, and recruitment of CX3CR1 expressing lymphocytes in murine hearts immediately following reperfusion. Analysis of tissue immunostaining showed that 75% of recruited CD3+ lymphocytes at 2h post reperfusion expressed CX3CR1. Migration of CX3CR1 expressing cells from the circulation into the I/R injured heart is further supported by qPCR data showing dynamic changes in myocardial CX3CR1 gene expression immediately following reperfusion.

Conclusion: These results support the hypothesis that lymphocytes expressing the fractalkine receptor have a key role in myocardial I/R injury and their functional role is currently being evaluated using CX3CR1 knockout mice.
INVESTIGATING THE NITRIC OXIDE-INDEPENDENT ROLE OF ALPHACGRP IN L-NAME-INDUCED HYPERTENSION.

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AlphaCGRP (aCGRP) is a potent vasodilator and has recently been shown to play a protective role in murine models of hypertension. We are investigating the mechanisms involved. aCGRP acts via nitric oxide-dependent and -independent mechanisms. We have utilized an L-NAME-induced experimental hypertension model. The systolic blood pressure (SBP) of age-matched male C57BL/6J wild type (WT) and aCGRP knock-out (KO) mice were measured. Groups were randomly assigned to receive L-NAME (1mg/ml) water or tap water for 6 weeks. Furthermore, a full-field laser perfusion imager was used to assess the vascular properties of the peripheral microcirculation after L-NAME treatment. Data is given as mean ± SEM and analysis was performed using 2-way or repeated measures ANOVA followed by Bonferroni post hoc test. All groups showed normotensive SBP at baseline, which the vehicle-treated mice maintained for 6 weeks. L-NAME treatment induced hypertension in WT mice (130.27 ± 3.80 mmHg), which was statistically significant from vehicle-treated WT mice (p<0.001). aCGRP KO mice developed an increase in SBP (149.5 ± 5.29 mmHg), after only one week of receiving L-NAME, which was significantly different (p<0.001) to its WT counterpart. The results indicate that deletion of aCGRP further enhances the hypertension that results from blockade of endogenous nitric oxide, suggesting that aCGRP can mediate at least some of its anti-hypertensive effects independently of nitric oxide. However, the peripheral blood flow measured so far, was not markedly affected in the mice, despite aCGRP being a potent peripheral vasodilator.

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INTERACTIONS OF NEUROPILIN-1 AND VEGF ISOFORMS IN DEVELOPMENT OF VASCULAR-TARGETED TREATMENT STRATEGIES

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Vascular endothelial growth factor-A (VEGF) is a major target of FDA-approved anti-angiogenic therapies in soft tissue sarcoma (STS) and other cancers. Although these drugs have changed the outlook for many STS patients, response is variable and therefore, validated biomarkers are required to facilitate stratified cancer care. Recent clinical studies identified VEGF co-receptor neuropilin-1 (NRP1) and VEGF isoforms as potential biomarkers of response to anti-VEGF agents but the mechanisms involved remain elusive. Using mouse fibrosarcoma (fs) cells expressing single VEGF isoforms (fswt expressing all isoforms and fs120, fs164 and fs188 expressing VEGF120, VEGF164 or VEGF188 respectively), no functional VEGFR2 and different levels of NRP1, we developed a co-culture model with fs and human endothelial cells to study VEGF mediated 'juxtacrine' endothelial VEGFR2 activation. Endothelial VEGFR2 phosphorylation was strongest and most sustained when co-cultured with fs188 cells, correlating with higher NRP1 expression in fs188 cells than fs120/fs164 cells. NRP1 was successfully knocked down by ~80% using siRNA in fswt and fs188 cells, which led to a reduction of ~25% in fs cell migration and a tendency for increased adhesion to matrix proteins. Results suggest a central role for NRP1 in fs cell adhesion and migration. Dependence of endothelial VEGFR2 activation on NRP1 levels in adjacent fs cells suggests a role in tumour vascularisation. Therefore, it can be hypothesised that NRP1 expression is integral to the differing metastatic rates of the fs tumours in vivo. Elucidating the mechanisms involved will enable evaluation of NRP1/VEGF isoforms as predictive biomarkers of anti-VEGF therapies.
Phospholipase D (PLD) regulates biological functions including membrane trafficking and exocytosis via catalysis of membrane phosphatidyl choline to the signalling molecule phosphatidic acid. Its activity is implicated in the pathophysiology of disease from Parkinsons disease to cancer leading to the development of small molecule inhibitors. von Willebrand Factor (vWF) secretion from endothelial cells has been shown to be regulated by the PLD 1 isoform and because vWF is stored with the adhesion molecule P-selectin, a common PLD dependent mechanism for leukocyte recruitment was hypothesised. P-selectin is transiently expressed on acutely activated endothelial cells and captures flowing leukocytes requiring both exocytotic and endocytotic mechanisms in regulating its expression. Using isolated human endothelial cells, we investigated the constitutive and activated release of vWF and P-selectin expression via ELISA and video-microscopy of recruited neutrophils respectively. Histamine caused vWF release and P-selectin dependent neutrophil recruitment in a PLD 1 dependent manner. Time course analysis of neutrophil recruitment suggested a significant loss of P-selectin expression from the surface of endothelial cells via endocytosis by 30minutes post histamine stimulation. In histamine activated endothelial cells, this downregulation was prevented by both PLD 1 and PLD 2 specific inhibitors. This suggests that P-selectin is both expressed and removed from the endothelial cell surface in a PLD dependent manner. Regulation of PLD in acutely activated endothelium could therefore be used to reduce coagulation and leukocyte recruitment. Conversely, prolonging P-selectin expression may allow for tumour targeted immune cell recruitment and ultimately, their clearance.
OCCLUDIN SPLICE VARIANTS IN THE HUMAN PLACENTAL VASCULAR BED

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Occludin (OCLN) is a transmembrane tight junctional protein which contains two extracellular loops required for stable adhesion and a cytoplasmic tail that allows signaling. So far, NCBI database reports three mRNA OCLN splice variants. Variants 1 and 2 translate into a fully functional isoform A (60 kDa; full length), while variant 3 translates into a truncated isoform B (30 kDa; cytoplasmic tail only) which cannot translocate to tight junctions. Our aim was to determine if these splice variants are present in the human placenta. RNA and proteins were isolated from placental chorionic villous tree biopsies taken at full term from uncomplicated (9) pregnancies, after informed consent. Q-PCR amplification and immunoblotting were used to determine gene expression of OCLN splice variants and protein isoforms. All three OCLN splice variants were found in the term human placenta, with OCLN variant 2 showing the largest percentage of expression. Additionally, the protein expression of isoform A was 2.3-fold greater than isoform B (p < 0.05). This study is the first to show that OCLN variant 2 is the predominant splice variant in the human placenta. These results pave the way into investigations as to whether altered expression of these OCLN splice variants, with their resultant protein isoforms, may underlie endothelial tight junctional perturbations. Keywords: Occludin, splice variants, human placenta.
TETRASPANIN TSPAN18 PROMOTES HAEMOSTASIS AND THROMBO-INFLAMMATION THROUGH REGULATION OF ORAI1-DEPENDENT STORE-OPERATED CA2+ ENTRY

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The induced release of von Willebrand factor (vWF) endothelium is important for haemostasis, but also promotes thrombo-inflammation which can initiate deep vein thrombosis. The mechanism of induced vWF release is not fully understood, but is dependent on intracellular Ca2+ signalling. The tetraspanins are a superfamily of 33 four-transmembrane proteins which interact with specific 'partner proteins', and regulate their trafficking to the cell surface, clustering and lateral diffusion in the plasma membrane. The aim of this study was to perform the first characterisation of human and mouse tetraspanin Tspan18. Tspan18 was expressed at relatively high levels by endothelial cells, but also by platelets and T-lymphocytes. Tspan18-deficient mice had defective haemostasis due to the loss of Tspan18 from non-haematopoietic cells. The mice were protected from deep vein thrombosis, and histamine-induced release of vWF from endothelium was impaired. In model cell lines, over-expression of Tspan18, but not other tetraspanins, promoted Ca2+ signalling, and Tspan18 specifically interacted with the store-operated Ca2+ entry channel Orai1. Tspan18 in human umbilical vein endothelial cells was essential for Orai1 trafficking to the plasma membrane and Ca2+ signalling, and subsequent vWF release and platelet adhesion. Finally, a rare human Tspan18 M241V sequence alteration variant, found in two heterozygote patients with bleeding disorders, did not activate Ca2+ signalling or interact with Orai1. In summary, Tspan18 is a novel regulator of store-operated Ca2+ entry on endothelial cells by interacting with Orai1 and promoting its trafficking to the cell surface. Tspan18 may be important in human bleeding disorders and thrombo-inflammation.
During metastasis, cancer cells adhere to, and traverse endothelial barriers. The mechanism of tumour cell migration is similar to that used by leucocytes during inflammation and the cell surface adhesion molecule CD99 is known to play a role in this process. We have investigated the role of CD99 in the adhesion and transendothelial migration (TEM) of metastatic breast cancer cells. Data mining revealed that CD99 expression was associated with metastasis in various types of breast cancer and we have performed functional assays to investigate the role of CD99 in breast cancer TEM. Inhibition of CD99 expression in MDA-MB-231 breast cancer cells using RNAi, or blocking CD99 with antibodies, resulted in decreased adhesion to HUVEC endothelial monolayers. However, neither endothelial nor breast cancer expressed CD99 was required for TEM. Live cell imaging indicated that CD99 knockdown in the endothelial monolayer reduces the ability of MDA-MB-231 cells to disrupt endothelial monolayers and undergo cell spreading. Furthermore, co-culture of endothelial monolayers and MDA-MB-231 resulted in decreased EC CD99 surface expression. This indicates that surface CD99 may be internalized or degraded by metastatic breast cancer during cancer cell-endothelial cell interactions. This study suggests a novel role for CD99 in the adhesion of cancer cells to the endothelium and future work will explore whether other cancer types utilise CD99 in a similar manner.
CHARACTERISATION OF HUVEC EXPOSED TO PROLONGED SHEAR STRESS

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Studying endothelial cells (EC) in vitro has yielded important insights into EC activation and leukocyte recruitment. Many experiments employ EC cultured under static conditions. However, EC experience shear stress in vivo. Culture under shear stress for =24h has been shown to modulate expression of cell adhesion molecules involved in leukocyte adhesion and migration, including ICAM-1 and VCAM-1. The effect of prolonged culture under shear stress has been less well characterised. We aimed to establish the optimal time for EC exposure to arteriolar shear stress prior to experimentation, and examine the response of these EC to TNF-a. HUVEC were cultured under unidirectional laminar flow at a shear stress of 12dyn/cm2 for 24h or 72h, followed by treatment with 100U/ml TNF-a for 4h. EC were stained for f-actin and VE-cadherin, and morphology assessed using fluorescence microscopy. ICAM-1 and VCAM-1 surface expression was measured by flow cytometry. EC cultured at 12dyn/cm2 demonstrated alignment and f-actin organisation in the direction of flow after 72h but this was not readily apparent after 24h, suggesting that prolonged culture under shear stress induced further phenotypic changes. TNF-a increased ICAM-1 and VCAM-1 expression following static culture or 24h at 12dyn/cm2. TNF-a-induced increase in VCAM-1 expression was suppressed following 24h culture under shear, although no differences were observed in ICAM-1 expression between static and shear conditions. These data suggest that prolonged EC exposure to shear stress promotes further changes in phenotype, as despite suppressing VCAM-1 expression in response to TNF-a, 24h preconditioning was insufficient to induce EC alignment.
SIZE MATTERS - THE DEVELOPMENT AND TESTING OF THE PRIOR HYPOTHESIS

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Fluid balance and exchange in the body are driven by the balance of two opposing pressures, namely the blood pressure and the membrane osmotic pressure (MOP). The Prior Hypothesis suggests that the drop in blood pressure, from the arterial to the venous end of the capillary, matches the drop in MOP ensuring fluid balance. Fluid exchange is driven by the pulsing of the blood pressure. In order to test this hypothesis, we have measured the MOPs of the major components of human plasma at mid point plasma levels, across membranes with pore sizes of 1, 3, 10, 30, 50 and 100kDa. Summing the results enabled plasma MOP/ pore size plots to be drawn. Interpolation of these plots with plasma MOP’s, enables estimation of pore sizes. This technique has enabled us to estimate pore sizes at the arterial, mid-point and venous end of human capillaries, and the changes in permeability between lying to standing. Clinically, the same technique has enabled calculation of pore size during GI surgery, cardiac bypass and in pulmonary oedema patients. Changes in the latter were consistent with changes in the plasma levels of inflammatory mediators. Measurement of concurrent mean arterial pressure (MAP) and plasma MOP gives a simple diagnostic tool for heart failure, hypertension and low and high osmotic pressure shock. Interpolation of MOP/pore size plots enables estimation of capillary pore size. In the human body, pore size matters.
TIFY – A PREDICTED-QUALITY BASED FRAME REJECTION AND REGISTRATION TOOL FOR IMPROVING THE STABILITY OF CARDIAC INTRAVITAL MICROSCOPY OUTPUT

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An ability to image the microcirculation, and the complex intercellular relationships that occur within it, is critical to our understanding of microvascular dysfunction. Intravital microscopy (IVM) is an effective tool to allow visualisation of the microvascular bed within a wide variety of organs. Some tissues, due to either their anatomical location or normal physiological function, are in a state of constant motion. Although physical stabilising techniques exist, these often do not restrict all movement in a field-of-view resulting in out-of-focus frames and blurring. To assist in processing such images, we have developed a tool – named Tify – to help remove poor quality frames. Users manually provide Tify with image scores for a handful of frames (~20), and the application uses linear regression based techniques (specifically, the correlation coefficients) to calculate predicted quality scores for the remainder of the frames. We have been able to show that Tify is able to predict, with accuracy, the quality score that a user would assign to any frame from a given image stack. Furthermore, the accuracy increases with the number of manually scored frames. Interestingly, coefficients can be re-used between similar captures with no loss of accuracy. To retain some degree of temporal correlation between frames, Tify is able to perform frame windowing – retaining only the highest scoring frame within a rolling time window. In the case of cardiac IVM, we are generally able to apply windows as small as 250ms. Tify may be a useful tool for processing biomedical imaging.

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THE EFFECT OF HYPERGLYCAEMIC ENDOTHELIAL CELL-DERIVED EXTRACELLULAR VESICLES ON MONOCYTE FUNCTION

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Background: Diabetes increases the risk of cardiovascular disease through an increased atherosclerotic burden, however, mechanisms behind this are poorly understood. Recent work implicates extracellular vesicles (EV) in cell-to-cell communication and EVs have been implicated in diabetes. Here, we show that endothelial cell derived EV, under conditions of hyperglycaemia and inflammation, enhance monocyte responses, namely static adhesion and chemotaxis/chemokinesis.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured under euglycaemic (5mM) or hyperglycaemic (20mM) conditions +/- inflammation (TNF-a) for 16 hours. Endothelial cell derived EV (HUVEC-EV) were harvested using differential ultra-centrifugation and concentration/number were determined by Nanoparticle Tracking Analysis; typical EV markers were confirmed by western blot (TSG101, ALIX, CD9, HSP70). EVs were exposed to THP-1 monocytes and cell migration, static adhesion and atherogenic foam cell formation was assessed.

Results: HUVEC-EV significantly increased under co-stimulation conditions of hyperglycaemia + TNF-a (P<0.01); exposure of co-stimulated HUVEC-EV significantly enhanced monocyte chemokinesis and chemotaxis (P<0.01) and adhesion to activated endothelium (P<0.01), (compared to control conditions) but did not alter atherogenic foam cell formation.

Conclusion: We show exposure of EV to monocytes increase their ability to adhere to the endothelium and enhances cell migration, elucidating a possible mechanism for increased atherosclerotic burden in diabetes. Further work is needed to explore how EVs exert these effects.
Exploring Pro-Inflammatory HMGB-1 Release From Platelets

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Released HMGB-1 acts as damage-associated molecular pattern. HMGB-1 can be passively released from necrotic cells or actively secreted. Platelet-derived HMGB-1 can act as a pro-inflammatory signal, and exacerbate thrombosis. However, the mechanism of HMGB-1 release from platelets is unclear. In this study, we investigated HMGB-1 surface exposure in activated platelets. Washed platelets were stimulated with ADP, U46619, thrombin, cross-linked collagen-related peptide (CRP-XL), thrombin-plus-CRP-XL, or the calcium ionophore, A23187. Platelets were co-stained against HMGB-1, CD62P (a marker of a-granule secretion) and CD41, and analysed by flow cytometry. Platelet stimulation triggered HMGB-1 surface exposure, a-granule secretion and micro-particle release in an activator-dependent manner. The relatively weak activators, ADP or U46619, didn't trigger a detectable increase in HMGB-1 surface exposure. Under these conditions, ADP and U46619 triggered weak a-granule secretion or little micro particle release. Thrombin, Thrombin-plus-CRP-XL and A23187 all induced a greater increase in HMGB-1 surface exposure, while CRP-XL did not. However, all of these activators triggered increased maximal surface CD62P, indicating that HMGB-1 release does not correlate with a-granule secretion. Intriguingly, under stimulation conditions that led to substantial micro-particle release, HMGB-1 appeared to be restricted to the platelets and excluded from the micro-particle surface. This contrasts with CD62P, which was found on surface of platelets and micro-particles. In summary, strong platelet activation leads to increased HMGB-1 surface expression. However, the mechanism of this does not directly correlate with platelet a-granule secretion. The apparent exclusion of HMGB-1 from micro particles suggests that its surface exposure is regulated in a complex manner.
VOLUNTARY WHEEL RUNNING EXERCISE EFFECTS ON FAST AND SLOW TWITCH MUSCLES: CAPILLARITY AND FIBRE TYPE COMPOSITION CHANGES IN RAT

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Skeletal muscle plasticity may expand aerobic capacity by either increasing capillary supply or altering fibre type composition. In addition, fibre size changes may influence intramuscular diffusion distances thereby affecting efficiency of oxygen delivery / metabolite removal. The aim of this study was to determine the effect of training volume on gross and regional changes in phenotype of fast extensor digitorum longus (EDL) and slow soleus (Sol) skeletal muscles. Voluntary wheel running offers minimal stress on exercising animals. Wistar rats (n=9), 360 - 397g were divided into three groups: running wheel (RW), RW with fructose supplement (RWF) and control (C). EDL and Sol muscles were cryosectioned and immunohistochemistry used to quantify capillary supply, fibre type and mean fibre area. Exercise alone (RW) stimulated a higher capillary to fibre ratio (C:F) relative to control (1.95±0.07 vs. 1.48±0.05, P<0.05), but increased exercise volume (RWF) produced no further increase (1.97±0.10, n.s.). In contrast, RWF produced an increased capillary density (CD) relative to both RW and C (956±198 vs. 784±7 and 718±99 mm$^{-2}$, respectively, P<0.05). CD was significantly higher in the glycolytic region of RW compared to C (750±84 vs. 609±38 mm$^{-2}$, P<0.05), along with greater Type IIa numerical density (0.35±0.04 vs. 0.10±0.05, P<0.05). It is proposed that exercise-induced angiogenesis and muscle fibre transformation are region specific, and that the response is determined by initial muscle fibre type composition.

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REGULATION OF PLATELET ACTIVATION AND THROMBOSIS BY THE KINASE-PHOSPHATASE PAIR CSK-CD148

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Platelets contain high levels of Src family kinases (SFKs) that are essential for transmitting activation signals from a variety of receptors, including immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors, integrins and G protein-coupled receptors. However, it remains unclear how SFKs are regulated in platelets. In this study, we investigate the role of the kinase-phosphatase pair C-terminal Src kinase (Csk) and CD148 in regulating platelet SFK activity in thrombosis and haemostasis. Csk and CD148 conditional double-knockout (DKO) mice were generated by crossing Csk- and CD148-floxed mice with Pf4-Cre transgenic mice and analysed for platelet functional defects. Deletion of Csk-CD148 in the megakaryocyte lineage in mice resulted in a 4-fold increase in platelet SFK activity, but a paradoxical reduction in platelet reactivity to collagen due to down-regulation of the ITAM-containing collagen receptor complex GPVI-FcR ?-chain. Interestingly, the immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B and Csk-homologous kinase (Chk) were concomitantly up-regulated, contributing to the reduction in platelet reactivity. In vitro thrombus formation under flow on a collagen-coated surface was markedly impaired; however, tissue factor-mediated thrombus formation was normal. As a consequence, DKO mice exhibited increased bleeding in the tail bleeding assay and thrombus instability following laser injury of arterioles in the cremaster muscle. Findings from this study establish the kinase-phosphatase pair Csk-CD148 as a critical regulator of platelet SFK activity and reveal novel cell-intrinsic negative feedback mechanisms that prevent pathological thrombosis from occurring.

This work was funded by the British Heart Foundation.
Ageing is universally accepted as the primary risk factor for inflammatory disorders. Whilst many studies have investigated the impact of age on immune cell functions, less is known about the impact of age on vascular responses. The present work aimed to investigate the impact of ageing, and more specifically aged ECs, on neutrophil-microvessel wall interactions. Analysis of leukocyte trafficking in mouse cremaster muscles by intravital microscopy (IVM) revealed increased leukocyte rolling and adhesion in IL-1ß-stimulated tissues in aged mice (>16 months) as compared to young (2-4 months). To specifically investigate the impact of aged ECs in these responses, chimeric mice exhibiting young myeloid cells but aged vasculature were generated through bone-marrow transfer. For these studies, donor animals were young LysM-EGFP-ki mice with recipients either young wild-type (WT) C57BL/6 mice (controls; termed young chimeric mice) or aged WT C57BL/6 mice (termed aged chimeric mice). Analysis of these mice by IVM demonstrated similar responses between young and aged WT mice as compared to young chimeric and aged chimeric mice, respectively. Collectively these results suggest that aged ECs are a key factor in promoting elevated leukocyte-vessel wall interactions. Furthermore, analysis of the chimeric mice by confocal IVM demonstrated aberrant modes of neutrophil-vessel wall interactions, including enhanced neutrophil reverse transendothelial cell migration in the aged chimeric animals. Collectively, we have obtained evidence for the ability of aged ECs to support exaggerated/aberrant neutrophil-vessel wall interactions, responses that could contribute to elevated pathological inflammation noted with age.

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Albuminuria, a hallmark of diabetes, is caused by increased glomerular permeability. In culture vascular endothelial growth factor (VEGF)-C decreases glomerular endothelial cell (GEnC) protein passage. Therefore, VEGF-C was investigated as an anti-albuminuric agent. VEGF-C was tested under the following conditions 1) raised VEGF-A, 2) glomerular endothelial glycocalyx (E-GLX) dysfunction and 3) diabetes. Glomeruli were isolated from: 1) healthy mice (FVB/mixed) and stimulated ex vivo with VEGF-C (10nM) and/or VEGF-A (1nM); 2) healthy mice given chondroitinase (0.087μg/g) and hyaluronidase (0.015μg/g) i.v 30min before sacrifice or via osmotic minipump for 2wk, before being treated with VEGF-C ex vivo (acute) or via i.p. injections in the final week (chronic); and 3) Type-I diabetic (STZ) podocyte-specific inducible VEGF-C (podVEGFC) mice or Type II Diabetic (db/db) mice stimulated with VEGF-C ex vivo. Urine albumin creatinine ratios (uACR) and glomerular albumin permeability (Ps'alb) were determined. 1) VEGF-C significantly ameliorated the VEGF-A induced increase in Ps'alb (p<0.001). 2) VEGF-C significantly ameliorated Ps'alb induced by acute and chronic E-GLX dysfunction (p<0.01). 3) Overexpression of podocyte VEGF-C prevented and reversed albuminuria in Type I diabetic mice (p<0.05) and VEGF-C reduced Ps'alb in glomeruli isolated from Type II diabetic mice (p<0.01). These results demonstrate that VEGF-C can reduce an increase in glomerular permeability caused by excess VEGF-A, removal of the E-GLX and Type II diabetes. VEGF-C also directly ameliorates albuminuria in a model of Type I diabetes. This evidence highlights VEGF-C as a promising anti-albuminuric agent.
EFFECTS OF VEGF-A165B AND VEGF-A165A ON VASCULAR LEAKAGE IN THE EXTRA-CORPOREALLY PERFUSED HUMAN PLACENTA FROM NORMAL FULL TERM PREGNANCIES

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The human placenta expresses the pro-angiogenic /pro-permeability VEGF-A165a isoform and the anti-angiogenic VEGF-A165b isoform. Their effect on placental microvascular junctional integrity and permeability is not known. Using a dual perfusion system, the fetal microcirculation of placental lobules (12) were perfused with recombinant human VEGF-A165a (20ng/ml), VEGF-A165b (20ng/ml), or vehicle for 30 mins. In reversal experiments, the VEGF-A165a exposure was followed by VEGF-A165b (40 ng/ml). Dextran-TRITC (75kDa, 1mg/mL) was added for the last 10 mins. The % of vascular profiles showing disrupted junctional VE-cadherin and associated ‘hot-spots’ were quantified using unbiased sampling. In control perfusions, 30.2 ± 4.4% of profiles were associated with hotspots. VEGF-A165a resulted in a statistically significant increase with 71.2 ± 13.8% showing tracer leakage (p<0.01). No increases were seen with VEGF-A165b. In VEGF-A165a + VEGF-A165b perfusions, 9.4 ± 1.2% vascular profiles showed tracer leakage (p<0.05). Disruption of junctional VE-cadherin matched leakage. Only 49.3% of vessels showed full occupancy in VEGF-A165a perfusions. VEGF-A165b did not alter VE-cadherin profile (p<0.01). Addition of VEGF-A165b to VEGF-A165a perfusion resulted in 81 ± 5% of profiles showing continuous junctional VE-cadherin (p<0.05). Our data suggests that VEGF-A165b does not induce microvascular leakage in the human placenta and can inhibit or rescue VEGF-A165a induced junctional changes at a 1:2 concentration ratio. The ratio of these isoforms may be important in determining the placental barrier in normal and complicated pregnancies.

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oxLDL BOTH STIMULATES AND INHIBITS ENDOTHELIAL ANGIGENESIS AND MIGRATION POTENTIALLY VIA A SPHINGOSINE-1-PHOSPHATE / ERK DEPENDENT MECHANISM

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Oxidised lipids are known to promote inflammation and contribute to cardiovascular disease. Changes to circulating serum lipids particularly oxidative modifications can affect endothelial function. We therefore tested the hypothesis that serum lipids, LDL and its oxidation products can control angiogenesis. Using serum lipid depletion and oxidised lipids we assessed cell endothelial migration, angiogenesis, adhesion molecule and lipid receptor expression and also leukocyte trafficking. At relatively high concentrations, oxLDL inhibited angiogenesis and endothelial migration, potentially stimulated ICAM-1 expression and caused chronic recruitment of leukocytes. However, lower concentrations of oxLDL stimulated endothelial migration in a sphingosine-1-phosphate dependent manner. Native LDL was shown to have no effects at equivalent concentrations in these assays. Serum lipids were also shown to regulate endothelial function as their removal from cultures inhibited endothelial migration and angiogenesis. The ability of endothelial cells to mount an inflammatory response however was unaffected in serum lipid depleted cultures suggesting a function specific effect in response to serum lipids. Analysis of known oxLDL receptor-1 (LOX-1) and scavenger receptor B (CD36) on endothelial cells showed minimal expression. Blockade with a specific sphingosine kinase inhibitor or a S1P1 receptor antagonist inhibited oxLDL dependent migration while a S1P2 receptor antagonist potentially enhanced migration but had no effect on oxLDL stimulated migration. These data suggest an important role for circulating lipids and oxidised lipoproteins in the angiogenic and migratory response of endothelial cells via the sphingosine-1-phosphate pathway.
IDENTIFICATION OF SIGNALLING PATHWAYS MODULATED BY THE ENDOTHELIAL EXPRESSED Rho GTPase RhoJ

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RhoJ is a RhoGTPase expressed in endothelial cells which regulates cell motility and tube formation, processes critical to angiogenesis. We have shown that RhoJ is localised to focal adhesions and modulates their disassembly. RhoJ knockdown causes increased numbers of focal adhesions which disassemble more slowly, while a dominant active mutant of RhoJ (daRhoJ) causes the converse. RhoJ interacts with the GIT-PIX complex, a regulator of focal adhesion disassembly, with daRhoJ causing increased levels of GIT2 protein. More recently, others have shown a role for RhoJ in the polarized trafficking of podocalyxin, a process critical to lumen formation. In order to better understand the molecular function of RhoJ, and the signalling pathways with which it interacts, the effects of daRhoJ expression in human umbilical vein endothelial cells on a range of human kinases was assessed using a human phospho-kinase array. Results demonstrated that activation of RhoJ causes modifications of a number of pathways including the c-Jun N-terminal kinase, Signal Transducer and Activator of Transcription (STAT), AMP-activated protein kinase alpha-2 and beta-catenin. These data indicate that RhoJ activity affects a range of signalling proteins with a role in cell survival and adhesion.
INVESTIGATING THE PROTECTIVE ROLE OF EXOGENOUS TGFβ1 IN CARDIAC ISCHAEMIA REPERFUSION INJURY

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Background: Timely reperfusion of the occluded coronary artery in acute myocardial infarction patients rescues ischaemic myocardium but also leads to a major inflammatory response. This process, known as ischaemia reperfusion (I/R) injury, can contribute up to 50% of final infarct size. Anti-inflammatory therapies in the acute setting have potential to prevent this damage and reduce infarct size. Historical data have shown exogenous TGFβ1 in the acute setting in feline and rat models is protective, but the mechanisms remain poorly understood. Our aim is therefore to exploit murine Cre-LoxP technology to determine cell-types and mechanisms responsible. Methods: We used a mouse model of cardiac I/R injury with and without delivery of TGFβ1. At 24hr following reperfusion we assessed the infarcted region for (i) immune infiltrate using immunofluorescent staining, (ii) expression of inflammatory cytokines using qPCR, and (iii) infarct size using triphenyl-tetrazolium-chloride staining. We also quantified “mature” scar size after 4 weeks using Masson’s trichrome staining. Results: Exogenous TGFβ1 treatment resulted in (i) significantly reduced leucocytes infiltrating the injured myocardium, (ii) significantly reduced expression of IL1β and CCL2 in the infarct and (iii) significantly reduced infarct size at 24hours following reperfusion. Importantly we also showed that TGFβ1 treatment led to reduced “mature” scar size at 4 weeks. Conclusion: Having demonstrated a cardioprotective effect of exogenous TGFβ1 in mice post I/R, we will now use Cre/LoxP genetics to elucidate the cell-type-specific mechanisms involved. By improved understanding of TGFβ1 mediated cardiac protection, we will be able to develop this as an effective clinical therapy.
GENERATION OF ENDOGENOUS SOLUBLE PD1 AS A POTENTIAL THERAPEUTIC STRATEGY FOR CANCER

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Immune checkpoint inhibitors are novel immunotherapies that target the programmed death receptor (PD1). Blocking PD1 from binding to its receptor PDL1, present on cancer cells, restores T cell function. PD1 is alternatively spliced to a soluble variant (sPD1) that could inhibit PDL1 from acting on the receptor. Splicing is controlled by RNA binding proteins such as SRSF. Nuclear localisation of SRSF is regulated by phosphorylation of SR protein kinases such as Clk, DYRK and SRPK. However, PD1 splicing control is unknown. The aim of this study was to determine how splicing of PD1 is controlled. Bioinformatic search of SR protein binding sites was undertaken using ESE finder. RNA immunoprecipitation of SR proteins was undertaken by RNA pull down, followed by RT-PCR for PD1. Jurkat cells were transfected with nuclear localised SRSF plasmids and RT-PCR was carried out. Knock down of splicing factor kinases was undertaken by lentiviral transduction in Jurkat cells. Bioinformatic analysis indicated SRSF1 binding sites in intron 3 of the PD1 pre-mRNA. Jurkat cells expressed both sPD1 and flPD1 RNA. RNA immunoprecipitation of SRSF1 pulled down fl-PD1. Jurkat cells transfected with nuclear localised SRSF1 plasmid resulted in expression of only fl-PD1. Knock down of SRPK1 resulted in downregulation of fl-PD1, and increased sPD1. These results suggest that SRPK1 phosphorylation of SRSF1, resulting in its nuclear localisation, induces a switch in splicing from sPD1 to fl-PD1. This could be a novel mechanism of checkpoint inhibition.

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GLOMERULAR PERMEABILITY: CORRELATION OF FUNCTION AND STRUCTURE

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The glomerulus is a three-dimensional bundle of capillaries mainly designated to filter the blood for waste products but retain large proteins such as albumin. The filtration depends on the structure of the glomerular filtration currently thought of as many layer barrier including the glycocalyx, endothelial cells, glomerular basement membrane, slit diaphragms and the sup-podocyte space. To be able to quantify the macromolecular transport of macromolecules to this structure in health and disease would give both a valuable diagnostic tool and enable the study of therapeutic interventions. Here we make the first step to this correlative workflow. Determination of glomerular permeability was performed using isolated murine glomeruli harvested via sequential sieving of excised kidneys. The glomeruli were incubated overnight in % bovine serum albumin bound to fluorescein (FITC-BSA) on thickly coated poly-l-lycine gridded imaging dishes. The grids acting as a map for relocation. A mid-section within the glomeruli was found, and imaged at 4 frames a second using confocal microscopy. Once started the external FITC-BSA was quickly replaced with BSA of the same concentration without fluorescein. The drop in fluorescence in individual loops was quantified as albumin flux. The glomerulus was then fixed, embedded and sectioned for electron tomography. We were able to correlate vascular structure between the light and electron microscopy. This methodology pilot study gave comparable diffusive solute permeability with other groups working in the area of \((5.8 \pm 2.7) \times 10^{-7} \text{ cm.s}^{-1}\) and electron microscopy demonstrated the vascular damage to the same isolated glomerulus.
THE INHIBITORY EFFECTS OF EICOSAPENTAENOIC ACID (EPA) ON FOAM CELL FORMATION ARE LOST IN PATIENTS WITH ARTERIAL DISEASE

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In atherosclerosis, monocytes are recruited to the arterial intima where they form pro-inflammatory foam cells after uptake of oxidised Low Density Lipoproteins (OxLDL). O-3 polyunsaturated-fatty acids such as EPA are known for their anti-inflammatory properties. Here, we investigated the capacity of monocytes to form foam cells in the healthy and atherosclerosis patients and whether this is modulated by EPA. Monocytes were co-cultured with endothelial cells (EC) for 24 hours and the ability of EC to recruit flowing leukocytes was tested. We have shown that classical monocytes form foam cells more efficiently than non-classical/intermediate monocytes as they express higher levels of CD36, a scavenger receptor mediating OxLDL uptake. This correlated with their ability to promote EC activation and secondary recruitment of leukocytes. In healthy subjects, EPA reduced OxLDL content and inflammatory activity in classical monocytes, by down-regulating the expression of CD36. This effect of EPA was lost in foam cells derived from atherosclerosis patients. Monocytes from healthy subjects exposed to the serum of atherosclerosis patients had higher OxLDL content and abrogated the inhibitory effects of EPA on healthy monocytes. This suggests the presence of a circulating agent in patient serum that amplifies formation of foam cells and counteracts the anti-inflammatory effects of EPA. This study reveals that EPA can reduce foam cell formation in healthy subjects but highlights a disease-dependent change in monocyte fate that leads to exaggerated foam cell formation and increased inflammatory activity of these cells.
Endothelial properties including shape and biochemistry are altered by the force of the blood on the vascular wall. This force, known as Wall Shear Stress (WSS), is affected by cellular properties such as shape and depth of glycocalyx, as well as vascular properties such as plasma viscosity. Here we endeavour to use a novel technique to measure the WSS directly in vivo. The method is to use 900nm long 7nm diameter filamentous bacteriophages (M13) to act as a nano-string. The nano-string can be tethered by a link molecule from one end to a surface. By directional quantification of the M13 at video rate imaging the local shear force is compared to the local Brownian force. This method ensures that the force is measured such that assumptions of the viscosity are not required, moreover due to its relative size local topographical effects will be quantifiable. To address the 3D imaging concerns with tissue scatter and topography we also model the semi-rigid nano-string as a loosely-tethered straight rod, and by applying a Gaussian point spread function enable M13 orientation to be characterised as a mathematical inverse problem. We report point estimates and confidence ellipses for the three-dimensional M13 orientation at each imaging frame. Further we demonstrate using a single capillary cannulation in-vivo the nano-string can be attached to the vascular wall via a lectin link molecule and directions quantified. We believe that if the model and the imaging can be combined in vivo measurements will be practical for medical research purposes.
EXPANSION IN 3D CULTURE ALTERS THE MORPHOLOGY AND ADHESIVE CAPABILITY TO THE INJURED HEART OF MESENCHYMAL AND HAEMATOPOIETIC STEM CELLS

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Background: 2-dimensional (2D) monolayer stem cell (SC) culture is a convenient and conventional culture method. However, in vivo, SCs exist in a 3-dimensional (3D) environment alongside extracellular matrix and other cell types. Therefore, 2D cell culture does not represent the natural in vivo condition which may be better mimicked by 3D culture. In this study, we assessed whether the culture method impacted on the morphology and adhesive capability of human Wharton’s jelly mesenchymal SCs (MSCs) and murine hematopoietic SCs (HPC-7’s). Methods: HSCs/MSCs were cultured using routine 2D methods or a 3D hanging drop technique. Cell morphology and size was investigated microscopically. Adhesion to frozen sham and ischemic-reperfusion (IR) injured murine cardiac tissue was tested using a Stamper-Woodruff adhesion assay. Results: Although 3D cultured HSCs were slightly smaller, this did not attain statistical significance. However, 3D cultured MSCs were significantly (p<0.05) smaller than those grown in 2D culture. Both 2D (p<0.05) and 3D (p<0.05) cultured HSCs demonstrated significant adhesion to IR injured cardiac tissue when compared to sham tissue. This result was not mirrored when using MSCs. Furthermore, 3D HSCs showed a significantly (p<0.05) increased adhesion to injured tissue when compared to 2D HSCs. Similar results were also observed with 3D cultured MDCs. Conclusion: Since 3D cultured HSCs and MSCs demonstrate better adhesive properties in vitro, they may be more suited for systemic delivery to the injured heart in vivo. The smaller 3D MSC size may also impact positively on their homing capability to the heart.

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Quantifying Immunotherapeutic Potential with Anatomic Blood Flows

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Immunotherapeutic strategies to induce tumour regression rely on extravasation of effector cells such as cytotoxic T lymphocytes (CTLs) from circulation into the tumour microenvironment. CTLs migrate from tumour draining lymph nodes into the circulation, thus their flux through a tumour depends on organ perfusion. We produced a simple ordinary differential equation model analogous to those used for physiologically based pharmacokinetic models to quantify the maximum potential extravasation rate for each organ, using human blood flows and organ volumes [e.g. ICRP Pub. 89, 2002]. The organs predicted to have the highest extravasation rates include the lungs, kidneys and intestines: organs in which some of the first successful applications of immunotherapy were reported [Couzin-Frankel. Cancer Immunotherapy. Science 2013.]. The same model can be applied to other species such as mice. The predicted cross-species proportional rates of extravasation differ in a way that cannot be allometrically scaled, which has consequences for interpretation of preclinical observations. Exercise, consumption of food or temperature changes at the skin alter the distribution of blood flow and thus of maximum potential extravasation rate; these simple interventions could form parts of treatment strategies. Finally, T-cell mediated tumour killing reduces the size of a tumour and thus the rate of extravasation from the blood. A dynamic tumour model is used to show that persistent tumour size depends on organ perfusion. In summary, anatomical considerations alone can be used to predict maximum potential immunotherapeutic efficacy without considering detailed organ vasculature, which can elucidate potential reasons for clinical failure.
SYNTHESIS AND EVALUATION OF A NOVEL MITOCHONDRIA-TARGETED PEPTIDE BASED H2S DONOR COMPOUND (RTP-10) IN HYPERGLYCAEMIA-INDUCED MICROVASCULAR ENDOTHELIAL CELL DYSFUNCTION

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An overproduction of mitochondrial reactive oxygen species (ROS) in endothelial cells, is a major contributor to vascular endothelial dysfunction (VED) and angiopathy in diabetes. Hyperglycaemia (HG) induces metabolic changes in mitochondria, notably superoxide production, membrane hyperpolarisation and loss of ATP synthesis. Supplementation of cells and diabetic animals with very high concentrations of hydrogen sulfide (H2S) has been shown to prevent HG-induced metabolic changes in vascular endothelial cells. Given the predominantly mitochondrial effects of H2S against diabetic vascular damage, we previously described two mitochondria-targeted H2S (mtH2SD) donor organic compounds based around a triphenylphosphonium (TPP+) targeting scaffold, AP39 and AP123. These compounds have shown significant efficacy in animal models of mitochondrial dysfunction at very low doses (7-300 µg/kg). In this current study, we have used an alternative approach to target H2S to mitochondria using a novel H2S donor derivative of D-Arg-L-Tyr-L-Lys-L-Phe-NH2 (RTP-10). This approach may be advantageous over TPP+-based scaffolds in that mitochondrial accumulation is not dependent on mitochondrial ΔΨm. In this current study we exposed murine B.End3 brain microvascular endothelial cells to hyperglycaemia (HG) and after 7 days added RTP-10 and measured the reversal of HG-induced metabolic changes, specifically mitochondrial ΔΨm (JC-1), mitochondrial superoxide (mitosox), ATP synthesis (by luminescence). RTP-10 caused a concentration-dependent (0.1-30 µM) increase in mitochondrial H2S levels and reversed HG-induced mitochondrial hyperpolarisation and oxidant production, and restored ATP synthesis. This study further suggests that targeting H2S to mitochondria may be a useful therapeutic strategy for preventing/inhibiting HG-induced VED and angiopathy.
Diabetes is an increasingly prevalent condition. One hallmark of this disease is vascular endothelial dysfunction (VED) and angiopathy due at least in part to hyperglycaemia (HG)-induced overproduction of mitochondrial oxidants and accompanying mitochondrial damage. Hydrogen sulfide (H2S) is a physiological mediator shown to decrease mitochondrial reactive oxygen species during HG. Lower H2S bioavailability has been reported in patients with diabetes, as well as animal models of diabetes and supplementation of cells and diabetic animals with very high concentrations of hydrogen sulfide (H2S) has been shown to prevent HG-induced metabolic changes to mitochondria in vascular endothelial cells. We have hypothesised that diabetes is a disease of “H2S deficiency” and strategies to target mitochondria to overcome this “deficiency” may represent a novel approach to treat/prevent diabetic VED and angiopathy. To investigate this hypothesis, we have synthesised a novel mitochondria-targeted H2S donor (RT-01). We exposed murine B.End3 microvascular endothelial cells to HG for 7 days and then added RT-01 (3-300 nM) with HG for 3 further days. After this time, we determined the ability of RT-01 to reverse HG-induced metabolic changes; specifically mitochondrial \( \Delta m \) (JC-1), mitochondrial superoxide (mitosox), ATP synthesis (luminescence) and cell viability (LDH). Mitochondrial H2S production was also determined by fluorescence microscopy using AzMC and MitoTracker Red. RT-01 caused a concentration-dependent (3-300 nM) increase in mitochondrial H2S levels and reversed HG-induced mitochondrial hyperpolarisation and oxidant production, and restored ATP synthesis. This study suggests targeting H2S to mitochondria may be a useful therapeutic strategy for preventing/inhibiting HG-induced VED and angiopathy.
MICROVASCULAR RESPONSIVENESS TO LOCALLY DELIVERED LIRAGLUTIDE, A GLUCAGON-LIKE PEPTIDE-1 ANALOGUE, DECREASES WITH AGE

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Our previous research demonstrated that GLP-1 analogues, Exenatide and Liraglutide, are vasodilators and increase skin perfusion. However, whether age influences the microvascular responsiveness to GLP-1 analogues is not known. This study aims to examine whether skin microvascular responsiveness to Liraglutide is associated with age. 57 participants without diabetes were recruited (age range: 21-85 years, 45% male). Liraglutide at 1/10th minimum treatment dose (0.06mg) and saline (0.9%, microinjection control) were microinjected into the dermis of the forearm. Laser Doppler imaging assessed skin perfusion at baseline then every 30 seconds for 10 minutes following microinjection. Skin perfusion response was expressed as stabilised response (SR, mean perfusion between 7.5-10 minutes post injection). Participant characteristics: median age (25th, 75th quartiles): 55 (40, 69) years; waist: 87.0 (79.9, 95.0) cm; HbA1c: 38 (35, 39) mmol/mol; mean arterial pressure (MAP): mean (SD) 96 (11) mmHg. The response to Liraglutide was greater than the saline microinjection trauma control (median Liraglutide SR (25th, 75th quartiles: 1.36 (1.12, 1.68)V vs saline: 0.64 (0.51, 0.88)V, p<0.001 Wilcoxon signed rank). Age was negatively associated with the Liraglutide SR (Rs = -0.676, p<0.001). This association remained after adjustment for gender, waist, MAP and saline response (standardised beta: -0.545, p<0.001). This study suggests that microvascular responsiveness to Liraglutide decreases with age, and that this relationship is not dependent on gender, central obesity or blood pressure.
TARGETING VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 PREVENTS INTERCELLULAR ADHESION MOLECULE-1-DEPENDENT MONOCYTE ADHESION TO HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Rheumatoid arthritis (RA) affects ~1% of the UK population, is painful and significantly disrupts quality of life. Chronic pain can still develop, despite treatment-controlled inflammation. Vascular endothelial growth factor-A (VEGF-A) is increased in the serum of RA patients. Our recent studies indicate anti-VEGFR2 is anti-nociceptive and has a central site of action. In particular, anti-VEGFR2 reduced the number of ICAM-1-positive blood vessels and reactive microglia in the spinal cord indicating an inhibitory action on CNS endothelium and prevention of immune cell transmigration into the parenchyma. Hypothesis: targeting VEGFR2 affects monocyte adhesion to human brain microvascular endothelial cells (HBMEC) through an inhibitory action on ICAM-1.

Method: HBMEC monolayers were treated with TNFa or VEGF-A (24h) for endothelial activation. Fluorescently-labelled monocytes were allowed to adhere to the monolayer (2h), unattached monocytes washed off, cells fixed and fluorescence measured.

Results: VEGF-A or TNFa increased monocyte adhesion in a concentration-dependent manner. TNFa or VEGF-A-induced adhesion was significantly reduced by pre-treatment with: VEGFR2 inhibitor (ZM323881, reduced effect of TNFa by 69.1±10.1%, VEGF-A 66.2±7.8%) VEGFR1&2 inhibitor (PTK787, TNFa 58.3±10.1%, VEGF-A 58.4±17.5%) or a VEGF-receptor antagonist (VEGF-A165b, TNFa 65.2±8.6%, VEGF-A 57.5±8.1%). Furthermore blocking ICAM-1 similarly inhibited monocyte adhesion indicating ICAM-1-dependence (TNFa 71.4±6.5%, VEGF-A 84.0±9.2%). VEGFR2-mediated endothelial ICAM-1 expression and immune cell adhesion may play a role in central sensitization, contributing to secondary hypersensitivity in inflammatory pain. Targeting endothelial VEGFR2 may prevent/alleviate pain experienced in RA. Funded by Arthritis Research UK.
INVESTIGATION OF THE FEASIBILITY OF POST-OPERATIVE MONITORING OF TISSUE BLOOD FLOW AND TISSUE OXYGENATION FOLLOWING FREE-FLAP RECONSTRUCTIVE PLASTIC SURGERY

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Background: Devices for monitoring free flap breast reconstructions post-operatively allow early detection and salvage when perfusion is compromised. However, there is no consensus of clinical monitoring technique. This study aims (i) to assess the feasibility of using a combined white light spectroscopy (OXY) and Laser Doppler (LDF) device in monitoring oxygenation (SO2) and skin perfusion (Flux) of flaps and (ii) to examine the underlying physiology and potential clinical usefulness of monitored parameters. Patients and Methods: All identified patients were recruited into a single centre, observational pilot study. The feasibility of flap monitoring using a mooVMS OXY-LDF monitor during the first 72 hours post-operatively was assessed. The underlying physiology and potential clinical usefulness of monitored parameters were assessed by studying the data trend of parameters. Results: Five patients were recruited; three were suitable for OXY/LDF analysis (mean age=55yrs, 46–64yrs). Time of increase for SO2(%)/Flux(PU) for Patients 1, 2 and 3 were: SO2,18hrs/Flux,>24hrs; SO2,2hrs/Flux,>30hrs; SO2,12hrs/Flux,>45hrs, respectively. Similarly, the mean values of SO2(%)/Flux(PU) at the first 5 hours vs 5 hours at stabilised plateau (with relative increase, stabilised plateau/baseline) for Patients 1, 2 and 3 were: SO2,11vs39(~3-fold)/Flux,6vs26(~4-fold); SO2,18vs61(~3-fold)/Flux,20vs50(~2-fold); SO2,3vs38(~12-fold)/Flux,9vs12(~1.3-fold), respectively. Stable flap skin temperatures were observed throughout the 72 hours (Patients 1, 2 and 3: 33±4ºC (mean±SD), 35±2ºC and 35±2ºC, respectively). Conclusion: The feasibility of flap monitoring by OXY/LDF has been demonstrated in breast reconstruction patients. Significant increases in SO2/Flux occurred independently with different relative amplitudes post-operatively. This warrants further investigation to understand the underlying physiology during the flap recovery period.
INVESTIGATION OF MECHANISMS UNDERLYING THE BLUNTED ENDOTHELIUM-DEPENDENT VASODILATATION IN YOUNG SOUTH ASIAN (SA) MEN RELATIVE TO WHITE EUROPEAN (WE) MEN

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Cardiovascular disease is more prevalent in SAs than WEs. We recently reported endothelium-dependent dilator responses evoked in skin were attenuated by cyclooxygenase (COX) inhibition in WEs, but augmented in SAs. However, the COX and nitric oxide synthase (NOS) pathways inhibit one another and endothelium-dependent hyperpolarising factor (EDHF). Thus, in 10 WE and 11 SA normotensive men (18-25 years), we have recorded red cell flux in forearm skin during ACh iontophoresis (7x20s pulses at 100 µA, 1, 200 µA; 60s intervals) and reactive hyperaemia (arterial occlusion) without and after topical application of NOS inhibitor L-NAME (10nM), and after NOS inhibition followed by COX inhibition (600mg aspirin p.o; L-NAME/Asp). L-NAME attenuated ACh-evoked dilatation in WEs (179.9±5.4 vs 140.2±4.7 perfusion units*(PU),*:P<0.05), not in SAs (164.9±4.4 vs 166.4±4.8PU). Further, peak reactive hyperaemia was attenuated after L-NAME in WEs (86.5±2.8 vs 58.4±2.9PU*) not SAs (80.6±2.8 vs 74.8±2.5PU). Following L-NAME/Asp, ACh-evoked dilatation was augmented in WEs (195.0±5.5PU§: P<0.05 vs L-NAME), but attenuated in SAs (137.4±4.2PU§). Reactive hyperaemia was augmented in WEs (64.3±3.1PU§) but unchanged in SAs (71.5±2.8PU). These results suggest the contribution of NO to endothelium-dependent dilatation is blunted in young SA men. Removal of inhibitory influences of NO on COX allows a small dilator influence of COX products to be revealed in SAs, but also suggests the dilator influences of EDHF are much greater in WEs than SAs.
TUMOURICIDAL T LYMPHOCYTE HOMING IN A MURINE CANCER MODEL USING MICRO-PET/CT

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Introduction: Adoptive cell therapies using tumour-infiltrating lymphocytes (TILs) and chimeric antigen receptors (CARs) are being trialled in the clinic. Their success depends on recruitment of injected T cells from tumour blood vessels into solid cancers, which is not routinely measured. Non-invasive tracking of 99mTc-labelled leucocytes is used to diagnose inflammation but the short half-life of 99mTc precludes its’ use for long-term tracking of T cells. Aims: Using 89Zr-oxine to label tumour-specific T lymphocytes and PET/CT to follow their migration in tumour-bearing mice.

Materials and Methods: CD90.2 F5 CD8+ T cells were labelled using 20 MBq 89Zr-oxine for 15 min at 37°C, washed and viability assessed. Therapeutic doses of 89Zr-labelled and unlabelled T cells were injected intravenously into CD90.1 mice bearing B16-NP68 melanomas and imaged using Mediso PET/CT preclinical scanner for up to 17 days. Radioactivity was analysed by drawing regions of interest around tumour, lymphoid and non-lymphoid organs and compared with mice receiving free 89Zr-oxine. After radioactive decay, tissues were harvested and analysed by flow cytometry for donor cells. Results: 89Zr-T cell labelling was 18-20% efficient with low toxicity. After 16 hours 89Zr accumulated in spleen, liver, skeleton and tumour, not in the heart, kidneys or bladder. Donor T cells were found in the spleen and liver of post-mortem tissues. Free 89Zr-oxine distributed differently from 89Zr-labelled T cells. Conclusions: 89Zr-oxine cell-labelling can be used to track the migration pathways of immunotherapeutic cell grafts and differentiate between T lymphocyte accumulation inside solid tumours and tumour progression.
LYMPH NODE LYMPHATICS EXHIBIT ALTERED IMMUNE INTERACTIONS IN RESPONSE TO TUMOUR-DERIVED FACTORS

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Tumours evade immune attack by a variety of immune-suppressive mechanisms, many of which involve targeting of dendritic cells (DCs). To mount an immune response, DCs must capture and present antigen, migrate to draining lymph nodes (dLNs) and localise to T-cell zones in order to prime responsive T cells. A key step in this process is the migration of DCs along lymphatic vasculature connecting the periphery to dLNs. As expansion of the lymphatics in the tumour microenvironment (TME) is well characterised, and a new role in immune modulation has been described, we posed the question as to whether lymphatic endothelial cells (LECs) could modulate DC-mediated immunity, in favour of immune suppression. Using the B16F10-melanoma model, microarray analysis of LECs derived from TDLNs demonstrated significant deregulation of functional pathways, including immune cell trafficking. To assess lymphatic trafficking of tumour-derived DCs, confocal imaging and flow cytometry analysis of TDLNs of TRITC painted tumours was performed. Compared with resting LNs, DCs were frequently observed restricted to the lymphatic subcapsular sinus. Modelling this in vitro, DCs incubated with tumour-conditioned media (TCM) treated LECs exhibited altered adhesion and mobility. Our microarray and data from the Cancer Genome Atlas (TCGA) revealed a potential role for Podoplanin in mediating these interactions. Flow cytometry confirmed an upregulation in TDLN-LECs at the protein level. Furthermore, Podoplanin blocking assays show hindered DC adhesion to LECs in vitro. These findings begin to support the notion that LECs could play an active role in shaping the TME through direct modulation of DC-trafficking.
CATHEPSIN D INDUCES MIGRATION IN HUMAN OMENTAL MICROVASCULAR ENDOTHELIAL CELLS (HOMECs)

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Aim: Epithelial ovarian cancer frequently metastasizes to the omentum, a process that requires pro-angiogenic activation of human omental microvascular endothelial cells (HOMECs) by tumour-secreted factors. We have previously shown that ovarian cancer cells secrete the protease cathepsin D (CathD) which induces HOMEC proliferation in a non-proteolytic manner. We have also shown that CathD induced activation of ERK1/2 and AKT pathways in HOMECs. The aim of this study is to investigate whether CathD induces migration in HOMECs and examine the signalling pathways involved i.e. ERK1/2 and AKT.

Methods: HOMECs were treated with CathD in the absence or presence of ERK1/2 inhibitor (U0126) and AKT inhibitor (MK2206) and cell migration was assessed using the Boyden chamber transmigration assay. A cell based ELISA was used to validate the effect of the inhibitors.

ResultA: CathD (50ng/ml) significantly induced cellular migration via activation of both the ERK1/2 and AKT pathways. The MAPK inhibitor (10 µM, U0126) significantly reduced CathD-induced migration in HOMECs i.e. CathD alone= 136±26% (n=12), CathD+U0126= 92±8% (n=7) (all vs control (100%)). Similarly, cellular migration was inhibited by AKT inhibitor (5 µM, MK2206) i.e. CathD alone= 180±66% (n=12), CathD+MK2206= 106±46% (all vs control (100%)). The validity of the inhibitor concentrations were confirmed by a cell-based ELISA which showed that both inhibitors U0126 and MK2206 significantly inhibited CathD-induced phosphorylation of ERK1/2 and AKT kinases respectively.

Conclusion: CathD contributes to pro-angiogenic responses of the omental microvasculature by inducing migration in HOMECs via activation of the ERK1/2 and AKT pathways respectively.
PEPITEM INHIBITS ONSET OF COLLAGEN INDUCED ARTHRITIS, AND THERAPEUTICALLY REDUCES DISEASE SEVERITY

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Background: The inappropriate recruitment and retention of T-cells into the joint is a cardinal feature of RA, yet the molecular mechanisms underpinning this remain unclear. We recently showed that patients with Rheumatoid arthritis (RA) have a defect in a newly identified immuno-protective check-point (adiponectin-PEPITEM axis) that normally limits T-cell trafficking during inflammation. Here we examined the therapeutic potential of PEPTIEM in a murine model of arthritis. Methods: Collagen induced arthritis was trigger in DBA/1 mice by immunisation with bovine type II collagen. Synthetic PEPITEM was administered by daily injections starting at day 21 (prior to disease onset) or 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: Administration of synthetic PEPITEM prior to disease onset inhibited the development of arthritis. We observed a significant reduction in disease incidence, clinical score, leukocyte infiltration and bone erosion when compared to control mice. Whilst we observed fewer leukocytes in the synovium, we detected higher levels of the FOXP3 transcript in PEPITEM treated mice than in control animals. Excitingly, PEPITEM also reduced the clinical score, leukocyte count in the synovium and damage to the bone when administered at the first signs of inflammation. Once again, we detected increased expression of FOXP3 mRNA in synovial tissue of PEPITEM treated animals. Conclusions: Thus targeting PEPITEM offers an alternative therapeutic approach for treating T-cell mediated diseases, such as RA.
INSULIN RESISTANCE WITH OBESITY IS ASSOCIATED WITH DEFICIENT OXYGEN EXTRACTION IN SKELETAL MUSCLE

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Aim: Glucose uptake is impaired in skeletal muscle with obesity. Insulin regulates active influx of glucose along with glycogenesis and glycolysis; matching muscle energy needs. Potential mechanisms of insulin resistance (IR) with obesity include mitochondrial dysfunction, inhibited capillary recruitment and oxidative stress. This study investigates the role of oxygen extraction (OE) in IR, independent of blood flow.

Method: IR was assessed in 12 fasted lean (BMI<25kgm-2) and 12 age-matched obese males (BMI>30kgm-2) by hyperinsulaemic euglycaemic clamps at 0.4 and 1.5 mU insulin kg-1min-1 and control saline infusions. After one hour stabilisation, OE was derived in calf muscle by near infrared spectroscopy from rate of fall in mean oxygen saturation during a 4 minute below knee arterial occlusion.

Results: Fasting triglycerides were higher in obesity (1.59(1.22-2.58) vs 0.82(0.74-0.90)mmol.l -1, p<0.001). Glucose infusion rates were significantly lower with obesity at 0.4 mU insulin kg-1min-1 (2.6(1.7-3.0) vs 5.0(3.3-7.4) mgkg-1min-1, p<0.01) and 1.5 mU insulin kg-1min-1 (8.7(6.9-9.9) vs 13.4(10.8-16.2) mgkg-1min-1, p<0.01) confirming increased IR in this obese group. Muscle OE during saline infusion was increased with obesity (0.083 vs 0.062%s-1, p<0.012) however the significant increase in OE in lean subjects with increased glucose uptake at 1.5mU insulin kg-1min-1 was diminished in obesity (0.102 vs 0.160%s-1, p<0.017). Conclusion: In lean subjects increased glucose uptake is associated with an increase in oxygen uptake independent of blood flow; suggesting upregulated active glucose transport, glycogenesis and mitochondrial activity. Increased muscle OE in obesity during euglycaemic saline infusion suggests inefficient mitochondrial aerobic respiration which may contribute to increased IR.
PC49

VASCULAR MACROMOLECULAR TRANSPORT: IMPLICATIONS OF THE SUB-GLYCOCALYX SPACE

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The primary function of the vasculature is to transport molecules to the required tissue. Amongst other parameters, this transport depends on the structure of the blood vessel wall and the effective pressure across the wall. The glycocalyx is thought to cause the primary oncotic pressure difference due to its size selectivity. The revised Starling hypothesis implies that there is a space with effectively zero concentration of albumin under the glycocalyx, as limited amounts of albumin can reach this sub-glycocalyx space from the tissue or the lumen side of the wall. However, the size and structure of this space is unexplored and, moreover, is currently impossible to reliably determine with structural imaging. Mathematical modelling can provide predictive tools to help answer these questions. We develop and solve a mathematical model of fluid flow through the glycocalyx in the presence of a sub-glycocalyx space. This continuum model allows us to predict the effective permeability of the glycocalyx in terms of the system parameters, including the height of the gap. Our model suggests that the sub-glycocalyx space provides resistance to the flow, contributing to a slight decrease in the hydraulic conductivity, but also causes a more uniform flow through the glycocalyx. Moreover, we quantify each of these sentiments in terms of the system parameters using an asymptotic analysis. Finally, we discuss how current structural imaging techniques could be used in conjunction with our results to indirectly estimate the size of the sub-glycocalyx space.
MICE LACKING THE INHIBITORY COLLAGEN RECEPTOR LAIR-1 EXHIBIT A MILD THROMBOCYTOSIS AND HYPERACTIVE PLATELETS

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Inhibition of platelet activation is important for controlling pathological thrombosis and is emerging as an important regulatory pathway in platelet formation. Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) is a collagen receptor that belongs to the inhibitory immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor superfamily. It is expressed on hematopoietic cells including immature megakaryocytes, but is not detectable on platelets. Although the inhibitory function of LAIR-1 has been described in immune cells, its physiological role in megakaryocytes and platelet formation has not been explored. Here, we investigate the role of LAIR-1 in platelet production and function by generating LAIR-1 deficient mice. Mice lacking LAIR-1 exhibit a significant increase in platelet count, a prolonged platelet half-life in vivo and increased proplatelet formation in vitro. Interestingly, platelets from LAIR-1-deficient mice exhibit an enhanced reactivity to collagen and the GPVI-specific agonist collagen related peptide (CRP) despite not expressing LAIR-1. In addition, ferric chloride induced thrombus formation in vivo was increased in LAIR-1 deficient mice. Deletion of LAIR-1 in megakaryocytes results in increased signalling downstream of the GPVI-FcR ?-chain and integrin αIIbβ3 due to enhanced Src family kinase activity. Findings from this study demonstrate that ablation of LAIR-1 disrupts the homeostatic balance of megakaryocytes and platelets, and induces an increased platelet count and reactivity resulting in a prothrombotic state in vivo. This study represents the first demonstration of a cellular phenotype associated with LAIR-1-deficiency and adds to the growing evidence that ITIM-containing receptors are critical regulators of platelet production and function.
ASSOCIATION BETWEEN RENAL FUNCTION AND MMPS IN THOSE WITH AND WITHOUT TYPE 2 DIABETES

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Nephropathy [that is increased albumin excretion rate (AER) and reduced Glomerular Filtration Rate (GFR)] is a hallmark of clinical microvascular disease in those with diabetes. We aimed to explore the associations between AER, GFR and members of the Matrix Metalloproteinase (MMP) family. **Methods:** People with (DM=210) and without (NoDM=117) Type 2 diabetes had GFR (Cockcroft-Gault) and AER (overnight urine collection) measured. Plasma biomarkers [MMP1, MMP3, MMP7, MMP10 and MMP12] were measured on OLINK proteomics platform. **Results:** MMP3, MMP7, MMP10 were associated with GFR in DM but not in NoDM [For DM, Adjusted Standardised Beta(StdBeta) -0.324(p<0.001), -0.251(p<0.001) and -0.178 (p=0.002) respectively], independently of potential confounders (age, sex, blood pressure, BMI, cholesterol and HbA1c). There was an interaction between presence/absence of diabetes and MMP3 (p=0.007). AER was associated with MMP7, independently of confounders, in DM but not in NoDM [For DM Adjusted StdBeta 0.266(p<0.001)]. All other biomarkers were not associate with GFR or AER. **Conclusion:** Decreasing GFR is associated with increasing MMPS in DM but not in NoDM. Increasing AER is associated with increasing levels MMP7 in DM but not in NoDM. These results suggest MMPS may play a role in diabetic renal complications.
PC52

A MULTI-CENTRE RELIABILITY AND VALIDITY STUDY OF LASER SPECKLE CONTRAST IMAGING AND THERMOGRAPHY IN PATIENTS WITH RAYNAUD’S PHENOMENON SECONDARY TO SYSTEMIC SCLEROSIS

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Background: Objective and reliable outcome measures for clinical trials of novel drugs to treat systemic sclerosis (SSc) related Raynaud’s phenomenon (RP) are currently lacking. Laser speckle contrast imaging (LSCI) and thermography are two non-invasive measures of perfusion that show excellent potential but require further clinical assessment. This multi-centre study aimed to determine the reliability and validity of a cold challenge protocol using both LSCI and thermography.

Methods: Patients underwent cold challenge on 2 consecutive days; 15oC water submersion of gloved hands (1 minute), un-gloved reperfusion and rewarming (23oC room temperature, 15 minutes). Baseline and changes in perfusion/temperature were imaged simultaneously using LSCI (relative perfusion) and thermography (skin temperature). Area under the reperfusion/rewarming curve (AUC) and maximum reperfusion/rewarming (MAX) were calculated locally. Test-retest reliability was assessed using intra-class correlation coefficients (ICC). Estimated latent correlations assessed the convergent validity of the LSCI and thermography.

Results: 159 patients with RP secondary to SSc were recruited from 6 UK specialist SSc centres. Reliability (ICC [95% CI]) for thermography was AUC 0.68 (0.58-0.80) and MAX 0.72 (0.64-0.81), and for LSCI, AUC 0.67 (0.54-0.76) and MAX 0.64 (0.52-0.75). High latent correlations of LSCI and thermography were present for AUC 0.94 (0.87-1.00) and MAX 0.87 (0.77-0.95).

Conclusion: This is the first multi-centre study examining the reliability and validity of a cold challenge protocol using LSCI and thermography in patients with RP secondary to SSc. LSCI and thermography demonstrated moderate to strong reliability and have very high convergent validity.
PODOPLANIN EXPRESSION BY UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS ALTERS THEIR MIGRATORY CAPACITY

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**Introduction:** Podoplanin (gp38) is a mucin-type protein that is expressed by tissue resident stromal cells at sites of chronic inflammation, such as those found in rheumatoid arthritis and cancers. Recent evidence suggests that podoplanin can mediate anti-inflammatory functions at these sites in the presence of its ligand CLEC-2. Here we examined the expression pattern of podoplanin on mesenchymal stem cells from umbilical cord (UCMSC) and whether it influences MSC function. **Methods:** Human UCMSC were obtained from healthy donors and screened for the expression of podoplanin using immunohistochemistry, flow cytometry and qPCR. To determine the effects of podoplanin on UCMSC migration and proliferation, cells were treated with siRNA before seeding on 8um pore Transwell filters or 12-well plates respectively. **Results:** Podoplanin was differentially expressed by UCMSC in a donor dependent manner. Three types of expression patterns were observed: negative donors; positive donors; and bimodal samples with a proportion of cells positive and a proportion either expressing much lower levels of or no podoplanin. In podoplanin positive UCMSC, siRNA reduced podoplanin gene and protein expression by 61% and 34% respectively at 72h post-transfection. siRNA transfection had no effect on UCMSC proliferation compared to controls. Interestingly, siRNA significantly reduced UCMSC trans-filter migration compared to controls. **Conclusion:** Thus podoplanin expression appears to play an important role in the migration of UCMSC. Physiologically, expression of podoplanin could enhance the localisation of MSC to sites of tissue damage and inflammation, whereupon they can exert their reparative and immunomodulatory effects.
THE ROLE OF EphA1 IN BLOOD-BRAIN BARRIER PERMEABILITY TO NEUROTOXIC LEUCOCYTES

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Immune activation and compromised blood brain barrier (BBB) integrity are neuropathological hallmarks of late-onset Alzheimer’s disease (LOAD). Genome-wide association studies (GWAS) have identified the erythropoietin-producing hepatocellular carcinoma receptor A1 (EphA1) gene as a susceptibility locus for LOAD. Intronic EphA1 variants rs11771145 (p=1.1x10^-13) and rs10808026 (p=1.4x10^-11) show strong LOAD association. A nonsynonymous variant (P460L) has also been identified (p=2.6x10^-3). Eph receptors bind membrane-bound ligands (ephrins), stimulating signalling in both interacting cells. Activation of human brain microvascular endothelial cells (hBECs) using soluble EphA1 stimulates leucocyte adhesion independently of changes in levels of intercellular adhesion molecule-1 (ICAM1) or vascular adhesion molecule-1 (VCAM-1), suggesting a novel EphA1-dependant pathway may control leucocyte extravasation across the BBB. We hypothesise that EphA1 SNPs influence LOAD by affecting membrane EphA1 expression on peripheral leucocytes, ultimately jeopardising BBB integrity, allowing leucocyte extravasation. Immunoblotting of HEK293 cells expressing wildtype EphA1 has identified EphA1 fragments. We are assessing whether these fragments are proteolysis products. The cellular distribution of EphA1 appears to change in response to ligand engagement (i.e. intracellular accumulation) and cleavage induction (i.e. increase in membrane expression). These experiments will be replicated in HEK293 cells expressing EphA1 P460L. EphA1 P460L ectodomain has been expressed as a soluble, fusion protein and its ability to activate hBECs will be compared to wildtype EphA1 using quantitative leucocyte recruitment assays. Patient-derived leucocytes homozygous for risk and non-risk alleles will be analysed for EphA1 expression and interactions with hBECs. We also aim to identify pathways activated in hBECs by EphA1 stimulation using RNAseq.
Amongst the few universal biological findings, it has been established that all mammalian cells are covered with a dense layer of carbohydrate chains or glycans, also known as the glycocalyx. Composed of membrane-bound macromolecules (e.g. glycoproteins, sulphated proteoglycans and glycosaminoglycan side chains) this structure spans between several hundred nanometres to a few micrometres on the cell surface. The glycocalyx of blood vessels in particular, is known to form a barrier between the circulating blood and the venular wall by regulating numerous vascular functions such as vessel permeability, shear stress mechanotransduction and leukocyte recruitment from the circulation. Interestingly, little is known about the composition and role of the lymphatic vessel glycocalyx, the second vasculature of all vertebrates that plays a key role in tissue homeostasis and trafficking of leukocytes during adaptive immune responses. Using confocal microscopy on the mouse cremaster muscles, here we show that the lymphatic endothelial (LEC) glycocalyx is mainly composed of heparan sulfate (HS), α-D galactosyl moieties and α2,3-sialylated N-linked glycans as demonstrated by the capacity of an anti-HS Ab, Isolectin-B4 (IB4) and Maackia amurensis lectin-1 (MAL-1), respectively, to bind these vessels. Interestingly, during acute inflammation following antigen sensitisation or TNF-stimulation, the binding of IB4, but not MAL-1 or anti-HS, to the lymphatic vasculature was significantly reduced. Furthermore, the downregulation of α-D galactosyl moieties on lymphatic endothelium glycocalyx was correlated with neutrophil migration into these vessels. Collectively, we report here the first characteristics of the composition of the LEC glycocalyx and its regulation during inflammation in vivo.
Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are cholestatic liver diseases characterized by immune infiltration and damage to the liver bile ducts. Genome-wide association studies have characterized CD80 and CD28, surface molecules both of which are involved in T cell activation, to be risk loci for PBC and PSC respectively. In this current study, we phenotyped T and B lymphocytes as well as classical, intermediate, and non-classical monocytes using flow cytometry for the expression of CD28, CD80 and CD86 from the blood of healthy donors and from livers of patients with PBC, PSC and end-stage metabolic injury. Our results show an increase in CD4+CD28+ T cells from the blood compared to all liver diseases. Furthermore, CD3+CD4+ and CD3+CD8+ T cells from the blood express higher levels of CD28 compared to cells from the liver however, no differences are found in the CD28 expression across all liver diseases. Moreover, there are statistically fewer CD80 expressing T and B cells in the liver compared to blood. Finally, CD4+ and CD8+ T cells express higher levels of CD86 in liver cells compared to blood cells. Statistically fewer CD86+ B cells and all three monocyte populations are found in the liver compared to blood. None of these differences are found across the different liver diseases. These findings show a potential problem in monocytes in the liver to provide a signal necessary for the activation of T cells and provide the first phenotypic characterization of the CD28-CD80 pathway in cholestatic liver diseases.
INVESTIGATION INTO MEASUREMENTS OF FIBROSIS, MICROVASCULAR STRUCTURE AND PERFUSION, WITH OXIDATIVE STRESS AND OXYGENATION IN PATIENTS WITH SYSTEMIC SCLEROSIS AS MEASURED WITH NON-INVASIVE IMAGING TECHNIQUES

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Background: Measurements of skin thickening (ultrasound [US]), microvascular structural abnormality (nailfold capillaroscopy [NC]) and microvascular dysfunction (laser Doppler imaging [LDI]) differentiate between primary Raynaud’s phenomenon (PRP) and systemic sclerosis (SSc). This study aimed to examine these parameters and measures of oxidative stress (OS) and oxygenation (OX), implicated in SSc pathogenesis, in a cross-sectional study. Methods: Skin thickness measurements were taken from 9 sites of the body. Capillary density and width were calculated using automated software. Perfusion images were taken of the hand pre and post local heating. Distal dorsal difference (DDD) was calculated at baseline and perfusion increase (POST/PRE) calculated at digit and dorsum. OS measurements (spectroscopy) were taken with UV- and OX with white-light at US sites. Between group comparisons (ANOVA) were made with STATA. Results: 139 patients with SSc, 61 healthy controls (HC) were assessed. US showed increased thickness at 8/9 sites for SSc vs HC. NC density was lower and width higher in the SSc vs HC group. LDI showed lower DDD and POST/PRE digit and dorsum for SSc vs HC. OS was significantly increased in 7/9 sites in SSc vs HC groups. OX was lower at the digit but overall showed no trend for increase or decrease across the 9 sites in SSc vs HC group. Conclusion: Data confirms increased skin thickness, microvascular structural and functional changes and increased free radical stress (OS) in SSc in a large data set. Relationships need to be explored and changes to be measured prospectively and these studies are underway.
Neutrophils are the primary responders to microbial infection and tissue damage that actively remove pathogens as well as promote wound healing. In the tumour microenvironment, studies have shown that neutrophil persistence within tissue is associated with cancer progression, and neutrophils may polarise to either anti- or pro-tumourigenic phenotype depending on the cue. It is accepted that tumours utilise multiple strategies to avoid immune clearance with stromal cells emerging as key immune modulators. Thus, changes within stromal populations, in particular tissue-resident fibroblasts, may alter immune cell function in turn promoting tumour growth. As neutrophils are key immune mediators, we hypothesised that cancer-associated fibroblasts (CAFs) suppress neutrophil immune function. Treatment of neutrophils with CAF conditioned media (CMed) did not significantly alter activation marker expression (CD11b, CD18 and CD62L) or reduce the phagocytic capability of the cells. However, treatment of neutrophils with CAF-derived factors in vitro significantly increased their capacity to form neutrophil extracellular traps (NETs) compared to normal fibroblasts. NET formation in this context was found to be induced by increased reactive oxygen species produced in response to CMed treatment. Interestingly, treatment with B16 melanoma tumour cell-derived CMed did not induce NETosis in the same manner indicating that this is a CAF-specific effect on tumour associated neutrophils. As NETs have previously been shown to capture circulating tumour cells to promote metastasis, we propose that tumour microenvironment-associated NETs act to capture other leukocyte populations. The functional consequence of these interactions to both immune function and localisation within the tumour are under continuing investigation.
INVESTIGATING THE SPECIFIC EFFECTS OF AN ACUTE RENAL INJURY ON MYOCARDIAL INFLAMMATION – A ROLE FOR IL-36 IN RENO-CARDIAC SYNDROME?

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Background: Patients with renal disease often present with cardiovascular complications that contribute to increased morbidity and mortality. Indeed, several studies show an inverse correlation between eGFR and cardiovascular event rates. The exact mechanisms of this cardiac damage remain unknown. This study investigated whether inflammatory changes occurred in the heart following an acute renal injury. A potential role for interleukin-36 (IL-36) was also determined, a poorly investigated cytokine whose receptor (IL-1 Rrp2; IL36R) we recently identified in murine heart.

Methods: Mice underwent renal ischemia-reperfusion (IR) injury or sham surgery. Kidneys, heart and liver were removed for immunohistological analysis of neutrophils, platelets, oxidative stress (8-OHdG staining) and VCAM-1 expression. Serum was removed and incubated with isolated murine neutrophils, after which their adhesion to endothelium was tested in vitro. Results: Neutrophil infiltration, oxidative damage and VCAM-1 expression was increased in the heart and liver of mice undergoing renal IR injury. VCAM-1 staining was intense around larger coronary vessels. Platelet presence, although significantly increased in the kidney itself, did not increase in remote organs. IL-36R expression was observed in sham glomeruli and around large renal vessels and increased post-renal injury. Interestingly, IL-36R was not observed in the liver, but expression increased in the heart post-renal injury. Renal IR serum significantly (p<0.05) increased neutrophil adhesion to endothelium in vitro.

Conclusion: Our results suggest an acute renal injury releases inflammatory factors into the systemic circulation which then promote neutrophil infiltration in remote sites either through endothelial VCAM-1 up-regulation and/or microvascular oxidative stress. Since IL36R up-regulation was only observed in remote the heart and not the liver, it may be a novel candidate for explaining the reno-cardiac syndrome.

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TYPE 2 DIABETES IS ASSOCIATED WITH REDUCED EXERCISE CAPACITY, REDUCED OXYGEN CONSUMPTION AND POORER SKELETAL MUSCLE OXIDATIVE CAPACITY IN OLDER ADULTS

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Objectives: Diabetes is associated with metabolic disturbances in skeletal muscle and reduced exercise capacity. Near-Infrared Spectroscopy (NIRS) measures microvascular changes in oxygenated and deoxygenated haemoglobin and can be used to estimate oxidative capacity in skeletal muscle using an arterial occlusion. We compared oxidative capacity of skeletal muscle in a group of older adults with or without diabetes. Methods: Participants were recruited to undertake a 6-minute stepper test. Power during exercise was defined as the workload divided by the duration of stepping. Analysis of expired gases was carried out to measure total-body oxygen consumption (VO₂). NIRS measurements were performed on the left gastrocnemius during transient arterial occlusions during recovery from exercise and the recovery time constant (τ) representing muscle oxidative capacity was calculated. Data are mean(SD) or adjusted mean±SEM. Multivariable linear regression was used. Associations with stepping performance were adjusted for age and gender; associations with τ were additionally adjusted for BMI and power. A possible interaction between power and diabetes on τ was investigated. Results: 115 participants (mean age=72(7) years, male=87) performed the test. Participants with diabetes (n=20) achieved fewer steps (169±13.5 versus 231±6.2 steps, p<0.001) and lower VO₂ values (15.3±0.77 versus 18.4±0.35ml.min⁻¹.kg⁻¹, p=0.008) than people without. τ was significantly longer in participants with diabetes (56.3±5.3 versus 45.2±2.4 s, p=0.047) and there was a significant positive interaction between power and diabetes (b-coefficient (interaction) = 0.11±0.05, p=0.02). Conclusion: People with diabetes have slower metabolic recovery following exercise. This is most evident in those working at higher power. Impaired muscle oxidative capacity may play a role in the reduced exercise capacity and reduced VO₂ observed in people with diabetes.
ABOUT THE HOST AND SPONSORING INSTITUTES

This meeting was part sponsored by the Institute of Cardiovascular Sciences and the Institute of Inflammation and Ageing, both within the College of Medical and Dental Sciences (CMDS). The research interests of these Institutes align themselves very well with the interests of both the BMS and the UKCAS. Please find some information on the research activities of these Institutes which may help with establishing collaborations, identifying PhD/Postdoctoral supervisors or securing your next academic position.

Welcome from the Institute of Inflammation and Ageing (IIA)

We are the only centre in the UK to have brought together, under the same roof, a group of world-leading medical and scientific experts in human ageing, trauma and major illnesses with the same driver: inflammation. Our partnership between the NHS and University brings basic scientists, clinicians and patient partners together to break down traditional bench to bedside, bedside to bedside and primary care-secondary care divisions that have prevented a truly integrated, multidisciplinary, patient-centred approach to treating chronic inflammation and ageing, an approach highly endorsed by patients.

This enables us to transform the way these chronic, debilitating and life-threatening conditions are studied, prevented and treated. Patients often suffer from more than one chronic inflammatory condition and so instead of looking at them separately, we consider them collectively. In this way we identify the major common drivers of ill health that can then be tackled to improve overall health rather than treating each disease individually. By working side-by-side, our specialists not only learn from each other, but they are also able to provide a holistic approach to treating patients.

Our innovative work also takes in trauma – and we are at the vanguard of new ways to help patients of all ages to recover from serious injury. We have won four Centres of Excellence awards in as many years, the MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research, the Arthritis Research UK Centre for Rheumatoid Pathogenesis, the NIHR Surgical Reconstruction and Microbiology Research Centre and the Scar Free Foundation. In 2016 we were awarded over £12 million for an NIHR BRC in Inflammation research. In total these awards bring in over £30 million of vital funding. Our research focuses on 3 main areas:

- Chronic Inflammatory Diseases
- Trauma
- Ageing

Best Wishes

Janet Lord
Director of IIA, MRC-ARUK, CTIR - Professor of Immune Cell Biology
Welcome from the Institute of Cardiovascular Sciences (ICVS)

It is a pleasure for the College of Medical and Dental Sciences to host the 67th Annual Conference of the British Microcirculation Society & 29th Meeting of the UK Cell Adhesion Society. The University of Birmingham has a long history of collaboration with BMS and UKCAS, having provided many office holders and hosted meetings of each on several occasions over the years. This involvement dates back to the days of the Department of Physiology and happily continues now with the Institute of Cardiovascular Sciences (ICVS). The Institute was formed in October 2015 as part of a Life Sciences Strategy review and recognises the continuing success and strategic importance of Cardiovascular Sciences in Birmingham, including studies of microcirculation and cell adhesion in the vasculature. Our aim is to translate the very latest scientific research findings in cardiovascular sciences to benefit health.

The ICVS has two main themes: Clinical and Integrated Cardiovascular Sciences (CICS) and Vascular Inflammation, Thrombosis, and Angiogenesis (VITA). These are grouped around key researchers with internationally-recognised expertise in the pathways accelerating arterial and cardiac diseases, and in platelet, leukocyte, and vascular biology. The new Institute combines these themes to create an interdisciplinary academic environment conducive to collaborative research aimed at developing mechanism-based approaches to clinical problems. We use detailed genetic, molecular and cellular analyses, alongside morphological and functional study of heart and vessels in genetically modified animals, and robust clinical approaches ranging from epidemiological and population sciences to the design and conduct of clinical trials. In this way, we aim to tackle current and emerging cardiovascular epidemics, such as atrial fibrillation and heart failure (which often develop in interaction between cardiac dysfunction and concomitant conditions such as chronic kidney disease) and arterial and venous thrombosis.

Recent developments include new custom-built facilities for state-of-the-art imaging with a suite of intravital microscopes, including two-photon and Nipkow-disk confocal, and super-resolution fluorescent microscopy (PALM/dSTORM). These enable us to study circulating platelets, leukocytes and stem cells in models of cardiovascular disease, and to evaluate the kinetics of responses of receptors, cells and super-cellular cardiac and vascular structures. Investigations include live imaging of microvessels of the heart, as well as more standard preparations such as cremaster and mesenteric vessels, and analysis of thrombotic, inflammatory and angiogenic responses induced by vascular disturbances linked to small and large vessel disease.

We are very much open for business, and welcome attendees who would like to find out more about our facilities and activities.

Best wishes

Gerard Nash
Director of ICVS – Professor of Cardiovascular Rheology
A. dSTORM super-resolution imaging of collagen and LAT in platelets
B. State-of-the-art super-resolution Lattice sheet microscopy
C. Confocal image of endothelial tube formation
D. State-of-the-art spinning Nipkow confocal-based intravital microscope
E. Intravital imaging of thrombus formation in vivo post-laser injury
F. Intravital imaging of neutrophils and platelet thrombi in the beating murine heart in vivo
G. Lattice lightsheet imaging of actin and tubulin movement during proplatelet formation
H. Beating murine heart echocardiography
TRAVEL INFORMATION

From Birmingham International Airport to the University by taxi
We would recommend that you try to arrive at Birmingham International Airport. As a guide, the journey from the airport to the Medical School by taxi should take about 30 minutes (depending on traffic) and cost about £25-£30.

From Birmingham International Airport to Birmingham city centre by train
From the airport terminal building, you can take a free monorail connection to Birmingham International railway station. This operates every two minutes (journey time 90 seconds). Birmingham International railway station has frequent services to Birmingham New Street Station in the city centre (journey time around 15 minutes). From New Street Station it is possible to get a local train to Birmingham University station (second stop from New Street).

From London airports
If you are arriving at a London airport, there is a frequent train service from London Euston Railway Station to New Street Station. From Heathrow Airport, take the Heathrow Express train to Paddington Station and then the Underground or a taxi to Euston Station. Alternatively, an Airbus runs from Heathrow Airport direct to Euston Station. From Gatwick Airport, take the Airport Express train to Victoria Station and then the Underground or a taxi to Euston Station.

By rail
Most cross-country services to Birmingham arrive at Birmingham New Street Station. Up to six local trains an hour depart for the University, usually from platform 10 or 11 (final destination Longbridge or Redditch) with a journey time of 2-3 minutes. On leaving the University Station, turn right for the Medical School. Alternatively, there are taxi ranks at New Street Station and throughout the city centre. The journey to the University takes about 10 minutes.

Car parking
The University has a ‘Pay and Display’ system operating on the Edgbaston campus and visitors are advised to have change available. There are parking spaces available for visitors on Pritchatts Road Car Park (junction with Vincent Drive) the North Car Park and North East Car park (access via Pritchatts Road).

Visitor Parking rates:
up to 1 hour £2.00
1-3 hours £3.00
3-5 hours £4.00
5-8 hours £6.00
No cash, register with RingGo to pay via your credit/debit card via your mobile phone.

Please be advised that enforcement procedures are in place in all University parking areas and penalty charge notices will be issued to all vehicles that fail to comply with parking regulations.

Further Information is available at:
http://www.birmingham.ac.uk/facilities/mds-cpd/conferences/bms/venue.aspx
CONFERENCE DINNER

Banqueting Suite in the Birmingham Council House

The Council House is situated at the junction of **New Street, Paradise Street and Colmore Row** and is clearly marked by the monument of the Earth Goddess in front of it. This statue, officially called *The River*, is fondly called the *Floozie in the Jacuzzi* (although she now no longer sits in a fountain of water but in her own mini botanical garden!). Entrance to the building is in its exact centre, just behind the statue. Leaving New Street Station, it is advised to leave via the Pallisade exit rather than the Debenhams exit for an easier journey. If you chose to get a taxi to the Council House, the address is: Birmingham, B1 1BD. A sparkling drinks reception will be held here before the conference dinner at 7.00pm. **Music during the drinks reception and dinner will be provided by professional harpist, Angharad James.** The dinner will end with a disco – a late night bar will be available.
The meeting will be held in the Medical School – B1
Some of you may be staying at Lucas House – G16
University Station is opposite the Medical School – trains depart New Street Station in Birmingham city centre every 15 minutes for this station (approx. 2 minute journey).
Nearby attractions include the Winterbourne House and Gardens (G12) and the Barber Institute of Fine Arts (R14).